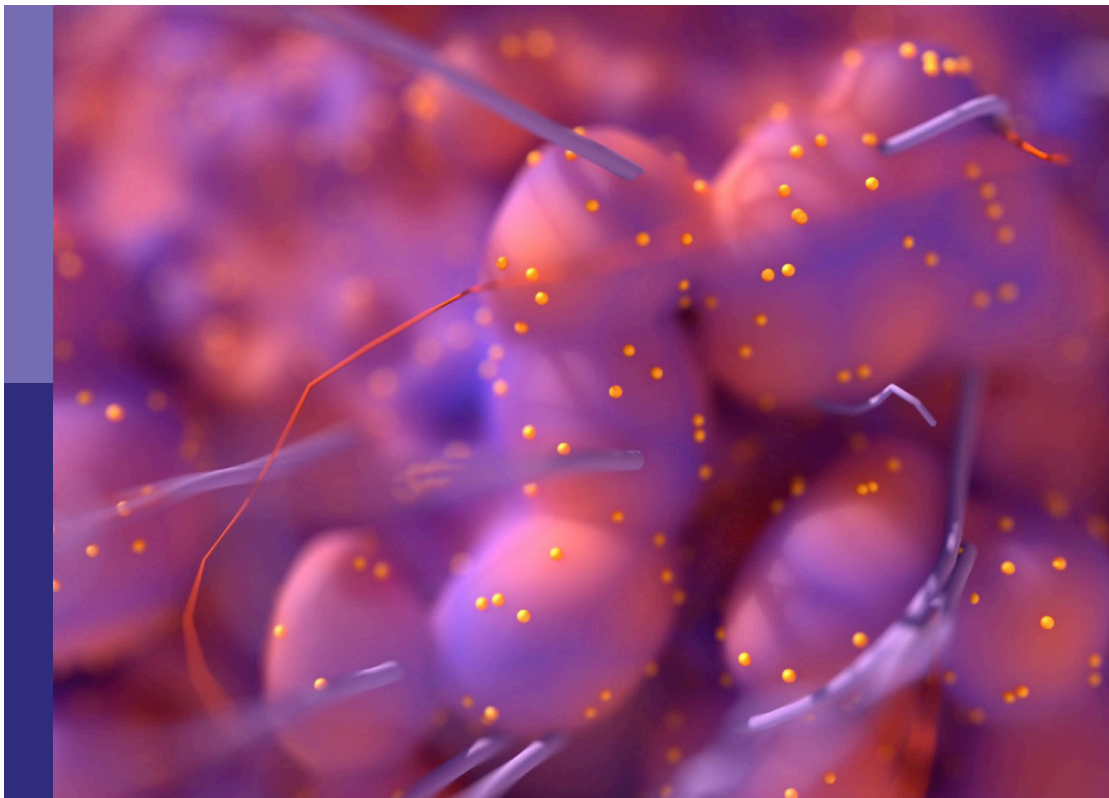
frontiers | Research Topics

Transplantation and cellular therapy in lymphomas and plasma cell disorders

Edited by
Saad Zafar Usmani, Nilanjan Ghosh, Edward Copelan and Peter Voorhees

Published in
Frontiers in Oncology



ISSN 1664-8714
ISBN 978-2-8325-5844-7
DOI 10.3389/978-2-8325-5844-7

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Transplantation and cellular therapy in lymphomas and plasma cell disorders

Topic editors

Saad Zafar Usmani — Levine Cancer Institute, United States
Nilanjan Ghosh — Levine Cancer Institute, United States
Edward Copelan — Atrium Healthcare, United States
Peter Voorhees — Levine Cancer Institute, United States

Citation

Usmani, S. Z., Ghosh, N., Copelan, E., Voorhees, P., eds. (2025). *Transplantation and cellular therapy in lymphomas and plasma cell disorders*.
Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-5844-7

Table of contents

05 Editorial: Transplantation and cellular therapy in lymphomas and plasma cell disorders
Saad Z. Usmani, Nilanjan Ghosh, Peter Voorhees and Edward Copelan

07 Autologous hematopoietic stem cell transplantation for multiple myeloma in the age of CAR T cell therapy
Charlotte F. M. Hughes, Gunjan L. Shah and Barry A. Paul

17 Novel and multiple targets for chimeric antigen receptor-based therapies in lymphoma
Yifan Pang and Nilanjan Ghosh

36 Beyond BCMA: the next wave of CAR T cell therapy in multiple myeloma
Kevin Miller, Hamza Hashmi and Sridevi Rajeeve

49 Mechanisms and management of CAR T toxicity
Christopher J. Ferreri and Manisha Bhutani

68 The treatment of follicular lymphoma with CD19-directed chimeric antigen receptor T-cell therapy
Ryan Jacobs and Caron Jacobson

76 Treatment of AL amyloidosis in the era of novel immune and cellular therapies
Caitlin Sarubbi, Hesham Abowali, Cindy Varga and Heather Landau

84 Chimeric antigen receptor T-cell therapy for aggressive B-cell lymphomas
Bei Hu, Victoria Korsos and M. Lia Palomba

97 Overview of infectious complications among CAR T- cell therapy recipients
Swarn Arya and Zainab Shahid

112 "Off-The-Shelf" allogeneic chimeric antigen receptor T-cell therapy for B-cell malignancies: current clinical evidence and challenges
Razan Mohty and Aleksandr Lazaryan

122 Obstacles to global implementation of CAR T cell therapy in myeloma and lymphoma
Fernando J. Medina-Olivares, Andrés Gómez-De León and Nilanjan Ghosh

131 Chimeric antigen receptor T-cells: a review on current status and future directions for relapsed/refractory multiple myeloma
Issam S. Hamadeh, Reed Friend, Sham Mailankody and Shebli Atrash

144 Re-examining the role of hematopoietic stem cell transplantation in relapsed large B-cell lymphoma in the era of chimeric antigen receptor (CAR) T-cell therapy
Tamara K. Moyo and Rakhee Vaidya

153 Survival outcomes of patients with diffuse large B-cell lymphoma undergoing autologous stem cell transplantation in Germany: real-world evidence from an administrative database between 2010 and 2019
Jan-Michel Heger, Peter Borchmann, Sybille Riou, Barbara Werner, Michael S. Papadimitrious and Jörg Mahlich

frontiers | Frontiers in Oncology

TYPE Editorial
PUBLISHED 10 December 2024
DOI 10.3389/fonc.2024.1527836

OPEN ACCESS

EDITED AND REVIEWED BY
Alessandro Isidori,
AORMN Hospital, Italy

*CORRESPONDENCE
Edward Copelan
edward.copelan@atriumhealth.org

RECEIVED 13 November 2024
ACCEPTED 20 November 2024
PUBLISHED 10 December 2024

CITATION
Usmani SZ, Ghosh N, Voorhees P and Copelan E (2024) Editorial: Transplantation and cellular therapy in lymphomas and plasma cell disorders.
Front. Oncol. 14:1527836.
doi: 10.3389/fonc.2024.1527836

Editorial: Transplantation and cellular therapy in lymphomas and plasma cell disorders

Saad Z. Usmani[1], Nilanjan Ghosh[2], Peter Voorhees[2] and Edward Copelan[2]*

[1]Myeloma Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, United States, [2]Atrium Health Levine Cancer Institute, Wake Forest University School of Medicine, Charlotte, NC, United States

KEYWORDS

hematopoietic cell transplantation, CAR T cells, lymphomas, multiple myeloma, cellular therapy

Editorial on the Research Topic
Transplantation and cellular therapy in lymphomas and plasma cell disorders

We, the editors of this Research Topic of Frontiers in Oncology, invite readers to examine the 13 expert perspectives provided on these timely topics. Hematopoietic cell transplantation, particularly autologous transplantation, has long been a standard of care in the treatment of many individuals with lymphomas or multiple myeloma. The safety and effectiveness of the procedure has improved in recent years and in this issue authorities in these malignancies detail its evolving, but critical, role in the context of the growing application of chimeric antigen receptor (CAR)-based therapies.

While allogenic transplantation was the first successful cellular therapy to treat hematologic malignancies, it is a rather blunt instrument and associated with significant toxicities and only modest effectiveness in lymphomas and multiple myeloma. Allotransplant's effectiveness relies on donor cells recognizing and attacking antigens on tumor cells which do not invoke autoreactivity. Most antigens expressed on malignant cells are also expressed on normal cells. The immune system has developed to avoid autoimmune reactions. Incorporation of synthetic receptors, derived from immunoglobulin to redirect T cell specificity, and the zeta chain from CD3, to activate function, produced first generation CAR T-cells (1), which evaded this tolerance (2).

The addition of costimulatory domains provided for expansion of functional CARs and persistence of these tumor-fighting cells (3). Clinical trials with CD19-directed CAR Ts showed durable complete responses in substantial proportions of patients with B cell malignancies including non-Hodgkin lymphoma (4) and subsequent trials confirmed these exceptional responses. Subsequently, dramatic responses to BCMA CAR T cells in multiple myeloma were demonstrated (5). In both lymphomas and multiple myeloma, the role of CAR Ts has assumed a growing role and moved steadily towards an earlier role in management.

This Research Topic details the appropriate role of CAR Ts and also details their toxicities, including B cell aplasia, cytokine release syndrome, neurotoxicity, and infections.

Perhaps most importantly, we include discussions of the obstacles to global implementation of CAR T therapy with a focus on disparities in access and of challenges to development of off-the-shelf CAR Ts. We hope this Research Topic provides better understanding of the appropriate use of transplantation and CART Ts at present and insight into the future.

Author contributions

SU: Writing – original draft, Writing – review & editing. NG: Writing – original draft, Writing – review & editing. PV: Writing – original draft, Writing – review & editing. EC: Writing – original draft, Writing – review & editing.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Eshhar Z, Waks T, Gross G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the gamma or zeta subunits of the immunoglobulin and T-cell receptors. *Proc Natl Acad Sci*. (1993) 90:720–4. doi: 10.1073/pnas.90.2.720

2. June CH, Sadelain M. Chimeric antigen receptor therapy. *N Engl J Med*. (2018) 379:64–73. doi: 10.1056/NEJMra1706169

3. Krause A, Guo HF, Latouche JB, Tan C, Cheung NK, Sadelain M, et al. Antigen-dependent CD28 signaling selectively enhance survival and proliferation in genetically modified activated human primary T lymphocytes. *J Exp Med*. (1998) 188:619–26. doi: 10.1084/jem.188.4.619

4. Kochenderfer JN, Wilson WH, Janik JE, Dudley ME, Stetler-Stevenson M, Feldman SA, et al. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood*. (2010) 116:4099–102. doi: 10.1182/blood-2010-04-281931

5. Ali SA, Shi V, Maric I, Wang M, Stroncek DF, Rose JJ, et al. T cells expressing an anti-B-cell maturation antigen chimeric antigen receptor cause remission of multiple myeloma. *Blood*. (2016) 128:1688–700. doi: 10.1182/blood-2016-04-711903

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 27 March 2024
DOI 10.3389/fonc.2024.1373548

OPEN ACCESS

EDITED BY
Pasquale Niscola,
Sant'Eugenio Hospital of Rome, Italy

REVIEWED BY
Joselle Cook,
Mayo Clinic, United States
Guillermo José Ruiz-Argüelles,
Clínica Ruiz, Mexico

*CORRESPONDENCE
Barry A. Paul
Barry.paul@atriumhealth.org

RECEIVED 19 January 2024
ACCEPTED 15 March 2024
PUBLISHED 27 March 2024

CITATION
Hughes CFM, Shah GL and Paul BA (2024) Autologous hematopoietic stem cell transplantation for multiple myeloma in the age of CAR T cell therapy.
Front. Oncol. 14:1373548.
doi: 10.3389/fonc.2024.1373548

Autologous hematopoietic stem cell transplantation for multiple myeloma in the age of CAR T cell therapy

Charlotte F. M. Hughes[1], Gunjan L. Shah[1,2] and Barry A. Paul[3]*

[1]Adult Bone Marrow Transplant Service, Memorial Sloan Kettering Cancer Center, New York, NY, United States, [2]Cellular Therapy Service, Memorial Sloan Kettering Cancer Center, New York, NY, United States, [3]Department of Hematologic Oncology and Blood Disorders, Levine Cancer Institute, Atrium Health/Wake Forest Baptist, Charlotte, NC, United States

Chimeric antigen receptor (CAR) T cell therapy has revolutionized the management of relapsed and refractory myeloma, with excellent outcomes and a tolerable safety profile. High dose chemotherapy with autologous hematopoietic stem cell transplantation (AHCT) is established as a mainstream of newly diagnosed multiple myeloma (NDMM) management in patients who are young and fit enough to tolerate such intensity. This standard was developed based on randomized trials comparing AHCT to chemotherapy in the era prior to novel agents. More recently, larger studies have primarily shown a progression free survival (PFS) benefit of upfront AHCT, rather than overall survival (OS) benefit. There is debate about the significance of this lack of OS, acknowledging the potential confounders of the chronic nature of the disease, study design and competing harms and benefits of exposure to AHCT. Indeed upfront AHCT may not be as uniquely beneficial as we once thought, and is not without risk. New quadruple-agent regimens are highly active and effective in achieving a deep response as quantified by measurable residual disease (MRD). The high dose chemotherapy administered with AHCT imposes a burden of short and long-term adverse effects, which may alter the disease course and patient's ability to tolerate future therapies. Some high-risk subgroups may have a more valuable benefit from AHCT, though still ultimately suffer poor outcomes. When compared to the outcomes of CAR T cell therapy, the question of whether AHCT can or indeed should be deferred has become an important topic in the field. Deferring AHCT may be a personalized decision in patients who achieve MRD negativity, which is now well established as a key prognostic factor for PFS and OS. Reserving or re-administering AHCT at relapse is feasible in many cases and holds the promise of resetting the T cell compartment and opening up options for immune reengagement. It is likely that personalized MRD-guided decision making will shape how we sequence in the future, though more studies are required to delineate when this is safe and appropriate.

KEYWORDS

autologous transplant, CAR T cell therapy, multiple myeloma, newly diagnosed multiple myeloma, relapsed refractory multiple myeloma

1 Introduction

Multiple myeloma (MM) is a cancer of terminally differentiated plasma cells in the bone marrow. For 2023, an estimated 35,730 new cases will be diagnosed in the United States which represents 1.8% of all cancers and 19% of all hematologic malignancies (1). The advent of high doses of melphalan with autologous hematopoietic stem cell transplantation (AHCT) was a major advance and led to improved response rates (RR), progression free survival (PFS), and, in some trials, prolonged overall survival (OS) in patients with newly diagnosed MM (NDMM) and has been a cornerstone of treatment in eligible patients for the last 20 years (2–6). While there are no universally agreed upon transplant eligibility criteria and several risk stratification tools have been proposed, factors such as age, baseline performance status, and comorbidities are important tenets in determining a patient's eligibility (7–10). Recently, adoptive cell therapy using BCMA-directed autologous chimeric antigen receptor (CAR) T therapies have been tested in patients with relapsed refractory multiple myeloma (RRMM) and have shown unprecedented response rates, depth of response, and improved PFS when compared to standard of care (SOC) regimens (11–14). Currently, CAR T cells are under investigation for use as consolidation after induction therapy in transplant ineligible patients and are being compared head-to-head against high-dose melphalan and AHCT in large phase III trials of transplant eligible patients (15, 16). While these trials are yet to report, there is significant reason to believe that CAR T therapy may lead to improved outcomes as T-cell fitness - which is a prime driver of CAR T success - has been shown to decline with increasing lines of MM directed treatment (17–20). This also leads to the question of whether these therapies should be sequenced or are mutually exclusive. In this manuscript, we review the current data for each of these modalities and discuss trials currently evaluating AHCT and CAR Ts for patients with MM. Finally, we provide our thoughts on the role of each of these treatments in MM therapy in the United States where both options are commercially available.

2 CAR T in MM

CAR T cell therapies have revolutionized the treatment of RRMM. Most CAR T target B cell maturation antigen (BCMA) also known as TNFRSF17 or CD269; a type III transmembrane glycoprotein and non-tyrosine kinase receptor in the tumor necrosis factor receptor (TNFR) superfamily (21, 22). Expression of BCMA is selectively induced during plasma cell differentiation. Expression is nearly absent on naïve and memory B cells but is ubiquitously expressed on plasmablasts and plasma cells (23–25). BCMA expression is rare in other tissues with only low-level BCMA mRNA and protein expression seen in areas with endogenous plasma cell populations (i.e., the testes, gastrointestinal tract and trachea) (25). Additionally, expression of plasma cell BCMA increases with progression from monoclonal gammopathy of undermaintained significance (MGUS), to smoldering multiple myeloma (SMM) and MM. Higher levels of soluble BCMA has been associated with shorter time to progression in MGUS and SMM patients, and higher levels surface BCMA is associated with worse prognosis in MM patients (26–31). Several different modalities (antibody drug conjugates, bispecific antibodies, and CAR T) have been designed to target BCMA. Two BCMA CAR T: idecabtagene vicleucel (ide-cel) and ciltacabtagene autoleucel (cilta-cel) have been FDA approved for RRMM based on outcomes seen in large phase 2 trials.

2.1 Idecabtagene vicleucel

Ide-cel is a second-generation CAR which uses a lentiviral vector to transduce a BCMA targeting scFv fused to a 4-1BB co-stimulatory and CD3 signaling domains (32, 33). The pivotal phase II KarMMa study evaluated ide-cel at various doses in 128 RRMM patients who had previously received ≥ 3 prior lines of therapy including an immunomodulatory drug (IMiD), proteasome inhibitor (PI), and an anti-CD38 monoclonal antibody. Patients were infused with 150×10^6 to 450×10^6 CAR T cells. Overall response rate (ORR) was 73%, with 42 (33%) patients achieving a complete response (CR) or better. Measurable residual disease (MRD) negativity (at 10^{-5}) was achieved in 26% of all patients, but 79% of patients who achieved a ≥ CR. Notably, these response rates were relatively preserved across patients with high-risk features including penta-refractory disease, extramedullary disease, and high-risk cytogenetics. Median PFS was 8.8 months, but was 20.2 months in patients who achieved ≥ CR (11). OS for the KarMMa study was 24.8 months, but interestingly, was lower in the cohort of patients who had previously been treated with 3 prior lines (median OS 22 months) versus those who had been treated with 4 or more prior lines (median OS 25.2 months) (34). Based on these findings, ide-cel was approved for the treatment of adults with RRMM after four or more prior lines of therapy including an IMiD, PI, and anti-CD38 monoclonal antibody by the US Food and Drug Administration (FDA) in March 2021. In a *post-hoc* analysis of the KarMMa trial, lower levels of serum soluble BCMA, more robust blood and bone marrow CAR T expansion, and an increased ratio of naive and early memory CD4 T cells compared to senescent CD3 and CD8 T cells in the apheresis product prior to manufacturing were associated with improved response to ide-cel (35). Subsequently, real-world data for patients treated with commercial ide-cel outside the context of a clinical trial showed very similar efficacy. ORR in this population was 84% with 42% achieving ≥ CR. Median PFS (8.5 months) at 6.1 months of follow-up was similar to the KarMMa data. Notably, 75% of the patients included in this cohort would have been deemed ineligible to enroll on the KarMMa trial (36). Predictors of poor response to ide-cel in the real-world cohort included prior BCMA therapy, high-risk cytogenetics, elevated baseline ferritin level, and younger age (36, 37).

More recently ide-cel was evaluated against investigator's choice of one of 5 SOC regimens: daratumumab, pomalidomide, and dexamethasone; daratumumab, bortezomib, and dexamethasone; ixazomib, lenalidomide, and dexamethasone; carfilzomib and dexamethasone; or elotuzumab, pomalidomide, and dexamethasone in RRMM patients who had received 2-4 prior

lines of therapy including an IMiD, PI, and an anti-CD38 monoclonal antibody in the phase III KarMMa-3 trial. This trial enrolled a substantial population (43%) of patients with high-risk cytogenetics (defined as presence of del17p, t[4;14], or t[14;16]) which were evenly distributed across both arms. The ORR was 71% for ide-cel and 42% for SOC. Median PFS in the intention-to-treat population was substantially higher in the ide-cel arm (13.3 months vs 4.4 months). The hazard ratio for disease progression or death for ide-cel vs SOC was 0.49 (95% CI 0.38 - 0.65). Ide-cel showed similar improved hazard ratios for disease progression or death in patients with high-risk cytogenetics (HR 0.61; 95% CI 0.41-0.90), extramedullary disease (HR 0.40; 95% CI 0.25-0.65), and disease refractory to at least one IMiD, PI, and anti-CD38 monoclonal antibody (HR 0.46; 95% CI 0.34-0.62) (14, 38). The FDA is currently reviewing a supplemental biologics license for the approval of ide-cel in this less heavily pretreated population based on the data from the KarMMa-3 trial. Additionally, cohort 2c of the phase II KarMMa-2 trial is evaluating the efficacy of ide-cel in patients with newly diagnosed MM (NDMM) who had an inadequate response to frontline AHCT. In this population, 77% of patients achieved ≥ CR, and median PFS has not be reached at a median follow-up of 39.4 months (39).

2.2 Ciltacabtagene autoleucel

Similar to ide-cel, cilta-cel uses a lentiviral vector to create a construct with a CD3ζ activation domain, and 4-1BB costimulatory domain. Cilta-cel's antigen binding domain contains bispecifc scFvs targeting two distinct BCMA epitopes, VHH1 and VHH2 (40). This bi-epitope binding confers higher avidity and specificity to BCMA. Cilta-cel was evaluated in the phase Ib/II CARTITUDE-1 trial RRMM patients who had previously been treated with ≥ 3 or were double refractory to an IMiD and a PI, and had previously received an anti-CD38 antibody. Ninety-seven patients were treated with cilta-cel (29 in the phase Ib portion, 68 in the phase II portion). The population was heavily pretreated (median of 6 prior lines; 84% penta-exposed) and included 42% who were penta-refractory (12). ORR was 98%, with 95% of patients achieving a VGPR or better and 82.5% of patients achieving a sCR. MRD negativity was evaluated in 61 patients at 10^{-5} and 52 patients at 10^{-6}. MRD negativity rates were 92% and 75% at 10^{-5} and 10^{-6} respectively (41). Median PFS was 34.9 months; median OS has not been reached at 27.7 months of follow-up (42). Based on these data cilta-cel was approved for the treatment RRMM patients following 4 or more prior lines of therapy, including an IMiD, PI, and an anti-CD38 monoclonal antibody in Feb 2022. Real-world data for cilta-cel is not as mature as that for ide-cel. However, in an early analysis a multi-institutional cohort of 143 patients infused with commercial cilta-cel-of whom 57% would have been ineligible for participation in the CARTITUDE-1 trial-ORR was 84% with 53% ≥ CR. Notably, 22% of patients included in this dataset were infused with an out of specification (OOS) cilta-cel product. The presence of high-risk cytogenetics (defined as the presence of del17p, t[4;14)], or t[14;16]) was associated with poorer ORR, PFS, and OS in multivariate analysis (43).

Cilta-cel was also evaluated in less heavily pretreated patients in the phase III CARTITUDE-4 trial. This trial randomized 419 RRMM patients previously treated with 1-3 prior lines to either cilta-cel or investigator's choice of 2 SOC regimens: pomalidomide, bortezomib, dexamethasone, or daratumumab, pomalidomide, dexamethasone. The trial strongly favored the cilta-cel arm with ORR of 84.6% vs 67.3% for SOC. Responses were deeper in the cilta-cel arm (73.1% vs 21.8% ≥ CR) which translated into improved PFS (median PFS not reached for cilta-cel, 11.8 months for SOC; HR 0.26; 95% CI, 0.18 -0.38). Subgroup analysis also favored cilta-cel for all parameters tested including high-risk cytogenetics, prior lines of therapy, degree of refractoriness, and presence of extramedullary disease (13, 44). Cilta-cel is currently under evaluation for patient with suboptimal response to frontline transplant, in treatment naïve high-risk and standard risk NDMM in cohorts D, E, F of the mutli-cohort CARTITUDE-2 trial.

2.3 CAR T toxicities

While these data illustrate the efficacy of both ide-cel and cilta-cel, both of these agents have important toxicities that need to be factored into any suitability discussion. Cytokine release syndrome (CRS) is a systemic inflammatory response thought to be secondary to activation of bystander immune and nonimmune cells resulting in significant cytokine release-especially IL-1, IL-2, IL-6, GM-CSF, and IFN-g (45–48). This inflammatory storm typically manifests as fever, fatigue, headache, arthralgias, and myalgias, but higher-grade manifestations including hypotension, shock, disseminated intravascular coagulation, and multiorgan system failure can occur (49, 50). Treatment of CRS ranges from supportive care with antipyretics, intravenous fluids, and supplemental oxygen for lower grade symptoms to vasopressors, the anti-IL-6 antibody tocilizumab, and high-dose corticosteroids for higher grade symptoms (50, 51). While low grade CRS is very common with both ide-cel and cilta-cel the timing of CRS onset varies for each CAR-T product (see Table 1). Ide-cel was associated with 84% CRS in the KarMMa trial with the majority being low grade (5% grade 3 or 4). Median onset of CRS with ide-cel in the KarMMa trial, the KarMMa-3 trial, and the real-world dataset was reliably 1 day (range 0-14) (11, 14, 36). In the CARTITUDE-1 trial 95% of patients experienced CRS with 4% grade 3 or 4. Median onset of CRS with cilta-cel in the CARTITUDE-1 trial was 7 days, while it was 8 days in the CARTITUDE-4 trial, and 9 days in the real-world cohort (12, 13, 43). These data are summarized in Table 1.

Immune effector cell-associated neurotoxicity syndrome (ICANS) is another adverse effect common to CAR-T therapy. ICANS is a toxic encephalopathy thought to be related to endothelial cell activation and disruption of the blood brain barrier mediated by inflammatory cytokines and chemokines, which results in direct neuronal cell injury. Mild symptoms include headache, confusion, focal neurologic deficits, and impaired fine motor skills. Higher grade ICANS can manifest with aphasia, seizure, cerebral edema, and coma (49). The mainstay of ICANS management is high-dose corticosteroids; additional supportive measures including mechanical ventilation

TABLE 1 Selected Toxicities in BCMA CAR-Ts.

Toxicity	Ide-cel (KarMMa)	Ide-cel (KarMMa-3)	Ide-cel (Real World)	Cilta-Cel (CARTITUDE-1)	Cilta-Cel (CARTITUDE-4)	Cilta-Cel (Real World)
Number of prior Lines	≥3	2-4	≥4	≥3	1-3	≥4
ORR (%)	73	71	84	97	85	84
Median PFS (months)	8.8	13.3	8.5	34.9	NR	NR
% CRS (all; grade 3/4)	84, 5	88, 4	82, 3	95, 4	76, 1	80, 5
Median Onset of CRS (range)	1 day (1-12)	1 day (1-14)	1 day (0-14)	7 days (5-8)	8 days (1-23)	9 days (1-23)
% Neurotoxicity (all; grade 3/4)	18, 3	15, 3	18, 6	17, 2	21, 3	18, 6
Median Onset of ICANS (range)	2 days (1-10)	3 days (1-317)	3 days (0-15)	8 days (6-8)	9.5 days (1-6)	9.5 days (1-6)
% Delayed Neurotoxicity (all; grade 3/4)	0	0	1	12, 8	NR	12, NR

ORR, Overall response rate; CRS, Cytokine release syndrome; ICANS, Immune effector cell associated neurotoxicity syndrome; NR, Not reached.

(if evidence of airway compromise) may be needed. Tocilizumab is generally only used if patients have coexisting CRS (50, 51). ICANS typically manifests later than CRS (around day 2-3 with ide-cel and days 8-10 with cilta-cel) (11–14, 36, 43). An additional neurologic toxicity seemingly unique to cilta-cel is less well understood. Termed movement and neurocognitive treatment-emergent adverse events (MNTs), they compromise a cluster of movement (e.g., micrographia, tremors), cognitive (e.g., memory loss, disturbance in attention), and personality changes (e.g. reduced facial expression, flat affect) which typically manifest after symptoms of CRS and ICANS have resolved, and unlike ICANS, are generally not responsive to steroids (52, 53). While the unique AEs associated with CAR-Ts are certainly cause for concern, with increasing ubiquity of this class of therapy and earlier recognition of, and intervention for, CRS and ICANS the rates of higher-grade AEs are decreasing in more recent trials (summarized in Table 1). Ideally, less high-grade CRS and ICANS will lead to less delayed neurotoxicity and MNT events.

Finally, the risk of secondary primary malignancies, long known to be an adverse effect of several myeloma therapies is largely unknown post-CAR T. However, recent data does raise concern for a possible increased risk. Specifically, rare reports of T-cell lymphomas derived from CAR T cells have been reported in several CAR T recipients. To date, only 12 cases have been reported out of the 7946 patients infused with CAR Ts, indicated predominately for B-cell lymphomas (54). Only 1 case has been reported in a myeloma patient who was treated with cilta-cel, but analysis of the patient's apheresis product (prior to CAR T manufacture) suggested that they had several genetic mutations present at baseline and the role of the CAR is unclear (55). Similarly, myeloid malignancies have been reported with post CAR T. In the long-term follow-up of the CARTITUDE-1 trial 9 patients (9%) have been diagnosed with MDS or AML (42). However, it is unclear whether this a result of the CAR T or lymphodepleting chemotherapy. To that effect, a recent analysis of 4 patients who developed MDS after treatment with an investigational anti-BCMA CAR T showed that while none of the patients had morphologic changes consistent with MDS prior to CAR T infusion, all four patients exhibited molecular alterations associated with MDS in their pre-CAR T as well as post-CAR T therapy bone marrow with no new mutations observed after CAR T (56). Clearly further follow-up is warranted.

3 Autologous hematopoietic stem cell transplant

High-dose chemotherapy with melphalan followed by AHCT is well established as standard of care for patients with NDMM who are sufficiently fit to tolerate intensive therapy, ie, are transplant eligible. The principle of this practice is to induce disease control and collect a clean stem cell product following a limited induction, then administer powerful anti-myeloma therapy which would only be feasible with a stem cell rescue. It was theorized and then confirmed that this would provide a PFS benefit if offered early in the treatment course, as opposed to deferring it until a later relapse. Foundational studies showed improved PFS and OS and were a gamechanger in the natural history of myeloma (2, 57, 58). Short-term treatment related adverse events include obligate cytopenias, as well as infections, gastrointestinal upset, and mucositis (5, 59, 60). Despite these acute effects, treatment-related mortality remains low, and though quality of life is impacted in the short-term, these effects appear to be transient and recover post AHCT (59, 61, 62).

When PI-IMiD combination therapy became standard care (63), new studies were required to update our understanding of the true benefit of upfront AHCT with modern regimens and are summarized in Table 2. The IFM 2009 trial published in 2017 (5), included induction with three cycles of lenalidomine, bortezomib, dexamethasone (RVd) and 1 year of maintenance lenalidomide after consolidation and demonstrated a PFS benefit, which was sustained in the updated long-term follow up data (64). No difference in OS was noted at 4 years. The DETERMINATION trial included maintenance until disease progression and demonstrated a greater PFS than its IFM precursor, and confirmed the PFS benefit of AHCT when added to RVd, but again did not show an OS advantage at 72 months follow-up (59). The FORTE study looked at new generation PI carfilzomib(K)-based regimens as induction and confirmed the PFS benefit of

TABLE 2 Recent trials comparing upfront versus delayed AHCT in patients with NDMM.

	Induction regimen in AHCT group	PFS (months) (Upfront AHCT vs control)
IFM-2009	VRd	Median: 50 vs 36
EMN-02/H095	VCd	Median: 56.7 vs 41.9
FORTE	KRd*	3-year PFS: 56% vs 33%
DETERMINATION	VRd	Median: 67.5 vs 46.2
Cardamon	KCd	Median: 42.4 vs 33.8

VRd, bortezomib, lenalidomide, dexamethasone; VCd, bortezomib, cyclophosphamide, dexamethasone; KRd, carfilzomib, lenalidomide, dexamethasone; KCd, carfilzomib, cyclophosphamide, dexamethasone.
*Other arm (KCd) not presented here.

upfront AHCT with both KRd and KCd induction (65). In the CARDAMON trial published in 2022, investigators determined that PFS of KCd alone was not non-inferior to upfront AHCT following KCd induction at 2 years (66).

Newer studies which have included the addition of anti-CD38 antibody agents to a triplet backbone have led to deep responses when used together with AHCT and are summarized in Table 3. Of note, none of these quad-therapy studies have compared upfront AHCT to a deferred- or non-AHCT control arm. In CASSIOPEA, Dara-DTd plus AHCT induced excellent response rates and MRD negativity compared to DTd plus AHCT alone, with a 93% 2-year PFS in the quad-containing arm (67). The phase II GRIFFIN trial tested Dara-VRd as induction with AHCT and showed similarly excellent 2-year PFS at 95.8% (68). The recently published phase III PERSEUS trial compared Dara-VRd vs VRd in induction and post-AHCT consolidation, and demonstrated a significant improvement in PFS with the addition of Dara (84.3% 4-year PFS, vs 67.7% in the VRd only group) (6). Importantly PERSEUS reported a powerful depth of response with 75.2% of patients in the D-VRd group achieving MRD-negativity. Similarly, the phase III IsKia trial compared the combination of Isatuximab-KRd to KRd alone with AHCT and consolidation showed significant improvement in MRD negativity rates with the quadruplet (MRD at 10^{-5} 77% vs 67%; MRD at 10^{-6} 67% vs 48% respectively) (69). It is important to consider whether the benefits seen from these therapies are attributable more to the addition of the anti-CD38 agent itself, as opposed to the quad-agent nature of combination. Indeed, more drugs are not necessarily better, as demonstrated in trials of other four-drug inductions outside the anti-CD38 setting, suggesting they may perform as well as three-drug combinations (70). Additionally, the MAIA trial showed a significant PFS and OS with the addition of daratumumab to Rd in transplant ineligible patients (71), though this abridged regimen has not been studied in the AHCT setting.

3.1 Impact of disease risk

Cytogenetics, and more recently MRD status, have been shown to correlate with survival. In a 2022 evaluation of the impact of AHCT with quad-therapy induction in NDMM, MRD was assessed by NGS pre and post AHCT, and the group with the greatest reduction in MRD burden had high-risk cytogenetics (HRCG) demonstrating a 'dose effect' with stepwise greater reduction in those with 0, 1 or 2+ HRCG abnormalities (72). Those with more than 2 HRCG abnormalities – so called ultra-high risk – have worse outcomes as demonstrated in subgroup analysis of MASTER and GRIFFIN trials (73). Though among ultra-high risk patients, those who achieve MRD negativity prior to or after AHCT have improved outcomes (74). In IFM 2009 long term follow up subgroup analysis, PFS (HR 0.28, p<0001) and OS (HR 0.35, p<0.001) was longer in patients who became MRD negative (64), and in DETERMINATION, there was no PFS difference between AHCT and non-AHCT therapy in patients who achieved MRD negativity (59). (59). In the CARDAMON trial, of the 22.8% of patients who achieved MRD negativity following induction, analysis suggested there was no benefit from AHCT gained in this group (66). A large retrospective study of NDMM patients who achieved a VGPR or greater after induction therapy assessed the MRD status by next-generation flow cytometry and found pre-AHCT MRD positivity was

TABLE 3 Benefit of anti-CD38 containing quad-therapy in newly diagnosed myeloma.

	Use of AHCT	Induction regimen	PFS	OS	MRD-Negative Rate (%, time-point, sensitivity)
CASSIOPEA	All arms received upfront AHCT	Dara-VTd	93% 2-year PFS	Not reported	64% at 100 days post-AHCT (10^{-5})
GRIFFIN	All arms received upfront AHCT	Dara-VRd	95.8% 2-year PFS	92.7% 4-year OS:	Post-induction: 22%/1% Post-consolidation: 50%/11% Post-1-year-maintenance: 59%/21% End of study: 64%/36% (10^{-5}/10^{-6})
PERSEUS	All arms received upfront AHCT	Dara-VRd	84.3% 4-year PFS	Not reported	75%/65% any timepoint during study (10^{-5}/10^{-6}) 64.8% sustained negativity for ≥12months (10^{-5})
MASTER	All arms received AHCT	Dara-KRd	87% 2-year PFS	94% 2-year OS	81%/71% at post-consolidation (10^{-5}/10^{-6})
MANHATTAN	No AHCT	Dara-KRd	98% 1-year PFS	100% 1-year OS	71% post-cycle 8 (10^{-5})

Dara-VRd, daratumumab, bortezomib, lenalidomide, dexamethasone; Dara-VTD, daratumumab, bortezomib, thalidomide, dexamethasone; Dara-KRd, daratumumab, carfilzomib, lenalidomide, dexamethasone.

associated with a shorter PFS (48.2 months vs 80.1 months, p<0.001) (75). Finally, the single-arm MASTER trial attempted to use MRD negativity to guide decision making in patients receiving Dara-KRd induction followed by upfront AHCT and Dara-KRd consolidation, ceasing treatment when a patient achieved two consecutive MRD-negative readings. This strategy showed promising PFS and rates of MRD negativity (76). HRCG patients in the MASTER trial had far poorer PFS, especially when their therapy was stopped-and achievement of MRD negativity.

3.2 Role of autograft at relapse

There have been several retrospective studies evaluating responses to a second or third AHCT in the setting of RRMM, demonstrating this a feasible and safe approach, which may provide PFS benefit (77–82). Further retrospective subgroup analysis studies have demonstrated the benefit of salvage AHCT is greater in those who had a longer duration of response with their first AHCT (83, 84). This was recently called into question when long term follow-up of the GMMG ReLApsE trial did not show a difference in PFS or OS, but patients were not allowed onto the study if lenalidomide refractory, and therefore likely not generalizable to the current RRMM population (85). The interim analysis of the prospective single arm Second Chance trial shows deep responses with median PFS not reached when using Dara-KRD with salvage AHCT in the early relapse setting (86).

Melphalan retains its potent disease control even in the post CAR T setting. In a recent assessment of salvage therapies after relapse following BCMA-directed CAR T cell treatment, there appeared to be a reasonable response with 71.4% ORR, and an OS of 23.2 months in those who underwent AHCT or allogeneic HCT. Many of these patients were refractory to multiple lines of therapy (median 5 lines prior to CAR T) and the vast majority (94.9%) had had a prior AHCT (87). Salvage AHCT holds theoretical appeal in augmenting the biology of relapsed myeloma to wipe the slate clean of a heavily exposed patient. The rationale here is twofold: to gain clonal control and reset the immune milieu (88). There is a pattern of immune dysregulation and microenvironment abnormalities seen in myeloma patients with reduced NK and T cells and increased immunosuppressive cells, particularly T regulatory cells (89). This dysfunction worsens with exposure to anti-myeloma agents (90, 91). With an infusion of relatively chemotherapy naïve autologous stem cells, there opens up an opportunity for myeloma-specific immunity to be regained. In particular, the pattern of dynamics of T cell reconstitution after AHCT with a favorable ratio of T regulatory to T effector cells (92–94), may be able to be harnessed to leverage the sensitivity to immune therapies including CAR T (95). This is being tested prospectively prior to CAR T cells in NCT05393804 with the hypothesis that "fresh" non-exhausted T cells will lead to better expansion and persistence of the CAR T cell made from these cells. Furthermore, the early recovery of NK cells after AHCT may provide an opportunity to maximize potency of NK cell-therapies in this window (96).

4 Discussion: CAR T or AHCT or both?

It remains very difficult to show OS benefit in any modern comparative trial for MM given the median 7-10 year survival quoted for standard risk patients and significant crossover that occurs in many trials. Increasingly, patients' OS is based on sequential progression free intervals in which the optimal sequence is unclear and ever changing due to newer data, approvals, and guidelines (97–99). The considerations we present in this section presume the indications approved in the United States in early 2024, and we acknowledge that in other parts of the world, these discussions differ based on availability and cost (100–102).

Firstly, studies of delayed AHCT, performed >12 months after diagnosis, suggested a reasonable response to this approach with a similar median time to progression and no difference in OS rates (5, 59). A major issue seen in the IFM2009 study was that 21% of those randomized to delayed AHCT -and deemed transplant eligible at randomization - were not able to later receive a salvage AHCT (5). A more recent retrospective comparison of upfront or delayed AHCT, found that delayed AHCT did not result in worse OS or PFS even when adjusting for age, disease risk, or depth of response at time of collection, but interestingly highlighted that those who underwent delayed AHCT frequently received a lower melphalan dose, reflective of mounting medical complexity with the passage of time and disease evolution (103). Data on the outcomes and safety of CAR T in frail patients suggests a relatively tolerable profile in this group, giving some weight to the argument that reserving CAR T for later in a patient's course may be a more deliverable sequence (104, 105).

Second, some believe that the post CAR T cell journey is much easier than after AHCT, but this may not always be the case. Prolonged cytopenias, immune compromise, CRS, ICANS, MNTs, and infection risk, and the requirements to stay within a certain distance of the treating facility can impact qualify of life (QoL) after CAR T infusion. Comparisons show that the recovery to baseline may not be that different between the two modalities (106, 107).

Increasingly concerning is the risk of secondary malignancies. A CIBMTR analysis recently reported a risk of 4% at a median of 37 months of follow-up after AHCT, and though most of these patients eventually died from their myeloma rather than the secondary malignancy, these patients had a reduced PFS and OS (108). However, studies have also demonstrated that melphalan exposure and AHCT (+/- lenalidomide exposure) increase the mutational burden in patients with MM (109, 110). On the CAR T side, the updated analysis of CARTITUDE-1 showed 16/97 (16%) had a secondary malignancy with 9 (9%) being myelodysplastic syndrome or acute myeloid leukemia (42). It true risk of CAR T derived T cell lymphoma is not yet clear, and impacts on monitoring guidelines yet to be established (55, 111). The etiology of these findings, and whether it may manifest with earlier use of CAR T are not yet known.

Practical and financial considerations will inevitably shape the uptake of these therapies, and incremental cost effectiveness analysis should be factored into paradigm development. CAR T therapy costs

are known to be dependent on rates of CRS and ICANS, and resource requirements may be prohibitive in some settings (112, 113). AHCT and CAR T costs may be reduced with utilization of outpatient care packages, however institutions need to have the resources and quality systems in place in order to safely facilitate the delivery of outpatient care, which can be a limiting factor particularly in low- and middle-income countries (LMIC) (114). In the LMIC setting, uptake of more efficacious practice may be limited at least in the short-term by costs, and we should be mindful of the increasing gap of resource-intensive and high-cost practices between high-, middle- and low-income settings (115, 116). Short-term focus can be premature however, and recent analyses have suggested more intensive therapies upfront may not only offset costs but leads to a long-term cost savings (117). Given the chronic nature of MM, our continued improvement in managing side effects, shortening hospital length of stay, and generally improving safety of both AHSCT and CAR T will be increasingly important to consider when evaluating the economic and quality of life impact. This will be especially important as CAR T migrates into less academic institutions where the systems to ensure adequate supportive care may need to be optimized. Additionally, when considering the prospect of bringing CAR T therapy to earlier lines of treatment, we will need to understand the value beyond traditional efficacy alone, with demonstration of quality-adjusted life years and other patient-reported outcomes, and the cost (both short- and long-term) to the healthcare system (97).

Overall, patients with MM will likely have both CAR T and AHCT during their treatment course. Sequencing depends on approvals and availability of the options, and will change over time as more treatments are available in earlier lines and with the results for the frontline prospective studies mentioned above. Prior toxicities and comorbidities, as well as concerns for future determents to quality of life and risk of secondary malignancies, allow for discussion and personalization of treatment. Optimizing both of these very effective modalities can allow patients to have long progression free remissions, which may even allow for a yet undescribed curative mechanism of action therapy to be approved.

Author contributions

CH: Writing – original draft, Writing – review & editing. GS: Conceptualization, Supervision, Writing – review & editing. BP: Conceptualization, Supervision, Writing – review & editing, Writing – original draft.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

GS received research funding from Janssen, Amgen, Bristol Myers Squibb, Beyond Spring, and GPCR, and served on the data safety monitoring board for Arcellx. BP has research funding from Bristol Myers Squibb, and served as a consultant or in an advisory role for Janssen and Abbvie.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. *CA Cancer J Clin*. (2023) 73:17–48. doi: 10.3322/caac.21763

2. Child JA, Morgan GJ, Davies FE, Owen RG, Bell SE, Hawkins K, et al. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. *N Engl J Med*. (2003) 348:1875–83. doi: 10.1056/NEJMoa022340

3. Blade J, Rosinol L, Sureda A, Ribera JM, Diaz-Mediavilla J, Garcia-Larana J, et al. High-dose therapy intensification compared with continued standard chemotherapy in multiple myeloma patients responding to the initial chemotherapy: long-term results from a prospective randomized trial from the Spanish cooperative group PETHEMA. *Blood*. (2005) 106:3755–9. doi: 10.1182/blood-2005-03-1301

4. Palumbo A, Cavallo F, Gay F, Di Raimondo F, Ben Yehuda D, Petrucci MT, et al. Autologous transplantation and maintenance therapy in multiple myeloma. *N Engl J Med*. (2014) 371:895–905. doi: 10.1056/NEJMoa1402888

5. Attal M, Lauwers-Cances V, Hulin C, Leleu X, Caillot D, Escoffre M, et al. Lenalidomide, bortezomib, and dexamethasone with transplantation for myeloma. *New Engl J Med*. (2017) 376:1311–20. doi: 10.1056/NEJMoa1611750

6. Sonneveld P, Dimopoulos MA, Boccadoro M, Quach H, Ho PJ, Beksac M, et al. Daratumumab, bortezomib, lenalidomide, and dexamethasone for multiple myeloma. *N Engl J Med*. (2023) 390(4):301–13. doi: 10.1056/NEJMoa2312054

7. Saad A, Mahindra A, Zhang MJ, Zhong X, Costa LJ, Dispenzieri A, et al. Hematopoietic cell transplant comorbidity index is predictive of survival after autologous hematopoietic cell transplantation in multiple myeloma. *Biol Blood Marrow Transplant*. (2014) 20:402–8.e1. doi: 10.1016/j.bbmt.2013.12.557

8. Palumbo A, Bringhen S, Mateos MV, Larocca A, Facon T, Kumar SK, et al. Geriatric assessment predicts survival and toxicities in elderly myeloma patients: an International Myeloma Working Group report. *Blood*. (2015) 125:2068–74. doi: 10.1182/blood-2014-12-615187

9. Engelhardt M, Dold SM, Ihorst G, Zober A, Moller M, Reinhardt H, et al. Geriatric assessment in multiple myeloma patients: validation of the International Myeloma Working Group (IMWG) score and comparison with other common comorbidity scores. *Haematologica*. (2016) 101:1110–9. doi: 10.3324/haematol.2016.148189

10. Larocca A, Dold SM, Zweegman S, Terpos E, Wasch R, D'Agostino M, et al. Patient-centered practice in elderly myeloma patients: an overview and consensus from the European Myeloma Network (EMN). *Leukemia*. (2018) 32:1697–712. doi: 10.1038/s41375-018-0142-9

11. 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) 384:705–16. doi: 10.1056/NEJMoa2024850

12. 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) 398:314–24. doi: 10.1016/S0140-6736(21)00933-8

13. San-Miguel J, Dhakal B, Yong K, Spencer A, Anguille S, Mateos MV, et al. Ciltacel or standard care in lenalidomide-refractory multiple myeloma. *N Engl J Med*. (2023) 389:335–47. doi: 10.1056/NEJMoa2303379

14. 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) 388:1002–14. doi: 10.1056/NEJMoa2213614

15. Dytfeld D, Dhakal B, Agha M, Manier S, Delforge M, Kuppens S, et al. Bortezomib, lenalidomide and dexamethasone (VRd) followed by ciltacabtagene autoleucel versus vrd followed by lenalidomide and dexamethasone (Rd) maintenance in patients with newly diagnosed multiple myeloma not intended for transplant: A randomized, phase 3 study (CARTITUDE-5). *Blood*. (2021) 138:1835–. doi: 10.1182/blood-2021-146210

16. Boccadoro M, San-Miguel J, Suzuki K, Van De Donk NWCJ, Cook G, Jakubowiak A, et al. DVRd followed by ciltacabtagene autoleucel versus DVRd followed by ASCT in patients with newly diagnosed multiple myeloma who are transplant eligible: A randomized phase 3 study (EMagine/CARTITUDE-6). *Blood*. (2022) 140:4630–2. doi: 10.1182/blood-2022-157021

17. Neri P, Maity R, Tagoug I, McCulloch S, Duggan P, Jimenez-Zepeda V, et al. Immunome single cell profiling reveals T cell exhaustion with upregulation of checkpoint inhibitors LAG3 and tigit on marrow infiltrating T lymphocytes in daratumumab and IMiDs resistant patients. *Blood*. (2018) 132:242–. doi: 10.1182/blood-2018-99-117531

18. Rytlewski J, Madduri D, Fuller J, Campbell TB, Mashadi-Hossein A, Thompson EG, et al. Effects of prior alkylating therapies on preinfusion patient characteristics and starting material for CAR T cell product manufacturing in late-line multiple myeloma. *Blood*. (2020) 136:7–8. doi: 10.1182/blood-2020-134369

19. Cooke RE, Quinn KM, Quach H, Harrison S, Prince HM, Koldej R, et al. Conventional treatment for multiple myeloma drives premature aging phenotypes and metabolic dysfunction in T cells. *Front Immunol*. (2020) 11:2153. doi: 10.3389/fimmu.2020.02153

20. Rytlewski J, Fuller J, Mertz DR, Freeman C, Manier S, Shah N, et al. Correlative analysis to define patient profiles associated with manufacturing and clinical endpoints in relapsed/refractory multiple myeloma (RRMM) patients treated with idecabtagene vicleucel (ide-cel; bb2121), an anti-BCMA CAR T cell therapy. *J Clin Oncol*. (2022) 40:8021. doi: 10.1200/JCO.2022.40.16_suppl.8021

21. Tai YT, Acharya C, An G, Moschetta M, Zhong MY, Feng X, et al. APRIL and BCMA promote human multiple myeloma growth and immunosuppression in the bone marrow microenvironment. *Blood*. (2016) 127:3225–36. doi: 10.1182/blood-2016-01-691162

22. Madry C, Laabi Y, Callebaut I, Roussel J, Hatzoglou A, Le Coniat M, et al. The characterization of murine BCMA gene defines it as a new member of the tumor necrosis factor receptor superfamily. *Int Immunol*. (1998) 10:1693–702. doi: 10.1093/intimm/10.11.1693

23. Ryan MC, Hering M, Peckham D, McDonagh CF, Brown L, Kim KM, et al. Antibody targeting of B-cell maturation antigen on Malignant plasma cells. *Mol Cancer Ther*. (2007) 6:3009–18. doi: 10.1158/1535-7163.MCT-07-0464

24. O'Connor BP, Raman VS, Erickson LD, Cook WJ, Weaver LK, Ahonen C, et al. BCMA is essential for the survival of long-lived bone marrow plasma cells. *J Exp Med*. (2004) 199:91–8. doi: 10.1084/jem.20031330

25. Carpenter RO, Evbuomwan MO, Pittaluga S, Rose JJ, Raffeld M, Yang S, et al. B-cell maturation antigen is a promising target for adoptive T-cell therapy of multiple myeloma. *Clin Cancer Res*. (2013) 19:2048–60. doi: 10.1158/1078-0432.CCR-12-2422

26. Novak AJ, Darce JR, Arendt BK, Harder B, Henderson K, Kindsvogel W, et al. Expression of BCMA, TACI, and BAFF-R in multiple myeloma: a mechanism for growth and survival. *Blood*. (2004) 103:689–94. doi: 10.1182/blood-2003-06-2043

27. Lee L, Bounds D, Paterson J, Herledan G, Sully K, Seestaller-Wehr LM, et al. Evaluation of B cell maturation antigen as a target for antibody drug conjugate mediated cytotoxicity in multiple myeloma. *Br J Haematol*. (2016) 174:911–22. doi: 10.1111/bjh.14145

28. Bujarski S, Soof C, Chen H, Li M, Sanchez E, Wang CS, et al. Serum b-cell maturation antigen levels to predict progression free survival and responses among relapsed or refractory multiple myeloma patients treated on the phase I IRUX trial. *J Clin Oncol*. (2018) 36:e24313–e. doi: 10.1200/JCO.2018.36.15_suppl.e24313

29. Ghermezi M, Li M, Vardanyan S, Harutyunyan NM, Gottlieb J, Berenson A, et al. Serum B-cell maturation antigen: a novel biomarker to predict outcomes for multiple myeloma patients. *Haematologica*. (2017) 102:785–95. doi: 10.3324/haematol.2016.150896

30. Sanchez E, Li M, Kitto A, Li J, Wang CS, Kirk DT, et al. Serum B-cell maturation antigen is elevated in multiple myeloma and correlates with disease status and survival. *Br J Haematol*. (2012) 158:727–38. doi: 10.1111/j.1365-2141.2012.09241.x

31. Visram A, Soof C, Rajkumar SV, Kumar SK, Bujarski S, Spektor TM, et al. Serum BCMA levels predict outcomes in MGUS and smoldering myeloma patients. *Blood Cancer J*. (2021) 11:120. doi: 10.1038/s41408-021-00505-4

32. Friedman KM, Garrett TE, Evans JW, Horton HM, Latimer HJ, Seidel SL, et al. Effective targeting of multiple B-cell maturation antigen-expressing hematological Malignances by anti-B-cell maturation antigen chimeric antigen receptor T cells. *Hum Gene Ther*. (2018) 29:585–601. doi: 10.1089/hum.2018.001

33. Raje N, Berdeja J, Lin Y, Siegel D, Jagannath S, Madduri D, et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. *N Engl J Med*. (2019) 380:1726–37. doi: 10.1056/NEJMoa1817226

34. Larry D, Anderson J, Munshi NC, Shah N, Jagannath S, Berdeja JG, et al. Idecabtagene vicleucel (ide-cel, bb2121), a BCMA-directed CAR T cell therapy, in relapsed and refractory multiple myeloma: Updated KarMMa results. *J Clin Oncol*. (2021) 39:8016. doi: 10.1200/JCO.2021.39.15_suppl.8016

35. 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) 29:2286–94. doi: 10.1038/s41591-023-02496-0

36. Hansen DK, Sidana S, Peres LC, Colin Leitzinger C, Shune L, Shrewsbury A, et al. Idecabtagene vicleucel for relapsed/refractory multiple myeloma: real-world experience from the myeloma CAR T consortium. *J Clin Oncol*. (2023) 41:2087–97. doi: 10.1200/JCO.22.01365

37. Hashmi H, Hansen DK, Peres LC, Castaneda Puglianini OA, Freeman CL, De Avila G, et al. Factors associated with refractoriness or early progression after idecabtagene vicleucel (Ide-cel) in patients with relapsed/refractory multiple myeloma (RRMM): U.S. Myeloma CAR T consortium real world experience. *Blood*. (2022) 140:4642–5. doi: 10.1182/blood-2022-164828

38. Patel K, Rodríguez-Otero P, Manier S, Baz R, Raab MS, Cavo M, et al. S195: Idecabtagene vicleucel (ide-cel) vs standard regimens in patients with triple-class–exposed (tce) relapsed and refractory multiple myeloma (rrmm): a karmma-3 analysis in high-risk subgroups. *HemaSphere*. (2023) 7:e369897b. doi: 10.1097/01.HS9.0000967692.36989.7b

39. Dhodapkar MV, Alsina M, Berdeja JG, Patel KK, Richard S, Vij R, et al. Efficacy and safety of idecabtagene vicleucel (ide-cel) in patients with clinical high-risk newly diagnosed multiple myeloma (NDMM) with an inadequate response to frontline autologous stem cell transplantation (ASCT): karMMa-2 cohort 2c extended follow-up. *Blood*. (2023) 142:2101. doi: 10.1182/blood-2023-173970

40. Xu J, Chen LJ, Yang SS, Sun Y, Wu W, Liu YF, et al. Exploratory trial of a biepitopic CAR T-targeting B cell maturation antigen in relapsed/refractory multiple myeloma. *Proc Natl Acad Sci U S A*. (2019) 116:9543–51. doi: 10.1073/pnas.1819745116

41. 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) 41:1265–74. doi: 10.1200/JCO.22.00842

42. Lin Y, Martin TG, Usmani SZ, Berdeja JG, Jakubowiak AJ, Agha ME, et al. CARTITUDE-1 final results: Phase 1b/2 study of ciltacabtagene autoleucel in heavily pretreated patients with relapsed/refractory multiple myeloma. *J Clin Oncol*. (2023) 41:8009. doi: 10.1200/JCO.2023.41.16_suppl.8009

43. Hansen DK, Patel KK, Peres LC, Kocoglu MH, Shune L, Simmons G, et al. Safety and efficacy of standard of care (SOC) ciltacabtagene autoleucel (Cilta-cel) for relapsed/refractory multiple myeloma (RRMM). *J Clin Oncol*. (2023) 41:8012. doi: 10.1200/JCO.2023.41.16_suppl.8012

44. Dhakal B, Yong K, Harrison SJ, Mateos M-V, Moreau P, van de Donk NWCJ, et al. First phase 3 results from CARTITUDE-4: Cilta-cel versus standard of care (PVd or DPd) in lenalidomide-refractory multiple myeloma. *J Clin Oncol*. (2023) 41: LBA106–LBA. doi: 10.1200/JCO.2023.41.17_suppl.LBA106

45. Wang Z, Han W. Biomarkers of cytokine release syndrome and neurotoxicity related to CAR-T cell therapy. *biomark Res*. (2018) 6:4. doi: 10.1186/s40364-018-0116-0

46. Norelli M, Camisa B, Barbiera G, Falcone L, Purevdorj A, Genua M, et al. Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. *Nat Med*. (2018) 24:739–48. doi: 10.1038/s41591-018-0036-4

47. Shimabukuro-Vornhagen A, Godel P, Subklewe M, Stemmler HJ, Schlosser HA, Schlaak M, et al. Cytokine release syndrome. *J Immunother Cancer*. (2018) 6:56. doi: 10.1186/s40425-018-0343-9

48. Cosenza M, Sacchi S, Pozzi S. Cytokine release syndrome associated with T-cell-based therapies for hematological Malignancies: pathophysiology, clinical presentation, and treatment. *Int J Mol Sci*. (2021) 22(14):7652. doi: 10.3390/ijms22147652

49. Lee DW, Santomasso BD, Locke FL, Ghobadi A, Turtle CJ, Brudno JN, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. *Biol Blood Marrow Transplant*. (2019) 25:625–38. doi: 10.1016/j.bbmt.2018.12.758

50. Neelapu SS, Tummala S, Kebriaei P, Wierda W, Gutierrez C, Locke FL, et al. Chimeric antigen receptor T-cell therapy - assessment and management of toxicities. *Nat Rev Clin Oncol*. (2018) 15:47–62. doi: 10.1038/nrclinonc.2017.148

51. Santomasso BD, Nastoupil LJ, Adkins S, Lacchetti C, Schneider BJ, Anadkat M, et al. Management of immune-related adverse events in patients treated with chimeric antigen receptor T-cell therapy: ASCO guideline. *J Clin Oncol*. (2021) 39:3978–92. doi: 10.1200/JCO.21.01992

52. Cohen AD, Parekh S, Santomasso BD, Gallego Perez-Larraya J, van de Donk N, Arnulf B, et al. Incidence and management of CAR-T neurotoxicity in patients with multiple myeloma treated with ciltacabtagene autoleucel in CARTITUDE studies. *Blood Cancer J*. (2022) 12:32. doi: 10.1038/s41408-022-00629-1

53. Van Oekelen O, Aleman A, Upadhyaya B, Schnakenberg S, Madduri D, Gavane S, et al. Neurocognitive and hypokinetic movement disorder with features of parkinsonism after BCMA-targeting CAR-T cell therapy. *Nat Med*. (2021) 27:2099–103. doi: 10.1038/s41591-021-01564-7

54. Adminstration FaD. *FDA adverse event reporting system (FAERS) public dashboard*. (2023). Available at: https://www.fda.gov/Drugs/GuidanceComplianceRegulatoryInformation/Surveillance/AdverseDrugEffects/ucm070093.htm.

55. Harrison SJ, Nguyen T, Rahman M, Er J, Li J, Li K, et al. CAR+ T-cell lymphoma post ciltacabtagene autoleucel therapy for relapsed refractory multiple myeloma. *Blood*. (2023) 142:6939. doi: 10.1182/blood-2023-178806

56. Vainstein V, Avni B, Grisariu S, Kfir-Erenfeld S, Asherie N, Nachmias B, et al. Clonal myeloid dysplasia following CAR T-cell therapy: chicken or the egg? *Cancers (Basel)*. (2023) 15(13):3471. doi: 10.3390/cancers15133471

57. Attal M, Harousseau JL, Stoppa AM, Sotto JJ, Fuzibet JG, Rossi JF, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. *Intergroupe Francais du Myelome. N Engl J Med*. (1996) 335:91–7. doi: 10.1056/NEJM199607113350204

58. Koreth J, Cutler CS, Djulbegovic B, Behl R, Schlossman RL, Munshi NC, et al. High-dose therapy with single autologous transplantation versus chemotherapy for newly diagnosed multiple myeloma: A systematic review and meta-analysis of randomized controlled trials. *Biol Blood Marrow Transplant*. (2007) 13:183–96. doi: 10.1016/j.bbmt.2006.09.010

59. Richardson PG, Jacobus SJ, Weller EA, Hassoun H, Lonial S, Raje NS, et al. Triplet therapy, transplantation, and maintenance until progression in myeloma. *N Engl J Med*. (2022) 387:132–47. doi: 10.1056/NEJMoa2204925

60. Grazziutti ML, Dong L, Miceli MH, Krishna SG, Kiwan E, Syed N, et al. Oral mucositis in myeloma patients undergoing melphalan-based autologous stem cell transplantation: incidence, risk factors and a severity predictive model. *Bone Marrow Transplant*. (2006) 38:501–6. doi: 10.1038/sj.bmt.1705471

61. Chakraborty R, Hamilton BK, Hashmi SK, Kumar SK, Majhail NS. Health-related quality of life after autologous stem cell transplantation for multiple myeloma. *Biol Blood Marrow Transplant*. (2018) 24:1546–53. doi: 10.1016/j.bbmt.2018.03.027

62. Roussel M, Hebraud B, Hulin C, Perrot A, Caillot D, Stoppa AM, et al. Health-related quality of life results from the IFM 2009 trial: treatment with lenalidomide, bortezomib, and dexamethasone in transplant-eligible patients with newly diagnosed multiple myeloma. *Leuk Lymphoma*. (2020) 61:1323–33. doi: 10.1080/10428194.2020.1719091

63. Durie BGM, Hoering A, Abidi MH, Rajkumar SV, Epstein J, Kahanic SP, et al. Bortezomib with lenalidomide and dexamethasone versus lenalidomide and dexamethasone alone in patients with newly diagnosed myeloma without intent for immediate autologous stem-cell transplant (SWOG S0777): a randomised, open-label, phase 3 trial. *Lancet*. (2017) 389:519–27. doi: 10.1016/S0140-6736(16)31594-X

64. Perrot A, Lauwers-Cances V, Cazaubiel T, Facon T, Caillot D, Clement-Filliatre L, et al. Early versus late autologous stem cell transplant in newly diagnosed multiple myeloma: long-term follow-up analysis of the IFM 2009 trial. *Blood*. (2020) 136:39. doi: 10.1182/blood-2020-134538

65. Gay F, Musto P, Rota-Scalabrini D, Bertamini L, Belotti A, Galli M, et al. Carfilzomib with cyclophosphamide and dexamethasone or lenalidomide and dexamethasone plus autologous transplantation or carfilzomib plus lenalidomide and dexamethasone, followed by maintenance with carfilzomib plus lenalidomide or lenalidomide alone for patients with newly diagnosed multiple myeloma (FORTE): a randomised, open-label, phase 2 trial. *Lancet Oncol*. (2021) 22:1705–20. doi: 10.1016/S1470-2045(21)00535-0

66. Yong K, Wilson W, De Tute RM, Camilleri M, Ramasamy K, Streetly M, et al. Upfront autologous haematopoietic stem-cell transplantation versus carfilzomib–cyclophosphamide–dexamethasone consolidation with carfilzomib maintenance in patients with newly diagnosed multiple myeloma in England and Wales (CARDAMON): a randomised, phase. *Lancet Haematology*. (2023) 10:e93–e106. doi: 10.1016/S2352-3026(22)00350-7

67. Moreau P, Attal M, Hulin C, Arnulf B, Belhadj K, Benboubker L, et al. Bortezomib, thalidomide, and dexamethasone with or without daratumumab before and after autologous stem-cell transplantation for newly diagnosed multiple myeloma (CASSIOPEIA): a randomised, open-label, phase 3 study. *Lancet*. (2019) 394:29–38. doi: 10.1016/S0140-6736(19)31240-1

68. Voorhees PM, Kaufman JL, Laubach J, Sborov DW, Reeves B, Rodriguez C, et al. Daratumumab, lenalidomide, bortezomib, and dexamethasone for transplant-eligible newly diagnosed multiple myeloma: the GRIFFIN trial. *Blood*. (2020) 136:936–45. doi: 10.1182/blood.2020005288

69. Gay F, Roeloffzen W, Dimopoulos MA, Rosiñol L, van der Klift M, Mina R, et al. Results of the phase III randomized iskia trial: isatuximab-carfilzomib-lenalidomide-dexamethasone vs carfilzomib-lenalidomide-dexamethasone as pre-transplant induction and post-transplant consolidation in newly diagnosed multiple myeloma patients. *Blood*. (2023) 142:4. doi: 10.1182/blood-2023-177546

70. Kumar S, Flinn I, Richardson PG, Hari P, Callander N, Noga SJ, et al. Randomized, multicenter, phase 2 study (EVOLUTION) of combinations of bortezomib, dexamethasone, cyclophosphamide, and lenalidomide in previously untreated multiple myeloma. *Blood*. (2012) 119:4375–82. doi: 10.1182/blood-2011-11-395749

71. Facon T, Kumar SK, Plesner T, Orlowski RZ, Moreau P, Bahlis N, et al. Daratumumab, lenalidomide, and dexamethasone versus lenalidomide and dexamethasone alone in newly diagnosed multiple myeloma (MAIA): overall survival results from a randomised, open-label, phase 3 trial. *Lancet Oncol*. (2021) 22:1582–96. doi: 10.1016/S1470-2045(21)00466-6

72. Bal S, Dhakal B, Silbermann RW, Schmidt TM, Dholaria B, Giri S, et al. Impact of autologous hematopoietic cell transplantation on disease burden quantified by next-generation sequencing in multiple myeloma treated with quadruplet therapy. *Am J Hematol*. (2022) 97:1170–7. doi: 10.1002/ajh.26640

73. Callander N, Silbermann R, Kaufman JL, Godby KN, Laubach JP, Schmidt TM, et al. Analysis of transplant-eligible patients (Pts) who received frontline daratumumab (DARA)-based quadruplet therapy for the treatment of newly diagnosed multiple myeloma (NDMM) with high-risk cytogenetic abnormalities (HRCA) in the griffin and master studies. *Blood*. (2022) 140:10144–7. doi: 10.1182/blood-2022-160451

74. Pasvolsky O, Ghanem S, Milton DR, Masood A, Tanner MR, Bashir Q, et al. Outcomes of autologous stem cell transplantation in patients with ultra-high-risk multiple myeloma. *Transplant Cell Ther*. (2023) 29:757–62. doi: 10.1016/j.jtct.2023.08.031

75. Pasvolsky O, Pasyar S, Bassett RL, Khan HN, Tanner MR, Bashir Q, et al. Impact of pretransplant minimal residual disease in patients with multiple myeloma and a very good partial response or better receiving autologous hematopoietic stem cell transplantation. *Cancer*. (2023). doi: 10.1002/cncr.35171

76. Costa LJ, Chhabra S, Medvedova E, Dholaria BR, Schmidt TM, Godby KN, et al. Minimal residual disease response-adapted therapy in newly diagnosed multiple myeloma (MASTER): final report of the multicentre, single-arm, phase 2 trial. *Lancet Haematol*. (2023) 10:e890–901. doi: 10.1016/S2352-3026(23)00236-3

77. Grovdal M, Nahi H, Gahrton G, Liwing J, Waage A, Abildgaard N, et al. Autologous stem cell transplantation versus novel drugs or conventional chemotherapy for patients with relapsed multiple myeloma after previous ASCT. *Bone Marrow Transplant*. (2015) 50:808–12. doi: 10.1038/bmt.2015.39

78. Garderet L, Iacobelli S, Koster L, Goldschmidt H, Johansson JE, Bourhis JH, et al. Outcome of a salvage third autologous stem cell transplantation in multiple myeloma. *Biol Blood Marrow Transplant*. (2018) 24:1372–8. doi: 10.1016/j.bbmt.2018.01.035

79. Manjappa S, Fiala MA, King J, Kohnen DA, Vij R. The efficacy of salvage autologous stem cell transplant among patients with multiple myeloma who received maintenance therapy post initial transplant. *Bone Marrow Transplant*. (2018) 53:1483–6. doi: 10.1038/s41409-018-0216-3

80. Khan AM, Ozga M, Bhatt H, Faisal MS, Ansari S, Zhao Q, et al. Outcomes after salvage autologous hematopoietic cell transplant for patients with relapsed/refractory multiple myeloma: A single-institution experience. *Clin Lymphoma Myeloma Leuk*. (2023) 23:e182–e9. doi: 10.1016/j.clml.2022.12.001

81. Tilmont R, Yakoub-Agha I, Eikema D-J, Zinger N, Haenel M, Schaap N, et al. Carfilzomib, lenalidomide and dexamethasone followed by a second ASCT is an effective strategy in first relapse multiple myeloma: a study on behalf of the Chronic Malignancies working party of the EBMT. *Bone Marrow Transplantation*. (2023) 58:1182–8. doi: 10.1038/s41409-023-02048-7

82. Hashmi H, Atrash S, Jain J, Khasawneh G, Mohan M, Mahmoudjafari Z, et al. Daratumumab, pomalidomide, and dexamethasone (DPd) followed by high dose chemotherapy-Autologous Stem Cell Transplantation leads to superior outcomes when compared to DPd-alone for patients with Relapsed Refractory Multiple Myeloma. *Transplant Cell Ther*. (2023) 29:262 e1– e6. doi: 10.1016/j.jtct.2023.01.013

83. Michaelis LC, Saad A, Zhong X, Le-Rademacher J, Freytes CO, Marks DI, et al. Salvage second hematopoietic cell transplantation in myeloma. *Biol Blood Marrow Transplant*. (2013) 19:760–6. doi: 10.1016/j.bbmt.2013.01.004

84. Lemieux E, Hulin C, Caillot D, Tardy S, Dorvaux V, Michel J, et al. Autologous stem cell transplantation: an effective salvage therapy in multiple myeloma. *Biol Blood Marrow Transplant*. (2013) 19:445–9. doi: 10.1016/j.bbmt.2012.11.013

85. Baertsch M-A, Schlenzka J, Hielscher T, Raab MS, Sauer S, Merz M, et al. Salvage autologous transplant and lenalidomide maintenance versus continuous lenalidomide/dexamethasone for relapsed multiple myeloma: long term follow up results of the randomized GMMG phase III multicenter trial relapse. *Blood*. (2023) 142:782. doi: 10.1182/blood-2023-178835

86. Shah GL, Bal S, Rodriguez C, Chhabra S, Bayer R-L, Costa LJ, et al. 534 - interim analysis of the 2nd chance protocol: A multicenter trial of daratumumab, carfilzomib, lenalidomide, & Dexamethasone for relapsed/refractory myeloma with salvage autologous hematopoietic cell transplantation. *Transplant Cell Ther*. (2022) 28:S415–S6. doi: 10.1016/S2666-6367(22)00693-5

87. Van Oekelen O, Nath K, Mouhieddine TH, Farzana T, Aleman A, Melnekoff DT, et al. Interventions and outcomes of patients with multiple myeloma receiving salvage therapy after BCMA-directed CAR T therapy. *Blood*. (2023) 141:756–65. doi: 10.1182/blood.2022017848

88. Janakiram M, Arora N, Bachanova V, Miller JS. Novel cell and immune engagers in optimizing tumor- specific immunity post-autologous transplantation in multiple myeloma. *Transplant Cell Ther*. (2022) 28:61–9. doi: 10.1016/j.jtct.2021.10.001

89. Tamura H. Immunopathogenesis and immunotherapy of multiple myeloma. *Int J Hematol*. (2018) 107:278–85. doi: 10.1007/s12185-018-2405-7

90. Davies FE, Raje N, Hideshima T, Lentzsch S, Young G, Tai YT, et al. Thalidomide and immunomodulatory derivatives augment natural killer cell cytotoxicity in multiple myeloma. *Blood*. (2001) 98:210–6. doi: 10.1182/blood.V98.1.210

91. Krejcik J, Casneuf T, Nijhof IS, Verbist B, Bald J, Plesner T, et al. Daratumumab depletes CD38+ immune regulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma. *Blood*. (2016) 128:384–94. doi: 10.1182/blood-2015-12-687749

92. Douek DC, Vescio RA, Betts MR, Brenchley JM, Hill BJ, Zhang L, et al. Assessment of thymic output in adults after haematopoietic stem-cell transplantation and prediction of T-cell reconstitution. *Lancet*. (2000) 355:1875–81. doi: 10.1016/S0140-6736(00)02293-5

93. Chung DJ, Pronschinske KB, Shyer JA, Sharma S, Leung S, Curran SA, et al. T-cell exhaustion in multiple myeloma relapse after autotransplant: optimal timing of immunotherapy. *Cancer Immunol Res*. (2016) 4:61–71. doi: 10.1158/2326-6066.CIR-15-0055

94. Rueff J, Medinger M, Heim D, Passweg J, Stern M. Lymphocyte subset recovery and outcome after autologous hematopoietic stem cell transplantation for plasma cell myeloma. *Biol Blood Marrow Transplant*. (2014) 20:896–9. doi: 10.1016/j.bbmt.2014.03.007

95. 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) 3:2812–5. doi: 10.1182/bloodadvances.2019000600

96. Porrata LF, Gastineau DA, Padley D, Bundy K, Markovic SN. Re-infused autologous graft natural killer cells correlates with absolute lymphocyte count recovery after autologous stem cell transplantation. *Leuk Lymphoma*. (2003) 44:997–1000. doi: 10.1080/1042819031000077089

97. Anderson LD Jr., Dhakal B, Jain T, Oluwole OO, Shah GL, Sidana S, et al. Chimeric antigen receptor T cell therapy for myeloma: where are we now and what is needed to move chimeric antigen receptor T cells forward to earlier lines of therapy? Expert panel opinion from the american society for transplantation and cellular therapy. *Transplant Cell Ther*. (2024) 30:17–37. doi: 10.1016/j.jtct.2023.10.022

98. mSMART. Treatment guidelines: multiple myeloma(2023). Available online at: https://www.msmart.org/mm-treatment-guidelines.

99. Dhakal B, Shah N, Kansagra A, Kumar A, Lonial S, Garfall A, et al. ASTCT clinical practice recommendations for transplantation and cellular therapies in multiple myeloma. *Transplant Cell Ther*. (2022) 28:284–93. doi: 10.1016/j.jtct.2022.03.019

100. Kumar SK, Lacy MQ, Dispenzieri A, Buadi FK, Hayman SR, Dingli D, et al. Early versus delayed autologous transplantation after immunomodulatory agents-based induction therapy in patients with newly diagnosed multiple myeloma. *Cancer*. (2012) 118:1585–92. doi: 10.1002/cncr.26422

101. Beinfeld M, Lee S, McQueen B, Fluetsch N, Pearson SD, Ollendorf DA. Anti B-cell maturation antigen CAR T-cell and antibody drug conjugate therapy for heavily pretreated relapsed and refractory multiple myeloma. *J Manag Care Spec Pharm*. (2021) 27:1315–20. doi: 10.18553/jmcp.2021.27.9.1315

102. Kapinos KA, Hu E, Trivedi J, Geethakumari PR, Kansagra A. Cost-effectiveness analysis of CAR T-cell therapies vs antibody drug conjugates for patients with advanced multiple myeloma. *Cancer Control*. (2023) 30:10732748221142945. doi: 10.1177/10732748221142945

103. Leng S, Moshier E, Tremblay D, Hu L, Biran N, Barman N, et al. Timing of autologous stem cell transplantation for multiple myeloma in the era of current therapies. *Clin Lymphoma Myeloma Leuk*. (2020) 20:e734–e51. doi: 10.1016/j.clml.2020.05.027

104. Davis JA, Dima D, Ahmed N, DeJarnette S, McGuirk J, Jia X, et al. Impact of frailty on outcomes after chimeric antigen receptor T cell therapy for patients with relapsed/refractory multiple myeloma. *Transplant Cell Ther*. (2023). doi: 10.1182/blood-2023-179981

105. Reyes KR, Huang CY, Lo M, Arora S, Chung A, Wong SW, et al. Safety and efficacy of BCMA CAR-T cell therapy in older patients with multiple myeloma. *Transplant Cell Ther*. (2023) 29:350–5. doi: 10.1016/j.jtct.2023.03.012

106. Sidana S, Dueck AC, Thanarajasingam G, Griffin JM, Thompson C, Durani U, et al. Longitudinal patient reported outcomes with CAR-T cell therapy versus autologous and allogeneic stem cell transplant. *Transplant Cell Ther*. (2022) 28:473–82. doi: 10.1016/j.jtct.2022.05.004

107. Delforge M, Shah N, Miguel JSF, Braverman J, Dhanda DS, Shi L, et al. Health-related quality of life with idecabtagene vicleucel in relapsed and refractory multiple myeloma. *Blood Adv*. (2022) 6:1309–18. doi: 10.1182/bloodadvances.2021005913

108. Ragon BK, Shah MV, D'Souza A, Estrada-Merly N, Gowda L, George G, et al. Impact of second primary Malignancy post-autologous transplantation on outcomes of multiple myeloma: a CIBMTR analysis. *Blood Adv*. (2023) 7:2746–57.doi: 10.1182/bloodadvances.2022009138

109. Maura F, Weinhold N, Diamond B, Kazandjian D, Rasche L, Morgan G, et al. The mutagenic impact of melphalan in multiple myeloma. *Leukemia*. (2021) 35:2145–50. doi: 10.1038/s41375-021-01293-3

110. Samur MK, Roncador M, Aktas Samur A, Fulciniti M, Bazarbachi AH, Szalat R, et al. High-dose melphalan treatment significantly increases mutational burden at relapse in multiple myeloma. *Blood*. (2023) 141:1724–36. doi: 10.1182/blood.2022017094

111. Levine BL, Pasquini MC, Connolly JE, Porter DL, Gustafson MP, Boelens JJ, et al. Unanswered questions following reports of secondary Malignancies after CAR-T cell therapy. *Nat Med*. (2024) 30:338–41. doi: 10.1038/s41591-023-02767-w

112. Hernandez I, Prasad V, Gellad WF. Total costs of chimeric antigen receptor T-cell immunotherapy. *JAMA Oncol*. (2018) 4:994–6. doi: 10.1001/jamaoncol.2018.0977

113. Hoda D, Richards R, Faber EA, Deol A, Hunter BD, Weber E, et al. Process, resource and success factors associated with chimeric antigen receptor T-cell therapy for multiple myeloma. *Future Oncol*. (2022) 18:2415–31. doi: 10.2217/fon-2022-0162

114. Lee SJ, McQueen RB, Beinfeld M, Fluetsch N, Whittington MD, Pearson SD, et al. *Anti B-cell maturation antigen CAR T-cell and antibody drug conjugate therapy for heavily pre-treated relapsed and refractory multiple myeloma; final evidence report.: institute for clinical and economic review*. (2021). Available at: https://icer.org/assessment/multiplemyeloma-2021/#timeline

115. Fiorenza S, Ritchie DS, Ramsey SD, Turtle CJ, Roth JA. Value and affordability of CAR T-cell therapy in the United States. *Bone Marrow Transplantation*. (2020) 55:1706–15. doi: 10.1038/s41409-020-0956-8

116. Choi G, Shin G, Bae S. Price and prejudice? The value of chimeric antigen receptor (CAR) T-cell therapy. *Int J Environ Res Public Health*. (2022) 19(19):12366. doi: 10.3390/ijerph191912366

117. Yamamoto C, Minakata D, Koyama S, Sekiguchi K, Fukui Y, Murahashi R, et al. Daratumumab in first-line therapy is cost-effective in transplant-eligible patients with newly diagnosed myeloma. *Blood*. (2022) 140:594–607. doi: 10.1182/blood.2021015220

frontiers | Frontiers in Oncology

TYPE Mini Review
PUBLISHED 22 April 2024
DOI 10.3389/fonc.2024.1396395

OPEN ACCESS

EDITED BY
Satoshi Yoshihara,
Hyogo Medical University, Japan

REVIEWED BY
Shinichi Makita,
National Cancer Center Hospital, Japan

*CORRESPONDENCE
Yifan Pang
yifan.pang@atriumhealth.org

RECEIVED 05 March 2024
ACCEPTED 08 April 2024
PUBLISHED 22 April 2024

CITATION
Pang Y and Ghosh N (2024) Novel and multiple targets for chimeric antigen receptor-based therapies in lymphoma. *Front. Oncol.* 14:1396395. doi: 10.3389/fonc.2024.1396395

Novel and multiple targets for chimeric antigen receptor-based therapies in lymphoma

Yifan Pang* and Nilanjan Ghosh

Department of Hematologic Oncology and Blood Disorders, Atrium Health Levine Cancer Institute, Wake Forest School of Medicine, Charlotte, NC, United States

Chimeric antigen receptor (CAR) T-cell therapy targeting CD19 in B-cell non-Hodgkin lymphoma (NHL) validates the utility of CAR-based therapy for lymphomatous malignancies. Despite the success, treatment failure due to CD19 antigen loss, mutation, or down-regulation remains the main obstacle to cure. On-target, off-tumor effect of CD19-CAR T leads to side effects such as prolonged B-cell aplasia, limiting the application of therapy in indolent diseases such as chronic lymphocytic leukemia (CLL). Alternative CAR targets and multi-specific CAR are potential solutions to improving cellular therapy outcomes in B-NHL. For Hodgkin lymphoma and T-cell lymphoma, several cell surface antigens have been studied as CAR targets, some of which already showed promising results in clinical trials. Some antigens are expressed by different lymphomas and could be used for designing tumor-agnostic CAR. Here, we reviewed the antigens that have been studied for novel CAR-based therapies, as well as CARs designed to target two or more antigens in the treatment of lymphoma.

KEYWORDS

chimeric antigen receptor, B-cell lymphoma, Hodgkin lymphoma, T-cell lymphoma, adoptive cellular immunotherapy

Introduction

CARs are synthetic molecules that are encoded by an antigen-binding domain – typically a monoclonal antibody-based single-chain fragment variable (scFv), an extracellular hinge to improve immune synapses formation, a transmembrane anchor, a costimulatory and intracellular domain for signal transduction (1). Once expressed by the transduced cells, most commonly T-cells, sometimes NK-cells, CARs improve the homing of T or NK cells to tumor to facilitate and enhance tumor-specific killing. Not all tumor antigens can become CAR targets, only the surface antigens with high densities can be recognized by CAR and fully activate modified immune cells (2, 3).

B-cell non-Hodgkin lymphoma

A rapidly growing list of indications underscores the success of CD19-targeted CAR T-cell therapy in B-cell non-Hodgkin lymphoma (NHL). Durable complete remission has been consistently reported in clinical trials utilizing CD19-CAR T in relapsed/refractory (R/R) B-NHL, indicating the curative potential of CD19-CAR T (4). However, for the more than 50% of patients who are refractory to or relapse after CD19-CAR T, there is an urgent need for alternative therapeutic options (1). The causes of resistance can be loosely divided into three categories: patient factors, CAR design factors, and target antigen modulation. Examples of patient factors include high disease burden, microbiome, and baseline cytokine milieu (5). CAR design factors, including transmembrane domain architecture, costimulatory domain, immune synapse spacing, affinity to antigen, are vital to the efficacy and safety of CAR T (1). For instance, high affinity of the CAR to CD19 antigen may not improve efficacy but promote T-cell exhaustion instead. Michelozzi, et al., recently demonstrated that low-affinity CD19-CAR T had enhanced *in vivo* expansion, prolonged persistence, and better tolerability, than traditional high-affinity CD19-CAR T (6). CAR integration using non-retroviral methods, such as using adeno-associated virus or non-viral gene editing to transfer a CD19-CAR into the T-cell receptor alpha constant (TRAC) region, has demonstrated improved functionality compared to traditional retroviral transduction (7). A novel TRAC-integrated CD19-targeted CAR T product is currently undergoing a phase 1 clinical trial in patients with R/R large B-cell lymphoma (NCT05757700). Target antigen modulation is one of the most common mechanisms of resistance. In different studies, CD19 antigen loss or down-regulation was seen in 25% to 33% of the relapsed cases (8–10). CAR-based therapies targeting other B-cell antigens hold the promise to improve the outcome of patients with R/R B-NHL. Active clinical trials of novel CAR-based therapy in B-NHL are listed in Table 1.

CD20

Anti-CD20 monoclonal antibody revolutionized the treatment of B-cell NHL and greatly improved patient outcomes. CD20 theoretically can elicit a more robust T-cell activation than CD19 because of slower endocytosis and stronger immunological synapses formation (11). However, the concern of developing resistance after recurrent rituximab exposure may have played a role in the delay in clinical development of CD20-CAR therapy (12). Reassuringly, CD20 antigen loss or mutation is uncommon in relapsed B- NHL and is not a significant cause of treatment failure (13, 14). The success of bispecific CD20 and CD3 T-cell engagers further confirm the utility of CD20-targeting in R/R B-NHL (15). *In vitro* studies demonstrated preserved cytotoxicity of CD20-CAR T in CD20-downregulated cancers (3).

Till, et al., treated seven patients with R/R follicular lymphoma (FL) or mantle cell lymphoma (MCL) with first generation CD20-CAR T (16). All patients had previous rituximab exposure. Responses included two complete remissions (CR) and three partial responses (PR). None had adverse events related to T-cell infusions. Later, Till, et al., treated three patients with a third generation CD20-CAR T with both CD28 and 4-1BB costimulatory domains, two achieved durable CR (17). Another third generation CD20-CAR T, MB-106, showed efficacy and tolerability in a single center clinical trial (NCT03277729) (18, 19). In 16 patients (12 FL, 2 MCL, 1 CLL, 1 diffuse large B-cell lymphoma), overall response rate (ORR) was 94%, CR 62%, and 90% of the CR was durable (duration of response, 3-18 months); no grade ≥ 3 cytokine release syndrome (CRS) or immune effector cell-associated neurotoxicity syndrome (ICANS) was observed. MB-106 is currently being tested in a multicenter phase 1/2 trial (NCT05360238) (20). Several other CD20-CAR Ts have also entered clinical trials for R/R B-NHL. C-CAR066 was previously tested in a phase 1 clinical trial in China. Fourteen patients were treated, including 11 with diffuse large B-cell lymphoma (DLBCL) and three with transformed FL; 12 previously received CD19-CAR T, one received bispecific CD19/20-CAR T and one received bispecific CD19/79b-CAR T. ORR was 92.9% and CR was achieved in 57.1%. Four remained in CR after 24 months. Grade ≥3 CRS only occurred in one patient and none experienced ICANS (21). C-CAR066 recently entered multicenter phase 1 studies in the US (NCT05784441). MB-CART20.1 is another CD20-CAR T which was tested in a Germany multicenter phase 1 trial (22). Durable CR was seen in 3 of the 10 treated patients. No dose-limiting toxicity (DLT) occurred in the trial, but the trial was stopped early due to COVID-19.

CD22

CD22 is a B-cell-specific transmembrane glycoprotein involved in B-cell survival, proliferation and function (23). Anti-CD22 antibody-drug conjugates (ADC) inotuzumab ozogamicin and moxetumomab pasudotox, were approved for B-cell acute lymphoblastic leukemia (ALL) and hairy cell leukemia, respectively (24, 25). Despite strong CD22 expression on mature B-cells, naked anti-CD22 antibody and ADC all failed to demonstrate significant benefit over standard of care chemoimmunotherapy in patients with CD22+ R/R B-NHL (26, 27). The challenges of CD22-targeted therapy in B-NHL include the higher variability of CD22 expression on lymphoma cells than CD19 and CD20, down regulation of CD22 and low antigen density (28, 29). CD22 has a bulky extracellular structure, making it difficult to target (30).

With the success of anti-CD22 ADC, CD22-CAR T was first developed for B-ALL. Haso, et al., found that CAR targeting the proximal domain of CD22 yielded superior antileukemic activity in preclinical models (31). Proximal-targeting CD22-CAR T was then tested in a phase 1 study in 21 children and adults with R/R B-ALL (32). Potent efficacy was seen even in patients with $CD19^{dim}$ or CD19-negative disease. Currently, various CD22-CAR T have entered phase 1 and/or 2 clinical trials, but no phase 3. In a meta-analysis with a data cutoff in March 2022, a total of seven CD22-CAR T clinical trials with 149 patients had primary efficacy data, but only two enrolled patients with lymphoma (33). A single

TABLE 1 Active clinical trials of novel CAR therapy for B-cell non-Hodgkin Lymphoma.

CAR Target	NCT number	Phase	Location	Notes
CD20	NCT03277729	1 and 2	US - Fred Hutch	CNS involvement eligible
	NCT05360238	1 and 2	US - multicenter	Cell name: MB-106
	NCT05784441	1	US - multicenter	Cell name: JNJ-90009530
CD22	NCT05972720	2	US - multicenter	Prior successful phase 1 NCT04088890 yielded the RP2D of 1 million cells/kg
	NCT04571138	1 and 2	US - multicenter	In both B-ALL and B-NHL
κLC	NCT04223765	1	US - UNC	B-NHL and CLL/SLL
	NCT00881920	1	US - Baylor	B-NHL, CLL, myeloma
CD79b	NCT05773040	1	US - MDACC	Cell-name: JV213
	NCT05312476	2	China	Inducible caspace-9 gene system
CD70	NCT05948033	1 and 2	China	Requires ≥ 10% CD70 antigen expression
ROR1	NCT05694364	1	US - Moffitt	PD-1 switchable
	NCT05588440	1 and 2	US - multicenter	Highly specific anti-ROR1 scFv with no preclinical evidence of on-target off-tumor toxicity
BAFF	NCT05312801	1	US - Cleveland	
CD19/CD20	NCT05149391	1	China	Cell name: C-CAR039
	NCT04007029	1	US - UCLA	
	NCT04186520	1 and 2	US - MCW	flexible manufacturing schema
	NCT04697940	1 and 2	China	decitabine-primed
	NCT05797233	1	US - NCI	
	NCT04989803	1	US - multicenter	Cell name: KITE-363 or KITE-753
	NCT06014762	1	US - multicenter	Allo-CAR with Rimiducid to reduce neurotoxicity
	NCT05826535	1 and 2	US - multicenter	Cell name: IMPT-314
	NCT05421663	1	US - multicenter	
	NCT04792489	2	US - multicenter	Cell name: MB-CART2019.1
CD19/CD22	NCT05091541	1 and 2	China	
	NCT05651100	1 and 2	China	Sequential CD19 and CD22 CAR T instead of bispecific CAR T
	NCT06005649	1 and 2	China	
	NCT03241940	1	US - Stanford	
	NCT03233854	1	US - Stanford	CAR T in combination with chemotherapy NKTR-255, CNS involvement eligible
	NCT05098613	1	US - University of Colorado	
	NCT03448393	1	US - NCI	All B-cell cancer in children and young adults
CD19/BCMA	NCT06097455	1	Spain	Cell name: ARI0003
CD19/CD70	NCT05436496	1 and 2	China	Sequential CD19 and CD70 CAR T instead of bispecific CAR T
CD20/CD22	NCT05607420	1 and 2	US - multicenter	Cell name: UCART20x22; Allo-CAR
CD20/CD79a	NCT05169489	1 and 2	US - multicenter	Cell name: bbT369
CD19/ CD20/ CD22	NCT05418088	1	US - OSU	

US, United States; Fred Hutch, Fred Hutchinson Cancer Center; CNS, central nervous system; RP2D, recommended phase 2 dose; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin Lymphoma; UNC, University of North Carolina; MDACC, MD Anderson Cancer Center; UCLA, University of California, Los Angeles; MCW, Medical College of Wisconsin; NCI, National Cancer Institute; OSU, Ohio State University.

center phase 1/1b study at Stanford University (NCT04088890) is one of the first trials using autologous CD22-CAR T to treat R/R LBCL (34, 35). Among 41 enrolled patients, 40 underwent leukapheresis and 38 (95%) had successful manufacturing of cells. Twenty-nine patients were treated at dose level (DL)1 (1 x 10^6/kg) and 9 at DL2 (3 x 10^6/kg); all but 1 progressed after prior CART19. ORR was 68%. Twenty (53%) entered CR. Response was similar between DL1 and DL2. At a median follow up of 18.4 months (range, 1.5-38.6), nineteen patients remained in CR. Median progression-free survival (PFS) was 2.9 months (range, 1.7-NR) and overall survival (OS) 22.5 months (range, 8.3-NR). CD22-CAR T was well tolerated especially at DL1, and proceeded to a phase 2 clinical trial (NCT05972720) with DL1 as the recommended phase 2 dose (RP2D) (35). Another CD22-CAR T, SCRI-CAR22v2, is being tested in a phase 1/2 multicenter clinical trial, PLAT-07, in pediatric and young adult patients with R/R CD22+ leukemia or lymphoma (NCT04571138). SCRI-CAR22v2 is an improved version of its predecessor SCRI-CAR22v1, with a shorter linker and transmembrane region, and better activity and survival than the latter (36). Allogeneic CD22-CAR T (alloCART22) has been developed. In preclinical models, alloCART22 demonstrated pharmacologic activity against CD22+ tumor and successful evasion of host innate and adaptive immune rejection (37). The safety and efficacy of alloCART22 are yet to be evaluated by clinical trials.

Hemophagocytic lymphohistiocytosis (HLH), a subtype of severe cytokine release syndrome, is associated with higher disease burden, pre-infusion NK-cell lymphopenia and persistent elevation of HLH-related cytokines including IFNγ, IL-1β and IL-18. HLH more common in patients receiving CD22-CAR T (35.6%) than CD19-CAR T (14.8%) (38, 39). On the other hand, ICANS is less common and less severe with CD22-CAR T, likely due to different cytokine milieu, and absence of CD22 expression on the blood brain barrier and oligodendrocyte precursor cells (40).

High peak CD22-CAR T expansion and high expression of the activator protein-1 Fos/Jun are associated with response and toxicity to CD22-CAR T (41). Fos/Jun heterodimer are important transcription factors in activated T-cells and enhances the transcription of inflammatory cytokines such as IL-2 (42). CD22-CAR T from patients who progressed do not have high Fos/Jun, instead, have higher proportion of terminal effector memory cells and higher expression levels of immunomodulatory killer cell immunoglobulin like receptors (41). Another main cause of relapse remains CD22 antigen loss (35, 43). Unlike CD19 antigen loss which is usually mediated by mutation and alternative splicing, down-regulation of CD22 on cell surface makes cells resistant to CD22 targeting (32). Upregulation of CD22 expression by bryostatin-1 pretreatment could potentially re-sensitize lymphoma cells to CD22-CAR T (44). Another major development to overcome antigen loss is bispecific or multi-specific CARs, which will be discussed in detail in later sections.

The natural ligand of CD22 is a cell surface trisaccharide (45). Transferring natural ligand-mimicking CD22-specific polysaccharide onto cell surface is a novel way of designing adoptive cell therapy. Wang, et al., designed CD22-targeting NK-cells by glycoengineering, making NK-cells present a modified polysaccharide ligand on their surface. Not strictly a CAR-NK, the engineered NK-cells could effectively bind to CD22-positive lymphoma cells and exert cytotoxicity in preclinical models (46). Traditional CAR-NKs expressing CD22-scFv have also been studied preclinically (47, 48).

CD19/CD20

Bispecific CAR T targeting two B-cell antigens can be activated upon binding with either antigens or both, thereby enhancing inflammatory cytokine production, and reducing resistance from single antigenic loss (49). Tandem CD19/20-CAR T targets both CD19 and CD20 antigens with a single CAR vector; the CD20 and CD19 binding domains are hinged with a flexible linker. Preclinical data of tandem CD19/20-CAR T revealed several important findings (50). First, CD19-scFv should be placed proximally and CD20-scFv distally to T-cell membrane, to allow optimal conformation of binding. Second, most of the cytokine release after CD19/20-CAR T is likely driven by CD20 recognition. Thirdly, tandem CD19/20-CAR T was more effective than a combination of CD19- and CD20-CAR T.

Based on supportive preclinical data, Shah, et al., conducted a first-in-human trial of bispecific CD19/20-CAR T, LV20.19 (MB CART2019.1, or zamtocabtagene autoleucel, zamto-cel) in patents with R/R B-NHL (51). In the first 22 patients treated, 18 (82%) responded at day 28, including 14 (64%) CR. Rates of grade 3-4 CRS and ICANS were 5% and 14%, respectively. For the patients who received a target dose of 2.5 million cells/kg (n=16), two-year PFS and OS were 44% and 69% at a median follow up of 31 months (range, 2-40) (52).

R/R MCL remains a challenging clinical entity, especially those cases with post-BTKi relapse and/or TP53 aberration. Tandem CD19/20-CAR T showed encouraging results in this hard-to-treat population. In a phase 1/2 clinical, 17 patients with R/R MCL received LV20.19, including 13 BTKi-refractory and seven TP53-mutated patients. The phase 2 portion utilized an adaptive manufacturing process to enhance the percentage of memory T-cells in the product. All patients responded at day 90, including 92% CR. Infection-related death occurred in two patients. Grade 3-4 CRS and ICANS occurred in zero and two patients, respectively. One-year PFS and OS rates were 77% and 84% (53).

The DALY I trial was a phase 1 clinical trial of zamto-cel in patients with R/R B-NHL (54). Twelve patients were enrolled and six received the recommended dose of 2.5 million cells/kg. ORR was 75% in the entire cohort and 5 patients achieved CR. Promisingly, all CRs were durable at 2-year follow up. No grade ≥ 3 CRS or ICANS were observed. Zamto-cel is currently undergoing phase 2 clinical trials, one as a 2nd line therapy for patients with R/R B-NHL who are transplant-ineligible (DALY 2-EU), and the other for patients with R/R DLBCL after ≥ 2 prior lines of systemic therapy (DALY II USA) (55, 56). The interim analysis for DALY II USA demonstrated good efficacy and tolerability of zamto-cel (56). In 22 evaluable patients, CR rate was 46% and PR 36%, 6-month PFS was 64%. The treatment was well-tolerated, only two had transient and reversible grade 3 ICANS and none had grade ≥ 3 CRS.

Other tandem CD19/20-CAR Ts have been developed and undergone early phase clinical trials. Tong, et al., designed several CD19/20-CAR T. They found that one of the constructs, TanCAR7, had the strongest immunological synapse formation and the most potent antitumor activity (57). TanCAR7 was subsequently studied in a phase 1/2a clinical trial for heavily pretreated R/R B-NHL (NCT03097770). Among 99 enrolled patients, 92 underwent leukapheresis, and 87 received TanCAR7 (58). Twelve (14%) had previous autologous stem cell transplant (SCT) and 9 (10%) had previous exposure to CD19-CAR T. Best ORR was 78% and 70% had CR. Median PFS was 27.6 months (95%CI, 11 months to not reached), and 76% of the responses were durable beyond 12-months. Majority of subjects (70%) developed CRS, including 8 (9%) with grade 3 and 1 (1%) with grade 4, ICANS grade ≥ 3 occurred in 2 (2%) patients. Three patients had treatment-related mortality due to pneumonia or CRS-induced lung injury. CAR expansion was associated with response, but CAR persistence did not significantly impact duration of response (DoR). C-CAR039 is another CD19/20-CAR T that underwent phase 1 study at multiple sites in China (59). Forty-eight patients received C-CAR039, including 44 with LBCL, 3 with FL and 1 with MCL. While the percentage of patients who had prior CD19-CAR T was unknown, while 8 (16.7%) had prior autologous SCT. Response to C-CAR039 was exceptionally high, with ORR 91.5% and CR 85.1%. Estimated 2-year PFS was 66% (95%CI, 53.2 – 81.9%) and OS 77.9% (95%CI, 66.6-91.1%). Although treatment was well tolerated with low rates of grade 3 CRS and no grade 3 ICANS, three patients developed secondary malignancy, including 2 AML and 1 T-cell lymphoma (CAR transgene negative) (60). C-CAR039 is undergoing a multicenter phase 1 study in the US (NCT05421663).

Naïve and memory T-cells-enrichment may improve *in vivo* CAR T function by reducing cell exhaustion and improving persistence (61–63). Regulatory T-cells (Treg, marked by CD25) and myeloid cells (marked by CD14) may cause immunosuppression and reduce CAR T function (64, 65). Larson, et al., conducted a phase 1 trial using autologous naïve and memory T (TN/MEM)-selected, Treg and myeloid cell depleted, tandem CD19/20-CAR T to treat R/R B-NHL (63). Among 17 patients screened, 10 received infusion, yielding a CR rate of 70%. At 17-month follow up, median PFS and OS were not reached. The therapy was safe, no neurotoxicity or grade > 1 CRS were seen. Relapse could be re-treated with CART19/20.

The antigen recognition process of tandem CAR is theoretically unpredictable, therefore bicistronic CAR-T, placing CD20 and CD19 CAR separately on the T-cell, is another approach. Bicistronic CD19/20-CAR T has been successfully manufactured and showed preclinical efficacy in CD19-negative or CD20-negative B-cell lymphoma (66). A phase 1 clinical trial testing this product in R/R B-NHL is under way (NCT05797233).

Although less effective than tandem CD19/20-CAR T in preclinical studies, sequential CD19-CAR T and CD20-CAR T may be easier to manufacture. Sequential infusion strategy was tested in a pilot trial in China (67). In this study, 21 patients with R/R DLBCL who received CD19-CAR T but had undetectable circulating cell levels received CD20-CAR T to prevent relapse. Median interval between cell infusions was 3.72 months (range, 2.56-9). Subsequent CD20-CAR T was well tolerated, with low risk and severity of CRS and ICANS, echoing the observation in other CD20-CAR T studies. Durable CR was seen in 15 (71.4%) patients at a median follow up of 24.7 months (range, 11.64-45.86). CD20-CAR T consolidation post-CD19-CAR T may be a valuable strategy for patients with high-risk DLBCL.

CD19/CD22

Dual CD19/CD22 targeting CAR T-cell therapy is a feasible method to bypass resistant mechanisms including antigen loss/mutation or down-regulation in B-cell malignancies. In B-ALL, CD19/22-CAR T showed good efficacy and DoR, but whether CD19/22-CAR T is better than CD19-CAR T alone remains debatable (68–70). Various strategies aiming to target both CD19 and CD22 have been developed, including CAR T cocktail, bicistronic or tandem CAR, some have entered clinical trials. Bispecific CD19/22-CAR-NK has proof-of-concept preclinical results (71).

CAR-T cocktail is a method of delivering multi-antigen targeting CAR-T cells based on the tumor's antigen expression. CAR-T cocktail could be made by transducing autologous T-cells with two lentiviral vectors at the same time (dual transduction) or separately, or give single antigen-specific CAR T sequentially. Gardner, et al., at the Seattle Children's Research Institute used lentiviral vectors to transduce either CD19 or CD22 CAR into autologous T-cells, resulting in pooled CD19-CAR T, CD22-CAR T, and cells with both CARs (72). However, early phase clinical trials using this pooled product in pediatric B-ALL demonstrated imbalanced CD19- and CD22-CAR T persistence, leading to antigen-negative relapse (73, 74). The Geno-Immune Medical Institute in China designed an autologous CAR-T platform, 4SCAR2.0, using an apoptosis-inducible intracellular domain CD28/CD27/CD3ζ-iCasp9 and variable extracellular domain (anti-CD19, CD11, CD30, CD70, etc) based on tumor antigen. 4SCAR2.0 is currently undergoing a multicenter clinical trial in China (NCT03125577). Each patient can receive multiple 4SCAR2.0 infusions that contains a single-targeting or dual-targeting CAR T. Preliminary results were available from 5 patients with refractory B-NHL, including 1 with PMBCL, 2 with DLBCL and 3 with FL. All received at least one infusion of 4SCAR-19 + 22, demonstrated durable remission, no ICANS and CRS as high as grade 1 (75, 76). It is plausible that repeated dosing improved the response rate and DoR, and low cell dose and the apoptosis-inducible domain reduced toxicity.

Sequential administration of CD19- and CD22-CAR T to treat R/R B-cell malignancies has been tested in China. In pilot study, Wang, et al., enrolled 89 patients, including 38 with R/R B-NHL and the rest with ALL. For the B-NHL cohort, each patient received around 5 x 10^6 cells/kg of CD19- and CD22-CAR T on successive days. ORR was 72.2% and CR rate 50%, median DoR was 15 months (range, 12-17). At a median follow up of 14.4 months, the median PFS was 9.9 months (95%CI, 3.3-NR) and median OS 18.0 months (95%CI 6.1-NR) (77). The group subsequently conducted another clinical trial, sequentially administering high-dose

chemotherapy followed by autologous SCT, CD22- and CD19-CAR T to patients with R/R B-NHL (78). In 49 consented patients, 42 completed sequential cellular therapy, including 23 with progressive disease and 9 with stable disease prior to SCT. Engraftment was not significantly impacted by post-SCT CAR-T. CRS was common (90%) but only 2 (5%) had grade ≥ 3 CRS. ICANS occurred in 9 (21%) and grade ≥ 3 in 2 (5%). High durable remission rates were reported, with 12-month PFS 95.7% (95%CI, 70.9-93.3%), 12-month OS 90.5% (95%CI. 76.6-96.3%), but those failed to respond by 3-month (n=4) had a dismal prognosis. Although response rate and survival appeared better than the previous study without SCT, the benefit of SCT should be carefully evaluated due to the financial toxicity of combination cellular therapy, and inherited risks of transplant such as hematologic toxicity, organ toxicity, secondary malignancies.

AUTO3 is a bicistronic CD19/22-CAR T previously tested in a phase 1 study in patients with R/R LBCL (ALEXANDER, NCT3289455) (79). The CD19 CAR has a OX40 costimulatory domain, and CD22 has a 4-1BB costimulatory domain (80). Pembrolizumab was used in combination, because PD-L1 upregulation was a possible resistance mechanism demonstrated by previous research. In the study, 52 patients received AUTO3 infusion and 48/52 received pembrolizumab (92.3%); treatment was administered as outpatient in 20 (38.5%) patients. None had previous exposure to CD19- or CD22-targeted therapies. In 47 evaluable patients, ORR was 66.0% and CR 48.9%. However, long-term outcome was not improved by neither AUTO3 nor pembrolizumab due to high rates of relapse, leading to a median PFS was 3.32 months. Response was not associated with AUTO3 expansion. Low serum cytokine levels were observed across the study cohort, correlating with low burden of CRS and ICANS. Previous study of AUTO3 in pediatric B-ALL also demonstrated disappointing results, indicating the necessity for CAR-T optimization (80). Immune checkpoint inhibitors had disappointing results in the treatment of R/R LBCL, which was reaffirmed by the ALEXANDER trial (81).

Tandem CD19/22-CAR T, with both anti-CD19 and anti-CD22 scFv on the extracellular domain, transduced by a single lentiviral vector, are also developed. Among all extracellular designs, tandem CD19/22-CAR with alternative sequence of scFv heavy and light chains, resulting in a loop structure of the extracellular domain, was the most potent one preclinically (82, 83). The loop CAR T subsequently underwent a phase 1 trial, 17 patients with R/R B-ALL and 21 with R/R LBCL received cell infusion. Rate of CRS was 76% (grade≥3, 5%), rate of ICANS was 37% (grade≥3, 10%) and two had laboratory evidence of HLH; all CRS and ICANS resolved. ORR in the LBCL cohort was 62%, half (29%) achieved a CR. Again, the response was short-lived, with median PFS 3.2 months (95%CI, 1.2-5.5). Main cause of resistance/relapse remained antigen loss, but authors also observed that the cells had suboptimal cytokine production against CD22, contributing to CD19-/CD22+ relapse (83). Alternative CD22 scFv and CAR design could potentially improve the efficacy of tandem CD19/22-CAR T (84, 85). Several small single-center clinical trials from China using different CAR constructs in patients with R/R aggressive B-NHL reported seemingly better results (86, 87). Another way to augment CD19/22-CAR T function is through epigenetic modification. Decitabine may enhance CAR-T function and reverse T-cell exhaustion by demethylating silenced genes, and may reverse antigen loss by upregulating antigen expression on lymphoma cells (88, 89). In a retrospective study, adding decitabine to lymphodepletion chemotherapy could improve response rate, DoR and survival in patients receiving CD19/22-CAR T (89).

CD20/CD22

CAR-T targeting both CD20 and CD22 is attractive for CD19-low or negative relapses. Off-the-shelf allogeneic CAR-T may avoid major hurdles in autologous CAR-T, such as T-cell exhaustion and length production time. Based on these premises, Aranda-Orgilles, et al., designed UCART20x22, an allogeneic, dual CD20 and CD22-targeting CAR-T (90). The allogenic T-cells underwent transcription activator-like effector nuclease (TALEN) mRNA-mediated *CD52* and *TRAC* gene knockout. *CD52*-knockout was to allow the addition of alemtuzumab into lymphodepletion, to avoid rejection; *TRAC*-knockout prevents graft-versus-host disease (91). UCART20x22 is currently being evaluated in NatHaLi-01 (NCT05607420), a phase 1/2 clinical trial for R/R B-NHL. Preliminary results from three treated patients were reported recently. All received DL1. No ICANS or grade ≥ 3 CRS have been observed. At day 28, two achieved CR and one PR (92). NatHaLi-01 has a target sample size of 80 patients and is estimated to complete by November 2027.

Other targets

BAFF and BAFF receptor

B-cell activating factor (BAFF) is crucial for the survival of mature B-cells (93). BAFF has three receptors – BAFFR, TACI, and BCMA – with varying but ubiquitous expression on a variety of B-cell NHLs (94). Due to the importance of BAFF to B-cell survival, antigen escape to BAFF or BAFFR is less likely than CD19 (95). CAR Ts designed against BAFF or BAFFR have both been constructed (95, 96). BAFFR-CAR T demonstrated effective antitumor effect in CD19-negative B-NHL and B-ALL in both *in vitro* cell line and *in vivo* xenograft studies (95). Compared with BAFFR-CAR T, BAFF-CAR T may minimize antigen escape more effectively with the ability to bind to three different receptors (96). Currently, BAFFR-CAR T is being tested in B-ALL or LBL (NCT04690595) while BAFF-CAR T is being tested in B-NHL (NCT05312801).

CLL-specific targets - CD23, FCμR, Siglec-6

The treatment paradigm of CLL has shifted dramatically since the development of targeted therapies. However, patients with risk factors such as TP53 mutation, who are refractory to BTKi or BCL2 inhibitors, or those with Richter's transformation (RT) still have a dismal prognosis. Two issues have hindered the development of CAR-T in CLL. One is the low efficacy of CD19-CAR T in CLL compared with other B-cell malignancies. Different studies reported

a CR rate of 20-70% and 18-month PFS of 25% (97). It is suspected that autologous CAR-T suffers from intrinsic T-cell exhaustion, but similar poor response has also been observed in allogeneic CAR-T (98, 99). Other factors may contribute to CLL's resistance to CAR-T therapy, such as high immune checkpoint protein expression, immune suppressive tumor microenvironment (TME), and high level of circulating inhibitory extracellular vesicles (100). The efficacy data of CD19-CAR T in RT remain scarce and conflicting (101). Some patients may even develop RT after CD19-CAR T, indicating the presence of intrinsic resistance mechanisms in RT to CD19-targeted therapy (102). The other issue with CAR-T development is the risk of B-cell aplasia and prolonged immunosuppression from the "on-target, off-tumor" effect of pan-B-cell targeting. Alternative targets to CD19 have been explored to spare the normal B-cell compartment. CD23, IgM Fc receptor (FCμR, other names include TOSO and Fas-inhibitory molecule 3), and Siglec-6 are proposed targets for CLL. CD23 is typically overexpressed in CLL but not in normal B-cells (103). Preclinical studies demonstrated the efficacy of CD23-CAR T in CLL, which could be enhanced by lenalidomide (104). Similar to CD23, FCμR is highly and consistently expressed by CLL cells but only marginally expressed by normal B-cells and hematopoietic stem cells (HSC) (105). In preclinical models, FCμR-CAR T could eliminate CLL cells while maintaining the number of healthy B-cells (105). Siglec-6 is a CLL surface antigen that is highly restricted in other tissues (106). Anti-Siglec-6 antibody JML-1 has the strongest CLL surface reactivity among detected antibodies in patients with CLL cured by allogeneic hematopoietic stem cell transplant (alloHSCT) (107). Kovalovsky, et al., constructed Siglec-6-CAR T using JML-1-derived scFv, and showed selective cytotoxicity against CLL cells in *in vitro* and *in vivo* xenograft murine models (106). The efficacy and safety of CLL-specific CAR-T need further clarification by clinical trials.

CD32B

CD32B is the predominant Fc receptor on B-cells and is expressed on a variety of B-cell malignancies (108). CD32B contributes to resistance development to targeted antibodies such as rituximab, by accelerating internalization of the antibodies (109). Therefore, CD32B is an attractive target for B-cell lymphoma not only from the abundance and specificity, but also for the potential reversal of CD20-resistance. In preclinical models, CAR-T targeting CD32B had effective cytotoxicity against CLL (110). Unlike CD23-CAR T, CD32B-CAR T may not avoid B-cell aplasia because CD32B expression is not limited to CLL. Of note, a small percentage of CD4+ or CD8+ T-cells also express CD32B, which is a T-cell activation suppressor (111). The implication of CD32B expression by T-cells on CD32B-CAR T is unclear.

CD70

The CD70-CD27 axis is involved in immune evasion and tumor progression. Aberrant co-expression of CD70 and CD27 has been observed in various B and T-NHL, and high CD70 expression but absence of CD27 has been seen in HL (112). Normal hematopoietic stem cells and most blood cells do not express CD70, making it a desirable CAR target (113). Meanwhile, CD70 contributes to T-cell exhaustion, therefore CD70-CAR T with CD70-knockout may have better performance than CD70-wildtype (114). CD70 is one of the targets of the aforementioned 4SCAR2.0 platform being studied in China (NCT05436496). Eight patients with heavily pretreated DLBCL have been treated with dual CD19 and CD70 targeting CAR T, with CR in 6 and ORR in 7 patients, and median PFS 10.5 months (115). One patient with R/R PCNSL enjoyed ongoing complete remission at 17-month post-4SCART19/70 (116). CD70 is also a viable CAR-NK target but requires CD70-knockout to prevent fratricide (117).

CD72

CD72 is a B-cell restricted, highly expressed surface antigen, and is upregulated in B-cell ALL and NHL. Down regulation or loss of CD19 do not impair CD72 expression (118). Different CD72-CAR Ts are undergoing preclinical testing in B-ALL and B-NHL models, showing promising efficacy and no off-target effect (119, 120).

CD79

CD79 is a B-cell restricted cell surface heterodimer (CD79a and CD79b, also known as Igα and Igβ) that participate in the B-cell receptor signaling pathway (121). CD79-targeting is an effective strategy in the treatment of B-NHL, exemplified by an anti-CD79b ADC polatuzumab-vedotin. CARs targeting either CD79a or CD79b are under development. JV213 is a CD79b-CAR T with a novel CD79 monoclonal antibody, a CD8α hinge/transmembrane domain, and an OX40 costimulatory domain. In preclinical testing, JV213 was superior to other CD79b-CAR Ts, and a phase 1 clinical trial using JV213 in R/R B-cell lymphomas was initiated (NCT05773040) (122). In a different group, Jiang, et al., introduced an inducible caspase-9 (iCas9) suicidal gene system into CD79b-CAR T to improve safety targets the B-cell receptor component Igβ to avoid antigen escape (123). The iCas9 CD79b-CAR T is undergoing early phase clinical trial in China (NCT05312476). bbT369 is a CD20 and CD79a dual-targeting CAR T which is current being evaluated a multicenter phase 1/2 clinical trial for R/R B-NHL (CRC-403, NCT05169489). In preclinical testing, bbT369 was more potent than CD19-CAR T and induced longer remission (124). Dual-targeting CAR T against CD19 and either CD79a or CD79b CARs are developed (125, 126). Leung, et al., demonstrated that CD19/79a(b)-CAR T induced longer tumor control than single-antigen targeting CAR T from preventing antigen-loss relapse, and targeting CD79a was more potent than C79b. However, bispecific CAR T with either tandem or bicistronic CAR structures had reduced activity against single-antigen positive cells due to compromised antigen binding and signaling, indicating the need to optimize structural design (126). Low-level aberrant CD79b expression monocytes, hematopoietic progenitor cells and T-cells could theoretically cause untoward hematologic toxicity, which will be elucidated by clinical trials (127).

ROR1

Receptor tyrosine kinase-like orphan receptor 1 (ROR1) is a cell surface protein overexpressed on various solid and hematological malignancies and minimally expressed on most adult tissues,

contributing to cancer stemness (128). To minimize toxicity on hematopoietic stem cells, different switchable CAR-ROR1 have been developed to allow tumor-restricted killing (129, 130). Early phase clinical trials are ongoing to study the efficacy and safety of ROR1-CAR T (ONCT-808) in patients with R/R BCL and/or advanced solid tumors, including those with RT (NCT05694364, NCT05588440) (130, 131). PRGN-3007 is a novel ROR1-CAR T that also includes membrane-bound IL15 for *in vivo* expansion and persistence, a kill switch for safety, and intrinsic PD-1 blockade to enhance cytotoxicity (132). PRGN-3007 is currently undergoing a phase 1/1b clinical trial for both R/R B-NHL and breast adenocarcinomas (NCT0569434) (130).

Immunoglobulin light chain CAR

Immunoglobulin is a part of the BCR and participates in BCR signaling upon biding to antigen. Normal B-cells have polyclonal surface immunoglobulin but malignant B-cells are monoclonal. Immunoglobulin light chain κ or λ-targeted CAR takes advantage of the light chain restriction of mature B-cell malignancies, and preserves humoral immunity by sparing normal B-cells with the reciprocal light chain (133). Circulating light chain could help improve CAR-T persistence (134). Dotti, et al, are conducting a phase 1 trial (NCT00881920) studying the safety of κ-CAR T in patients with R/R B-NHL/CLL or MM. Preliminary results on 16 treated patients (B-NHL/CLL = 9, MM = 7) demonstrated that κ-CAR T was well-tolerated without attributable toxicity. CR was achieved in two and PR in one in the B-NHL/CLL cohort (135). The group also constructed λ-CAR T, which in preclinical tests demonstrated light-chain restricted cytotoxicity (136).

B-cell maturation antigen

B-cell maturation antigen (BCMA) is not only expressed on multiple myeloma but also on a subset of B-NHL and CLL (137). The manufacture of bispecific CD19/BCMA CAR-T and CAR-NK are both feasible (138, 139). Early phase clinical trial demonstrated the efficacy of CD19/BCMA-CAR-T in multiple myeloma (138). The safety and utility of CD19/BCMA-CAR-T in aggressive B-NHL will be studied in a phase 1 clinical trial (NCT06097455).

Tri-specific CAR

CAR-T targeting CD19, CD20, and CD22 could potentially prevent relapse due to antigen loss or down-regulation more effectively. Zhou, et al., designed a tri-specific tandem CAR T that showed stronger cytolytic activity than mono- or bispecific CAR T in preclinical models (140). Schneider, et al., designed a tri-specific CAR T that contained a tandem CD20-CD19 CAR and a second CD22 CAR (141). Costimulatory domain derived from ICOS and OX40 or CD27 was more effective than CD28 or 4-1BB in tri-specific CAR, indicating the importance of optimizing costimulatory domains based on different single-chain variable fragment of the CAR. The tri-specific CAR T with OX40 costimulatory domain has been chosen for a phase 1 clinical trial in patients with R/R B-cell malignancies including NHL and CLL (NCT05418088) (142).

Classical Hodgkin lymphoma

Classical Hodgkin lymphoma (cHL) is characterized by a small percentage of malignant Hodgkin and Reed-Sternberg (HRS) cells that are of mature B-cell origin, embedded within a highly immunosuppressive, HRS-induced TME (143). Despite recent advances, patients with R/R cHL, especially those with prior exposure to CD30-ADC brentuximab vedotin and checkpoint inhibitors, still have a dismal prognosis. CAR-based therapy is a potential therapeutic option for these patients. The development of CAR in HL has been focused on targeting surface antigens of HRS cells and/or reversal of immune evasion (144). Active clinical trials of novel CAR-based therapy in cHL are listed in Table 2.

CD30

CD30 is one of the most extensively studied targets on cHL due to its strong and restricted expression on HRS. CD30 expression is upregulated on activated B- and T-cells (145). CD30 plays an anti-apoptotic and immunosuppressive role in lymphoma and TME, and may trigger chromosome instability and mutations in lymphoma cells (146). The study of CD30-CAR T in cHL began in the late 1990s, but the clinical application has been plagued by suboptimal response rate and duration of response, albeit generally good tolerance Based on pre-clinical data of a CD30-CAR T construct using a mouse-derived anti-CD30 monoclonal antibody as scFv, Ramos, et al., conducted a phase 1 clinical trial enrolling nine patients with heavily pretreated Epstein-Barr virus (EBV)-, CD30+ lymphoma, including seven with cHL (NCT01316146) (147, 148). Lymphodepletion chemotherapy (LDC) was not given. CD30-CAR T was well tolerated. Two patients with cHL had durable CR, the rest had transient SD. Subsequently, two parallel phase 1/2 studies of CD30-CAR T in patients with R/R CD30+ lymphoma were conducted, enrolling 41 adult patients with heavily pretreated cHL (NCT02690545, NCT02917083) (149). LDC regimens included bendamustine ± fludarabine, and fludarabine/cyclophosphamide. Among evaluable patients, ORR was 72% and CR 59%, 1-year PFS was 41%. Cutaneous and hematological toxicities were notable after CD30-CAR T infusion. No ICANS was seen and all CRS cases were low grade. Bendamustine/fludarabine LDC conferred the lowest rate of CRS and best response, and was selected for an ongoing multicenter phase 2 CHARIOT study evaluating CD30-CAR T in R/R cHL who failed at least 3 prior lines of therapy including chemotherapy, brentuximab-vedotin and PD-1 inhibitor (NCT02259556) (150).

Wang, et al., conducted a phase 1 clinical trial in China using a CD30-CAR T with different anti-CD30 scFv (NCT02259556) for patients with R/R CD30+ lymphoma (151). Eighteen patients were enrolled, including 17 with cHL and 1 with primary cutaneous anaplastic large-cell lymphoma (ALCL). Therapy was well tolerated however none of the patients achieved CR. PR rate was 39% and median PFS was 6 months (range, 3-14 months). Extranodal disease had poor response to CD30-CAR T. Another phase 1 study in China utilized a CD30-CAR T with dual CD28 and 4-1BB

TABLE 2 Active clinical trials of novel CAR therapy for Hodgkin Lymphoma and T-cell lymphoma.

CAR Target	NCT number	Disease	Phase	Location	Notes
CD4	NCT03829540	CD4-positive ALL and NHL	1	US - IU and Stony Brook Cancer Center	
CD5	NCT04767308	CD5-positive NHL	1	China	
	NCT03081910	CD5-positive T-cell leukemia and lymphoma	1	US - Baylor	Two arms: AutoCAR and AlloCAR; AlloCAR is from prior allogeneic transplant donor
	NCT05138458	CD5-positve T-cell lymphoma	1 and 2	US - multicenter	
CD7	NCT04599556	CD7-positive hematologic malignancy	1 and 2	China	
	NCT04840875	CD7 positive ATLL and T-ALL	1	China	
	NCT04823091	CD7-positive T-cell leukemia and lymphoma	1	China	AlloCAR
	NCT04689659	CD7-positive T-cell leukemia and lymphoma	2	China	AlloCAR
	NCT05059912	CD7-positive T-cell lymphoma	2	China	
	NCT05377827	CD7-positive malignancies including T-NHL or AML	1	US - Wash U	AlloCAR
	NCT03690011	CD7-positive T-cell lymphoma	1	US - Baylor	
CD30	NCT02259556	CD30-positive HL and NHL	1 and 2	China	
	NCT04653649	HL and CD30-positive ALCL and PTCL	1 and 2	Spain	Memory T-cells enriched, proximal target on CD30
CD30	NCT02917083	CD30-positive malignancy	1	US - Baylor	
	NCT02690545	CD30-positve HL and NHL	1 and 2	US - UNC	Flu/Benda LDC
	NCT06090864	CD30-positive HL	1 and 2	US - UNC	Co-expressing CCR4; Flu/Benda LDC
CD30	NCT03602157	CD30-postive HL and CTCL	1	US - UNC	Co-expressing CCR4; Flu/Benda LDC
	NCT04288726	CD30-positive HL, PTCL, other aggressive NHL	1	US - Baylor	AlloCAR using EBV-specific T-cells
CD37	NCT04136275	CD37-positive HL and NHL	1	US - MG	DLT at doses ≥ 100 million/kg, with bone marrow aplasia requiring allogeneic stem cell rescue
CD147	NCT05013372	CD147-positive T-NHL	1	China	AlloHSCT eligibility and donor availability are required
TRBC1	NCT04828174	TRBC1 positive T-cell malignancy	1	China	Suspended due to inability to enroll qualified patients
	NCT03590574	TRBC1 positive T-NHL	1 and 2	Europe - multicenter	

US, United States; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin Lymphoma; IU, Indiana University; AutoCAR, autologous CAR-T; AlloCAR, allogeneic CAR-T; Wash U, Washington University in St. Louis; HL, Hodgkin lymphoma; ALCL, anaplastic large cell lymphoma; PTCL, peripheral T-cell lymphoma; UNC, University of North Carolina; MG, Massachusetts General Hospital Cancer Center; DLT, dose-limiting toxicity; alloHSCT, allogeneic hematopoietic stem cell transplant.

costimulatory domains and post-CAR anti-PD-1 consolidation (152). Among 6 patients with R/R cHL, 1 died due from early post-CAR-T pleural hemorrhage, 5 achieved CR, but only 1 enjoyed ongoing remission beyond 3 years, the rest relapsed within 10 weeks to 28 months. Although the sample size was small, it appeared that dual costimulatory domain improved CD30-CAR T persistence but did not lead to increased toxicity.

Major barriers to the success of CD30-CAR T include off-target elimination of other CD30-expressing cells such as other T-cells, inefficient homing to tumor, intrinsic resistance of the tumor cells, and CD30-downregulation (153, 154). Whether soluble CD30 affect CAR function remains controversial (147, 148). A Spanish group developed a CD30-CAR T that targets a proximal epitope of CD30 to avoid interaction with soluble CD30 and enriched the product in memory T-cells. Preliminary results showed a 100% overall response rate and 50% durable CR rate in 10 patients who received therapy (155). To improve homing to lymphoma and persistence of CAR-T, strategies include co-expression of CCR4 or dual costimulatory domain of CD28/4-1BB (156, 157). CCR4-expressing CD30-CAR T is being evaluated in a phase 1 clinical trial (NCT03602157) (158). Preliminary data on 8 evaluable patients with cHL demonstrated 100% ORR with CR in 75%. Patient experienced no dose-limiting toxicity and the duration of response was prolonged – at a median follow up of 12.7 months, the median PFS was not reached and one

CR was ongoing beyond 2.5 years. To reverse intrinsic immune suppression in HL, post-CD30-CAR T PD-1 blockade has been studied in multiple studies showing improved expansion of CD30-CAR T and duration of response (152, 159, 160). Autologous stem cell transplant may have synergistical effects with CD30-CAR T and could consolidate and prolong the remission post-CD30-CAR T (161).

As previously tested CD30 scFv were derived from murine antibodies, there is a concern of human anti-mouse antibody causing resistance to CD30-CAR T. Fully-humanized CD30-CAR T was developed and tested in a phase 1 clinical trial at the NIH but with disappointing results (162). Among 21 patients treated, 20 had cHL; ORR was 43% and CR only occurred in 1 patient. Median duration of response was 8.9 weeks. The study was stopped early due to prolonged cutaneous and hematological toxicities. It was speculated that high disease burden and poor penetration of CAR-T to lymphoma due to immunosuppressive microenvironment were the main reasons for low efficacy.

Other targets

Several cell surface markers in the cHL TME have been proposed as CAR targets to break the immunosuppressive cycle. CD19+ B-cells are part of the cHL TME and some CD19+ B-cells may be HRS stem cells, making CD19 a putative target for CAR-T in cHL (163). Svoboda, et al., conducted a pilot study using a nonviral messenger RNA-vector transduced CD19-CAR T in patients with R/R cHL (NCT02277522 and NCT02624258) (163). Nonviral vector transduced CAR was only expressed for a few days as opposed to the persistent expression of viral-transduced CAR, potentially reducing the toxicity. The treatment was well tolerated but response was limited and short-lived. Of 4 treated patients, only 1 achieved CR but relapsed within 3 months. cHL positive for both CD19 and CD30 account for about 5% of all cases, making lentiviral-transduced sequential CD19-CAR T and CD30-CAR T is another therapeutic strategy (164). In a case report, one patient with CD19+CD30+ cHL had PR with CD19-CAR T and further response after subsequent CD30-CAR T (164). CD20 expression is more common in cHL, comprising around 20% of all cases and the prevalence is higher in EBV+ cHL (165, 166). Anti-CD20 therapy with rituximab is effective in CD20+ cHL (167). it is plausible that CD20 can become a CAR target for patients with CD20+ cHL.

CD123 is the α-subunit of interleukin-3 receptor and is widely expressed both on HRS and on the tumor-associated macrophages in the TME (168). In a preclinical study by Ruella, et al., CD123-CAR T may target HRS directly and overcome the immunosuppressive TME, killing cHL both *in vitro* and *in vivo*, and establish long-term memory (169). Due to expression of CD123 on normal hematopoietic cells and endothelial cells, off-target toxicity such as bone marrow failure is a valid concern for clinical application.

T-cell lymphoma

Development of CAR T-cell therapy in T-cell malignancies have been limited by fratricide, i.e., killing of sibling CAR and normal T-cells, leading to reduced efficacy and profound immune suppression (170). Identification of malignancy-specific T-cell surface antigen, such as C-C motif chemokine receptor 9, may circumvent fratricide (171). Some antigens are shared between B and T-cell lymphomas, such as CD37 and CD38, and no significant fratricide has been seen with CAR-T targeting CD37 or CD38 in preclinical experiments (172). Another hurdle in CAR T development is the risk of contamination by malignant T-cells in autologous products (173). Further, functional T-cells may be absent in patients with advanced T-NHL, leading to poor autologous T-cell quality (174). AlloCAR, on the other hand, may introduce GvHD. T-cell receptor alpha constant (TRAC)-knockout can prevent GvHD and is often employed in the construction of alloCAR (175). Tyrosine kinase inhibitors dasatinib and ibrutinib may suppress fratricide, enhance anti-tumor activity, and promote expansion (176, 177). Another promising development is CAR-NK which are non-GvHD inducing, off-the-shelf, and can be modified to prevent fratricide (174, 178). Active clinical trials of novel CAR-based therapy in TCL are listed in Table 2.

CD5

CD5 is present on at least 80% of T-cell malignancies, thymocytes, peripheral T-cells, and a small proportion of B-cells. Mamonkin, et al., constructed CD5-CAR T with CD28 costimulatory domain had attractive features in preclinical experiments, namely limited and transient fratricide and preserved immune response to viral antigens, but the cells failed to eliminate malignant T-cells *in vivo* and animals developed CD5+ relapse (179). CD5-CAR T with 4-1BB costimulatory domain exhibited better antitumor activity but with increased fratricide. To overcome fratricide, Mamonkin et al., designed reversible CAR expression system that could be suppressed with small molecules during *in vitro* cell culture, and restore CAR expression *in vivo* after drug withdrawal (180). Based on promising pre-clinical studies, Hill and Mamonkin, et al., conducted a phase 1 clinical trial applying autologous CD5-CAR T with CD28 costimulatory domain to patients with R/R T-cell malignancies (NCT0308190) (181). Among 17 enrolled patients with heavily pretreated TCL, 2 died before cell manufacture and 2 died prior to infusion, 2 received alternative therapy, I failed eligibility, and 1 production failed, rendering a total of 9 patients who received cell infusion. Therapy was well tolerated, with toxicity profile similar to commercial CD19-CAR T. Response was observed in 4 patients, including two CRs that lasted 6.4 and 7.2 months in the absence of consolidative alloHSCT (181). The study highlighted the challenges in timely manufacture of CAR-T in this heavily pre-treated population. Off-the-shelf alloCAR and CAR-NK may be more readily available for patients. Donor-derived CD5-knockout CD5-CAR T has been evaluated in a phase 1 clinical trial in patients with R/R T-ALL (182). All patients were previously treated with CART7 and had CD7-negative relapse. MRD-negative CR was achieved in all patients but the follow up was limited. Most of the side effects were hematological, but 1 patient developed lethal EBV infection with HLH at 2.7 months post therapy. NK-92 cells

transduced with CD5-CAR have demonstrated effective antitumor activity in murine T-ALL/TCL xenograft models (183, 184).

CD7

Autologous and allogeneic CD7-CAR Ts (autoCD7 and alloCD7, respectively) have both been developed. Most of the CD7-CAR T clinical trials have focused on T-acute lymphoblastic leukemia (ALL)/lymphoblastic lymphoma. An off-the-shelf alloCD7 product, WU-CART-007, utilized CRISPR/Cas9 deletion of CD7 and TRAC to minimize fratricide and GvHD (185). WU-CART-007 is undergoing a global phase 1/2 clinical trial in T-ALL/LBL (NCT04984356) (186). Pan, et al., conducted a phase 1 clinical trial in China focusing on T-ALL/LBL with post-alloHSCT relapse (187). T-cells were harvested from original donor (n = 12) or new donor (n = 8). The lentiviral vector had a CD7 binding domain to retain CD7 intracellularly and prevent fratricide. CRS and GvHD were common but mostly low grade. ICANS occurred in 15%, all < grade 3. All patients had grade 3-4 cytopenia likely related to the nature of disease. CR was seen in 90% of patients and 83% of the CR were durable at a median follow up of 6.3 months. New donor-derived CD7-CAR T did not cause higher rate of GvHD due to mixed chimerism. The authors also observed T-cell immune reconstitution from CD7-negative T-cells.

Another phase 1 study from China compared the outcomes of patients with T-cell malignancies, including 8 T-ALL/LBL and 2 PTCL, who received autoCD7 (n = 5) or alloCD7 (n = 5) (NCT04823091) (188). Notable toxicities included CRS in 8 patients, grade 3 CRS in 1, and HLH in 2. No ICANS was observed. Two patients experienced mild GvHD. Majority of patients had significant pancytopenia and/or infection complications. Unfortunately the two patients with PTCL did not respond. AlloCD7 was more readily available and did not require washout between chemotherapy and leukapheresis, benefiting patients with rapidly progressing refractory disease. Compared with autoCD7, alloCD7 was associated with higher response rate, less relapse, and better CAR T persistence (188). For patients with T-ALL/LBL Allogeneic stem cell transplantation post-alloCD7 was shown to be safe, and patients with CD7-positive relapse post-transplant could achieve remission again with alloCD7 re-treatment (186, 189).

Fratricide resistant CD7-CAR T may also be produced through natural selection of minimal CD7 epitope expressing T-cells from bulk T-cells either *in vitro* or *in vivo* (177, 190). Dasatinib and ibrutinib can temporarily inhibit fratricide and facilitate *ex vivo* expansion (177). CD7 is a viable target for CAR-NK and different NK-92-based CD7-CAR NKs have been developed in the lab pending clinical verification (191, 192).

Relapse after CD7-CAR T is usually from antigen escape which may be prevented by dual CD5/CD7 targeting (187, 193). In a preclinical study, Dai, et al., transduced CD5, CD7 or tandem CD5/CD7 scFv to CD5/CD7 knockout T-cells. CD5 and CD7 knockout did not change TCR structure and prevented fratricide. Tandem CD5/CD7 CAR-T had the best *in vivo* antitumor activity and prolonged the survival of mice bearing xenograft in murine xenograft models (193).

CD2

Costimulatory receptor CD2 is commonly expressed on T- and NK-cell surface and is involved in T-cell development and function (194). CD2 is expressed in about 90% adult peripheral T-cell lymphoma and about 70% in pediatric T-acute lymphoblastic leukemia/lymphoma (ALL/LBL) and PTCL, higher than CD7 (40-50%) (195). The development of CAR2 is potentially limited by fratricide, necessitating CD2-knockout in CAR-T. Recently, Angelos et al., tested CD2-knockout autologous CD2-CAR T in preclinical models, showing high antitumor activity even in post-CAR5 relapsed T-ALL xenograft mice (195). Xiang et al., developed a CD2 and TCR alpha subunit knockout allogeneic CAR T against CD2 (UCART2) to minimize fratricide and GvHD. CD2-knockout led to reduced CAR-T function, which could be overcome by coadministration of IL-7. Preclinical study demonstrated that the combination of UCART2 and IL-7 could effectively prolong the survival of xenograft mouse model with T-cell malignancy (196).

CD147

CD147 is highly expressed in several types of solid tumor and T-cell malignancies. Aside from promoting invasion and metastasis, CD147 is indispensable for T-cell differentiation at the thymus level (197). Several CD147-CAR Ts of various designs are undergoing different stages of clinical development in hepatocellular carcinoma and non-small cell lung cancer (198–200). CD147-CAR T exhibited potent efficacy and absent off-target effect in cell-line and xenograft models (201). An early phase clinical trial (NCT05013372) is being conducted in China to evaluate the safety and efficacy of CD147-CAR T in CD147-positive R/R T-NHL.

T-cell receptor-based therapy

T cell receptor β-chain constant domains 1 and 2 (TRBC1 and TRBC2) expression are mutually exclusive on T-cell surface. TRBC1 is expressed by around 40% of normal T-cells, and the incidence of TRBC1 positivity in T-cell malignancies is similar (202, 203). TRBC-targeting can eliminate the cancer and normal T-cells that express the specific TRBC but spare the other group of normal T-cells, thereby limiting fratricide, and rescuing patients from intolerable immunosuppression (204). An ongoing phase 1/2 clinical trial (NCT03590574) demonstrated that autologous TRBC1-CAR T was well-tolerated, and at a higher dose could induce response in patients with R/R TRBC1-positive PTCL (203). Initially, duration of response was short, with a lack of circulating CAR T expansion. The manufacturing process was modified to produce a more naïve phenotype, improving the duration of response (205). Another method that may improve TRBC1-CAR T function is to only transduce pre-selecting TRBC1-negative T-cells, to prevent fratricide and contamination of the product by TRBC1-positive malignant cells (202). Updated results from clinical trials are eagerly anticipated to further elucidate the utility of autologous TRBC1-CAR T.

TCR receptor variable region have also been proposed as potential CAR targets. T-cell malignancy typically exhibit TCR variable β-chain (Vβ) clonality, therefore targeting malignancy-specific Vβ may achieve cancer-specific cytotoxicity while sparing other normal T-cells. Vβ-targeting CAR-T and CAR-NK have both been generated, with proof-of-concept preclinical results (174, 206). TCR mutation or down-regulation may confer resistance to TCR-targeting therapy. Some T-NHLs do not have αβ TCR but have γδ TCR, and those would not respond to therapy against TRBC or Vβ.

Other targets

Pan-T antigen CD3 is a target of interest in T-cell malignancies but antibody-based therapies have been unsuccessful (207). Gene-edited T-cells with disrupted CD3 expression can be made into CD3 CAR-T that are resistant to fratricide. CD3 is also an appealing target for CAR-NK as NK-cells do not express CD3. Several pre-clinical studies have demonstrated strong antitumor activity of CD3-targeted, CD3-knockout CAR-T or CAR-NK in preclinical studies (208–210).

CD4 is a ubiquitous marker of mature T-cells. CD4-targeting CAR T with an alemtuzumab safety switch is currently in clinical trial for CD4-positive R/R T-cell NHL and ALL. Preliminary results demonstrated the efficacy and safety of CD4-CAR T (211). CD4 is also a potential CAR-NK target (212).

CD30 is ubiquitously expressed in systemic ALCL and variably expressed in other PTCL (213). Although CD30-CAR T is mainly studied in cHL, several patients with ALCL were enrolled in different trials, with good tolerance, minimal fratricide, and some patients enjoyed durable CR (147, 151, 152). Aside from ALCL, Voorhees, et al., reported durable CR after CD30-CAR T in a patient with heavily pre-treated CD30+ enteropathy-associated T-cell lymphoma (214). Of note, the patient previously received alloHSCT so the CD30-CAR T was of allogeneic origin.

NKG2D-ligand is highly expressed on cancer cells and rarely on healthy tissue. The NKG2D/NKG2D-ligand interaction is important for NK-cell mediated immune surveillance, but contributes to NK-cell exhaustion in cancer (215). T-cells transduced with NKG2D has become an exciting tumor-agnostic treatment for cancer. In NKG2D-ligand deficient tumor, NKG2D-T may also induce tumor-specific immunity by enhancing immune surveillance and modifying TME (216). Preclinical study demonstrated efficacy of NKG2D-T in T-NHL cell lines (216). Currently, several clinical trials are actively testing the efficacy and safety of NKG2D-T in solid tumors and AML/MDS (217).

CCR4 is a chemokine receptor expressed mainly in Tregs. Anti-CCR4 monoclonal antibody mogamulizumab has proven efficacy in T-reg-derived malignancies such as adult T-cell leukemia/lymphoma (ATLL) and CTCL. Perera, et al., demonstrated preclinical *in vitro* and *in vivo* efficacy of CCR4-CAR T in ATLL and other CCR4-positive TCL (218). Contrary to other CAR T, fratricide can improve the quality of CCR4-CAR T by eliminating Treg and type-2 helper T-cells, while sparing cytotoxic CD8+ and type-1 helper T-cells, enhancing antitumor efficacy (219).

Tumor-agnostic CAR

Certain molecules are expressed in different types of lymphoma and can become tumor-agnostic targets for lymphomas sharing the same marker. Besides CD30, which is shared by both cHL and TCL, other molecules of interest include CD37, CD38, and EBV-associated protein.

CD37 is highly expressed on universally all B-NHL and some cutaneous T-cell lymphoma (CTCL) and peripheral T-cell lymphoma (PTCL), while absent on hematopoietic stem cells, making it a potentially feasible CAR target (172). Clinical trials utilizing anti-CD37 monoclonal or bispecific antibodies and ADC in R/R B-NHL have been disappointing in general, likely due to the close association between CD20 and CD37 (220). In CD20-down regulated B-NHL, CD37 expression is also decreased, impairing the function of antibody-based therapy. Although CAR T typically requires high antigen expression, CD37 down-regulation do not seem to affect the function of CD37-CAR T (220). Preclinical study demonstrated potent cytotoxicity of CD37-CAR T against various CD37-expressing B- and T-NHL, without T-cell fratricide or detectable toxicity to other immune cells such as NK-cells and monocytes (29, 172). Structural modification, such as dual-costimulatory domain and novel linker design, may further improve CD37-CAR T function (221). A phase 1 clinical trial (NCT04136275) treated four heavily pre-treated patients (2 HGBCL, 1 CTCL, 1 HL) with CD37-CAR T, and achieved prolonged CR in three. However, two patients developed bone marrow failure unexpectedly, likely due to high T-cell dose (222). Bispecific CD19/37-CAR Ts were developed by several independent groups, yet to be tested in clinical trials (172, 223, 224).

CD38 signals multiple immunoregulatory pathways and is expressed by various hematological malignancies including MCL, LPL, Burkitt lymphoma, CTCL and NK/T-cell lymphoma (225–228). Lymphoma cells highly expressing CD38 respond well to CD38-CAR T, and those dimly expressing CD38 could be re-sensitized to CD38-targeted therapy by all-trans retinoic acid or panobinostat (228–230). Single targeting CD38-CAR T and dual targeting tandem CD38/latent membrane protein-1-CAR T all showed promising cytotoxicity against NK/T-cell lymphoma in *in vitro* and *in vivo* pre-clinical experiments (178). CD38-CAR NK has also been explored. Due to high CD38 expression on NK-cells, CD38-CAR NK needs simultaneous CD38-knockout to avoid fratricide (229, 231). CD38-CAR NK is effective against various CD38-expressing hematological malignancies in preclinical testing (229, 231).

Ebstein-Barr virus (EBV) is associated with various solid and hematologic malignancies. It is estimated that about 8% of lymphomas are EBV-positive, with the highest rate in angioimmunoblastic T-cell lymphoma, close to 50%, followed by about 30-40% in cHL; in immunocompromised hosts, lymphomas are almost universally positive for EBV (232–234). EBV contributes to pathogenesis and generation of immunosuppressive TME, and can be a marker of relapse (233, 235). Latent membrane proteins (LMP1 and LMP2A) are proteins encoded by EBV and participate in oncogenesis (236). A series of clinical trials were conducted using LMP1/2-specific cytotoxicity T-lymphocytes for cHL, demonstrating an ORR around 30-60% (237). CAR-T targeting

LMP showed preclinical efficacy in LMP-positive solid and hematological malignancies, such as ENKL (178, 238, 239). Gp350, an abundant EBV envelop glycoprotein, is another potential target to treat EBV lymphoproliferative disease (240).

Other strategies of tumor-agnostic CAR design pertain to improving cell trafficking to tumor and recognition of tumor antigen. For example, tumoral injection of a substance followed by administration of CAR-T specific to the substance can facilitate CAR-T homing (241). This strategy is more applicable to limited number of solitary lesions especially in solid tumor. Such substance could be small molecules that can be inserted into cell membrane by liposomal vector, or CD19 that can be transduced via oncolytic virus (241, 242). Another strategy is to improve T-cell function, to enhance host immunity against malignancy. Lai, et al., designed a CAR-T that secrets dendritic cell growth factor Fms-like tyrosine kinase 3 ligand (Flt3L) that can recruit host dendritic cells, increase T-cell activation, and induce epitope spreading towards otherwise unexposed tumor antigens (243).

CD16-expressing T-cells replaces the directly antigen-recognizing scFv with the extracellular portion of the Fc gamma receptor CD16, adding NK-cell like function to T-cells. When co-administered with tumor-specific antibody, the CD16-T recognizes the opsonized tumor cells and exerts cytotoxicity via antibody-mediated cellular cytotoxicity (244). When toxicity occurs, treatment can be aborted by withdrawal of antibody (245). While the presence of tumor antigen and respective monoclonal antibody are still required, CD16-T may overcome resistance mechanisms such as NK-cell exhaustion or lack of NK-cell infiltration in the TME (246). ATTCK-20-03 (NCT03189836) is a phase 1 clinical trial evaluated the combination of CD16-T and rituximab for R/R B-NHL (247). Among the 25 subjects treated, 14 (56%) responded, including 10 (40%) CR, with the longest duration of CR 586 days (range, 85-586). Treatment was well tolerated, resulting in no DLT, 1 case of grade 3 neutropenia, and 1 case of grade 2 CRS. ATTCK-20-03 demonstrated that antibody/CD16-T coupling is a feasible approach towards cancer treatment.

Conclusion

Identification of novel tumor antigens opens new therapeutic avenues for B-cell NHL beyond approved CD19-CAR T, and extends the application of CAR-based therapy to HL and TCL. Dual or multi-targeted CAR may lower the risk of antigen escape-mediated relapse. Tumor agnostic CAR may broaden the indication of adoptive cellular therapy across tumors of different cellular origin. Yet, finding new targets is not the only way to improve the feasibility and efficacy of CAR-based therapy. Modification of the non-antigen biding domains on a CAR may improve cell persistence and reduce toxicity (248). Genetic modification outside of the CAR molecule, such as adding Toll-like receptor, IL-18 expression, adding IL-7 and CCL19 expression, or administration of cytokines *in vivo*, may improve the activity of cells and prolong persistence (249–252). Vaccination combined with viral-specific CAR-T, immune checkpoint modification, pre-selection of memory- and naïve T-cells and elimination of Tregs, may reduce T-cell exhaustion and immunosuppression (63, 253–256). Advanced cell engineering enables the incorporation of inducible CAR expression switches to reduce toxicity (257). New manufacturing platforms reduce the cost and vein-to-vein time, improving the accessibility of CAR-T (258, 259). The rapidly evolving field of CAR-based therapy should hopefully deliver products with better efficacy, tolerability and accessibility, broader indication, and less physical and financial toxicity to patients with lymphoma.

Author contributions

YP: Writing – original draft, Writing – review & editing. NG: Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. We thank the Leon Levine Foundation and the Kerry and Simone Vickar Family Foundation.

Conflict of interest

NG has received research funding from Genentech, Pharmacyclics, Bristol Myers Squibb, AbbVie, Morphosys, Gilead/Kite Pharma; served as a consultant/advisor for AbbVie, AstraZeneca, Genentech, Beigene, Janssen, Lilly, Gilead/Kite Pharma, ADC Therapeutics, Novartis, Lava Therapeutics, Incyte.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Labanieh L, Mackall CL. CAR immune cells: design principles, resistance and the next generation. *Nature*. (2023) 614:635–48. doi: 10.1038/s41586-023-05707-3

2. Sadelain M, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. *Cancer Discovery*. (2013) 3:388–98. doi: 10.1158/2159-8290.CD-12-0548

3. Watanabe K, Terakura S, Martens AC, van Meerten T, Uchiyama S, Imai M, et al. Target antigen density governs the efficacy of anti–CD20-CD28-CD3 ζ Chimeric antigen receptor–modified effector CD8+ T cells. *J Immunol*. (2015) 194:911–20. doi: 10.4049/jimmunol.1402346

4. Cappell KM, Kochenderfer JN. Long-term outcomes following CAR T cell therapy: what we know so far. *Nat Rev Clin Oncol*. (2023) 20:359–71. doi: 10.1038/s41571-023-00754-1

5. Fischer JW, Bhattarai N. CAR-T cell therapy: mechanism, management, and mitigation of inflammatory toxicities. *Front Immunol*. (2021) 12:693016. doi: 10.3389/fimmu.2021.693016

6. Michelozzi IM, Gomez-Castaneda E, Pohle RVC, Cardoso Rodriguez F, Sufi J, Puigdevall Costa P, et al. Activation priming and cytokine polyfunctionality modulate the enhanced functionality of low-affinity CD19 CAR T cells. *Blood Advances*. (2023) 7:1725–38. doi: 10.1182/bloodadvances.2022008490

7. Kath J, Du W, Pruene A, Braun T, Thommandru B, Turk R, et al. Pharmacological interventions enhance virus-free generation of TRAC-replaced CAR T cells. *Mol Ther Methods Clin Dev*. (2022) 25:311–30. doi: 10.1016/j.omtm.2022.03.018

8. Duell J, Leipold AM, Appenzeller S, Fuhr V, Rauert-Wunderlich H, Da Via M, et al. Sequential antigen loss and branching evolution in lymphoma after CD19- and CD20-targeted T-cell–redirecting therapy. *Blood*. (2024) 143:685–96. doi: 10.1182/blood.2023021672

9. Neelapu SS, Rossi JM, Jacobson CA, Locke FL, Miklos DB, Reagan PM, et al. CD19-loss with preservation of other B cell lineage features in patients with large B cell lymphoma who relapsed post-axi-cel. *Blood*. (2019) 134:203. doi: 10.1182/blood-2019-126218

10. Plaks V, Rossi JM, Chou J, Wang L, Poddar S, Han G, et al. CD19 target evasion as a mechanism of relapse in large B-cell lymphoma treated with axicabtagene ciloleucel. *Blood*. (2021) 138:1081–5. doi: 10.1182/blood.2021010930

11. Cheng Q, Tan J, Liu R, Kang L, Zhang Y, Wang E, et al. CD20-specific chimeric antigen receptor-expressing T cells as salvage therapy in rituximab-refractory/relapsed B-cell non-Hodgkin lymphoma. *Cytotherapy*. (2022) 24:1026–34. doi: 10.1016/j.jcyt.2022.05.001

12. Smith MR. Rituximab (monoclonal anti-CD20 antibody): mechanisms of action and resistance. *Oncogene*. (2003) 22:7359–68. doi: 10.1038/sj.onc.1206939

13. Johnson NA, Leach S, Woolcock B, deLeeuw RJ, Bashashati A, Sehn LH, et al. CD20 mutations involving the rituximab epitope are rare in diffuse large B-cell lymphomas and are not a significant cause of R-CHOP failure. *Haematologica*. (2009) 94:423–7. doi: 10.3324/haematol.2008.001024

14. Sar A, Perizzolo M, Stewart D, Mansoor A, DiFrancesco LM, Demetrick DJ. Mutation or polymorphism of the CD20 gene is not associated with the response to R-CHOP in diffuse large B cell lymphoma patients. *Leuk Res*. (2009) 33:792–7. doi: 10.1016/j.leukres.2008.10.013

15. Budde LE, Olszewski AJ, Assouline S, Lossos IS, Diefenbach C, Kamdar M, et al. Mosunetuzumab with polatuzumab vedotin in relapsed or refractory aggressive large B cell lymphoma: a phase 1b/2 trial. *Nat Med*. (2023) 30:229–39. doi: 10.1038/s41591-023-02726-5

16. Till BG, Jensen MC, Wang J, Chen EY, Wood BL, Greisman HA, et al. Adoptive immunotherapy for indolent non-Hodgkin lymphoma and mantle cell lymphoma using genetically modified autologous CD20-specific T cells. *Blood*. (2008) 112:2261–71. doi: 10.1182/blood-2007-12-128843

17. Till BG, Jensen MC, Wang J, Qian X, Gopal AK, Maloney DG, et al. CD20-specific adoptive immunotherapy for lymphoma using a chimeric antigen receptor with both CD28 and 4-1BB domains: pilot clinical trial results. *Blood*. (2012) 119:3940–50. doi: 10.1182/blood-2011-10-387969

18. Shadman M, Gopal AK, Smith SD, Lynch RC, Ujjani CS, Turtle CJ, et al. CD20 targeted CAR-T for high-risk B-cell non-hodgkin lymphomas. *Blood*. (2019) 134:3235. doi: 10.1182/blood-2019-125102

19. Shadman M, Yeung C, Redman M, Lee SY, Lee DH, Ra S, et al. Safety and efficacy of third generation CD20 targeted CAR-T (MB-106) for treatment of relapsed/refractory B-NHL and CLL. *Blood*. (2021) 138:3872. doi: 10.1182/blood-2021-149181

20. Shadman M, Caimi PF, O'Brien SM, Reagan PM, Dezube B, Navaratnarajah P, et al. Efficacy and safety of a third generation CD20 CAR-T (MB-106) for treatment of relapsed/refractory indolent B-cell non-hodgkin lymphoma: phase-1 results from a multicenter trial. *Blood*. (2023) 142:2102. doi: 10.1182/blood-2023-175007

21. Li P, Liu W, Ye S, Zhou L, Zhu J, Huang J, et al. Two-year follow-up results of C-CAR066, a novel anti-CD20 chimeric antigen receptor cell therapy (CAR-T) in relapsed or refractory (r/r) large B-cell lymphoma (LBCL) patients after failure of CD19 CAR-T therapy. *Blood*. (2023) 142:2115. doi: 10.1182/blood-2023-181527

22. Kutsch N, Gödel P, Holtick U, Lohneis A, Vucinic V, Altefrohne FP, et al. A phase I dose finding trial of MB-CART20.1 in patients with relapsed or refractory B-cell non-hodgkin lymphoma. *Blood*. (2022) 140:12980–1. doi: 10.1182/blood-2022-168730

23. Shah NN, Sokol L. Targeting CD22 for the treatment of B-cell Malignancies. *Immunotargets Ther*. (2021) 10:225–36. doi: 10.2147/ITT.S288546

24. Dhillon S. Moxetumomab pasudotox: first global approval. *Drugs*. (2018) 78:1763–7. doi: 10.1007/s40265-018-1000-9

25. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. *New Engl J Med*. (2016) 375:740–53. doi: 10.1056/NEJMoa1509277

26. Fayad L, Offner F, Smith MR, Verhoef G, Johnson P, Kaufman JL, et al. Safety and clinical activity of a combination therapy comprising two antibody-based targeting agents for the treatment of non-hodgkin lymphoma: results of a phase I/II study evaluating the immunoconjugate inotuzumab ozogamicin with rituximab. *J Clin Oncol*. (2013) 31:573–83. doi: 10.1200/JCO.2012.42.7211

27. Dang NH, Ogura M, Castaigne S, Fayad LE, Jerkeman M, Radford J, et al. Randomized, phase 3 trial of inotuzumab ozogamicin plus rituximab versus chemotherapy plus rituximab for relapsed/refractory aggressive B-cell non-Hodgkin lymphoma. *Br J Haematol*. (2018) 182:583–6. doi: 10.1111/bjh.14820

28. Echeverri C, Fisher S, King D, Craig FE. Immunophenotypic variability of B-cell non-Hodgkin lymphoma: a retrospective study of cases analyzed by flow cytometry. *Am J Clin Pathol*. (2002) 117:615–20. doi: 10.1309/AAYH-1FK8-38PL-Q6DT

29. Köksal H, Dillard P, Josefsson SE, Maggadottir SM, Pollmann S, Fåne A, et al. Preclinical development of CD37CAR T-cell therapy for treatment of B-cell lymphoma. *Blood Advances*. (2019) 3:1230–43. doi: 10.1182/bloodadvances.2018029678

30. Kokalaki E, Ma B, Ferrari M, Grothier T, Hazelton W, Manzoor S, et al. Dual targeting of CD19 and CD22 against B-ALL using a novel high-sensitivity aCD22 CAR. *Mol Ther*. (2023) 31:2089–104. doi: 10.1016/j.ymthe.2023.03.020

31. Haso W, Lee DW, Shah NN, Stetler-Stevenson M, Yuan CM, Pastan IH, et al. Anti-CD22–chimeric antigen receptors targeting B-cell precursor acute lymphoblastic leukemia. *Blood*. (2013) 121:1165–74. doi: 10.1182/blood-2012-06-438002

32. Fry TJ, Shah NN, Orentas RJ, Stetler-Stevenson M, Yuan CM, Ramakrishna S, et al. CD22-targeted CAR T cells induce remission in B-ALL that is naive or resistant to CD19-targeted CAR immunotherapy. *Nat Med*. (2018) 24:20–8. doi: 10.1038/nm.4441

33. Fergusson NJ, Adeel K, Kekre N, Atkins H, Hay KA. A systematic review and meta-analysis of CD22 CAR T-cells alone or in combination with CD19 CAR T-cells. *Front Immunol*. (2023) 14:1178403. doi: 10.3389/fimmu.2023.1178403

34. Baird JH, Frank MJ, Craig J, Patel S, Spiegel JY, Sahaf B, et al. CD22-directed CAR T-cell therapy induces complete remissions in CD19-directed CAR–refractory large B-cell lymphoma. *Blood*. (2021) 137:2321–5. doi: 10.1182/blood.2020009432

35. Frank MJ, Baird J, Kramer A, Patel S, Sahaf B, Craig J, et al. S230: CD22 CAR T cell therapy is safe and effective in patients with large B cell lymphoma who have relapsed after CD19 CAR T cell therapy. *Hemasphere*. (2023) 7:e3362169. doi: 10.1097/01.HS9.0000967832.33621.69

36. Summers C, Baxter B, Annesley C, Yokoyama J, Rhea S, Huang W, et al. CD22 CAR optimization for improved in-human activity following inadequate CD22 CAR activity in phase 1 clinical trial PLAT-04. *Blood*. (2021) 138:403. doi: 10.1182/blood-2021-147928

37. Johnson A, Wright H, Hu X, Kinder J, van Hoeven N, Liang O, et al. Hypoimmune, allogeneic CD22-directed CAR T cells that evade innate and adaptive immune rejection for the treatment of large B cell lymphoma patients that are relapsed/refractory to CD19-directed CAR T cell therapy. *Blood*. (2023) 142:3437. doi: 10.1182/blood-2023-187096

38. Hines MR, Keenan C, Maron Alfaro G, Cheng C, Zhou Y, Sharma A, et al. Hemophagocytic lymphohistiocytosis-like toxicity (carHLH) after CD19-specific CAR T-cell therapy. *Br J Haematol*. (2021) 194:701–7. doi: 10.1111/bjh.17662

39. Lichtenstein DA, Schischlik F, Shao L, Steinberg SM, Yates B, Wang H-W, et al. Characterization of HLH-like manifestations as a CRS variant in patients receiving CD22 CAR T cells. *Blood*. (2021) 138:2469–84. doi: 10.1182/blood.2021011898

40. Jess J, Yates B, Dulau-Florea A, Parker K, Inglefield J, Lichtenstein D, et al. CD22 CAR T-cell associated hematologic toxicities, endothelial activation and relationship to neurotoxicity. *J ImmunoTherapy Cancer*. (2023) 11:e005898. doi: 10.1136/jitc-2022-005898

41. Kramer AM, Hamilton MP, Prabhu S, Desai M, Kuo A, Ehlinger Z, et al. Transcriptional profiling associated with CD22 CAR T cell clinical response in LBCL. *Blood*. (2023) 142:2091. doi: 10.1182/blood-2023-187615

42. Hussein MS, Li Q, Mao R, Peng Y, He Y. TCR T cells overexpressing c-Jun have better functionality with improved tumor infiltration and persistence in hepatocellular carcinoma. *Front Immunol*. (2023) 14. doi: 10.3389/fimmu.2023.1114770

43. Shalabi H, Kraft IL, Wang HW, Yuan CM, Yates B, Delbrook C, et al. Sequential loss of tumor surface antigens following chimeric antigen receptor T-cell therapies in diffuse large B-cell lymphoma. *Haematologica*. (2018) 103:e215–e8. doi: 10.3324/haematol.2017.183459

44. Ramakrishna S, Highfill SL, Walsh Z, Nguyen SM, Lei H, Shern JF, et al. Modulation of target antigen density improves CAR T-cell functionality and persistence. *Clin Cancer Res*. (2019) 25:5329–41. doi: 10.1158/1078-0432.CCR-18-3784

45. Chen WC, Completo GC, Sigal DS, Crocker PR, Saven A, Paulson JC. *In vivo* targeting of B-cell lymphoma with glycan ligands of CD22. *Blood*. (2010) 115:4778–86. doi: 10.1182/blood-2009-12-257386

46. Wang X, Lang S, Tian Y, Zhang J, Yan X, Fang Z, et al. Glycoengineering of natural killer cells with CD22 ligands for enhanced anticancer immunotherapy. *ACS Cent Science*. (2020) 6:382–9. doi: 10.1021/acscentsci.9b00956

47. Velasquez MP, Szoor A, Bonifant CL, Vaidya A, Brunetti L, Gundry MC, et al. Two-pronged cell therapy for B-cell Malignancies: engineering NK cells to target CD22 and redirect bystander T cells to CD19. *Blood*. (2016) 128:4560. doi: 10.1182/blood.V128.22.4560.4560

48. Tian X, Zhang R, Qin H, Shi X, Qi W, Jiang D, et al. Immunotherapy of B cell lymphoma with CD22-redirected CAR NK-92 cells. *Cent Eur J of Immunology*. (2023) 48:1–13. doi: 10.5114/ceji.2023.126672

49. Cronk RJ, Zurko J, Shah NN. Bispecific chimeric antigen receptor T cell therapy for B cell Malignancies and multiple myeloma. *Cancers (Basel)*. (2020) 12:2523. doi: 10.3390/cancers12092523

50. Schneider D, Xiong Y, Wu D, Nölle V, Schmitz S, Haso W, et al. A tandem CD19/CD20 CAR lentiviral vector drives on-target and off-target antigen modulation in leukemia cell lines. *J ImmunoTherapy Cancer*. (2017) 5:42. doi: 10.1186/s40425-017-0246-1

51. 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) 26:1569–75. doi: 10.1038/s41591-020-1081-3

52. Zurko JC, Fenske TS, Johnson BD, Bucklan D, Szabo A, Xu H, et al. Long-term outcomes and predictors of early response, late relapse, and survival for patients treated with bispecific LV20. *19 CAR T-cells Am J Hematol*. (2022) 97:1580–8. doi: 10.1002/ajh.26718

53. Shah NN, Furqan F, Szabo A, Kearl T, Zamora A, Neumann J, et al. Adaptive manufacturing of LV20.19 CAR T-cells for relapsed, refractory mantle cell lymphoma. *Blood*. (2023) 142:1024. doi: 10.1182/blood-2023-173954

54. Borchmann P, Lohneis A, Gödel P, Balke-Want H, Schmid C, Ayuk F, et al. P1184: Phase I trial of MB-CART2019.1 in patientes with relapsed or refratory B-cell non-hodgkin lymphoma: 2 year follow-up report. *HemaSphere*. (2022) 6:1070–1. doi: 10.1097/01.HS9.0000847600.12970.7d

55. Borchmann P, Vandenberghe P, Urbano-Ispizua A, Haiuon C, Griškevicius L, Lemonnier F, et al. A randomized phase II study of MB-CART2019.1 compared to standard of care in patients with relapsed/refractory DLBCL ineligible for ASCT – DALY 2-EU trial. *Hematological Oncol*. (2023) 41:840–1. doi: 10.1002/hon.3166_OT20

56. Shah N, Maziarz R, Jacobson C, Isufi I, Ghosh M, Ulrickson M, et al. P1134: Bispecific anti-CD20/19 CAR-T – zamtocabtagene autoleucel for relapsed/refractory DLBCL – interim analysis results of DALY-II-usa study. *Hemasphere*. (2023) 7:e07109dc. doi: 10.1097/01.HS9.0000971432.07109.dc

57. Tong C, Zhang Y, Liu Y, Ji X, Zhang W, Guo Y, et al. Optimized tandem CD19/CD20 CAR-engineered T cells in refractory/relapsed B-cell lymphoma. *Blood*. (2020) 136:1632–44. doi: 10.1182/blood.2020005278

58. Zhang Y, Wang Y, Liu Y, Tong C, Wang C, Guo Y, et al. Long-term activity of tandem CD19/CD20 CAR therapy in refractory/relapsed B-cell lymphoma: a single-arm, phase 1–2 trial. *Leukemia*. (2022) 36:189–96. doi: 10.1038/s41375-021-01345-8

59. Liang A, Zhou L, Li P, Yu W, Yang M, Xu Y, et al. Safety and efficacy of a novel anti-CD20/CD19 bi-specific CAR T-cell therapy (C-CAR039) in relapsed or refractory (r/r) B-cell non-Hodgkin lymphoma (B-NHL). *J Clin Oncol*. (2021) 39:2507. doi: 10.1200/JCO.2021.39.15_suppl.2507

60. Li P, Yu W-J, Zhou L, Yang M, Ye S, Zhu J, et al. C-CAR039, a novel anti-CD20/CD19 bi-specific CAR T-cell therapy shows deep and durable clinical benefits in patients with relapsed or refractory (r/r) B-cell non-hodgkin lymphoma (B-NHL) in long term follow up. *Blood*. (2023) 142:1025. doi: 10.1182/blood-2023-182817

61. Berger C, Jensen MC, Lansdorp PM, Gough M, Elliott C, Riddell SR. Adoptive transfer of effector CD8+ T cells derived from central memory cells establishes persistent T cell memory in primates. *J Clin Invest*. (2008) 118:294–305. doi: 10.1172/JCI32103

62. 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) 123:3750–9. doi: 10.1182/blood-2014-01-552174

63. Larson SM, Walthers CM, Ji B, Ghafouri SN, Naparstek J, Trent J, et al. CD19/CD20 bispecific chimeric antigen receptor (CAR) in naive/memory T cells for the treatment of relapsed or refractory non-hodgkin lymphoma. *Cancer Discovery*. (2023) 13:580–97. doi: 10.1158/2159-8290.CD-22-0964

64. 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) 28:1860–71. doi: 10.1038/s41591-022-01960-7

65. Luo W, Napoleon JV, Zhang F, Lee YG, Wang B, Putt KS, et al. Repolarization of tumor-infiltrating myeloid cells for augmentation of CAR T cell therapies. *Front Immunol*. (2022) 13. doi: 10.3389/fimmu.2022.816761

66. Lam N, Choi S, Yang S, Finney R, Cam M, Wu X, et al. Development of a bicistronic anti-CD19/CD20 CAR construct including optimization to abrogate retroviral recombination events. *Blood*. (2021) 138:4808. doi: 10.1182/blood-2021-150865

67. Zheng P, Xue F, Yang F, Feng S, Guo Y, Shi H, et al. P1168: Durable remission after sequential CD19/20 CAR T-cell "cake-icing" therapy occurred in refractory/relapsed DLBCL. *Hemasphere*. (2023) 7:e04478eb. doi: 10.1097/01.HS9.0000971568.04478.eb

68. Cordoba S, Onuoha S, Thomas S, Pignataro DS, Hough R, Ghorashian S, et al. CAR T cells with dual targeting of CD19 and CD22 in pediatric and young adult patients with relapsed or refractory B cell acute lymphoblastic leukemia: a phase 1 trial. *Nat Med*. (2021) 27:1797–805. doi: 10.1038/s41591-021-01497-1

69. Dai H, Wu Z, Jia H, Tong C, Guo Y, Ti D, et al. Bispecific CAR-T cells targeting both CD19 and CD22 for therapy of adults with relapsed or refractory B cell acute lymphoblastic leukemia. *J Hematol Oncol*. (2020) 13:30. doi: 10.1186/s13045-020-00856-8

70. Wang Y, Yang Y, Hong R, Zhao H, Wei G, Wu W, et al. A retrospective comparison of CD19 single and CD19/CD22 bispecific targeted chimeric antigen receptor T cell therapy in patients with relapsed/refractory acute lymphoblastic leukemia. *Blood Cancer J*. (2020) 10:105. doi: 10.1038/s41408-020-00371-6

71. Kim H, Han M, Kim M, Kim H, Im HJ, Kim N, et al. CD19/CD22 bispecific chimeric antigen receptor–NK–92 cells are developed and evaluated. *Oncol Lett*. (2023) 25:236. doi: 10.3892/ol.2023.13822

72. Gardner R, Annesley C, Finney O, Summers C, Lamble AJ, Rivers J, et al. Early clinical experience of CD19 x CD22 dual specific CAR T cells for enhanced anti-leukemic targeting of acute lymphoblastic leukemia. *Blood*. (2018) 132:278. doi: 10.1182/blood-2018-99-113126

73. Gardner RA, Annesley C, Wilson A, Summers C, Narayanaswamy P, Wu V, et al. Efficacy of SCRI-CAR19x22 T cell product in B-ALL and persistence of anti-CD22 activity. *J Clin Oncol*. (2020) 38:3035. doi: 10.1200/JCO.2020.38.15_suppl.3035

74. Annesley C, Summers C, Pulsipher MA, Skiles JL, Li AM, Vatsayan A, et al. SCRI-CAR19x22v2 T cell product demonstrates bispecific activity in B-ALL. *Blood*. (2021) 138:470. doi: 10.1182/blood-2021-148881

75. Lai X, Gu X, Tsao S-T, Zhang Q, Liu Y-C, Yaxian J, et al. Double CD19/CD22 chimeric antigen receptor-modified T cells for the treatment of stage IV relapsed and refractory follicular lymphoma. *Blood*. (2017) 130:5154. doi: 10.1182/blood.V130.Suppl_1.5154.5154

76. Jiao C, Zvonkov E, Lai X, Zhang R, Liu Y, Qin Y, et al. 4SCAR2.0: a multi-CAR-T therapy regimen for the treatment of relapsed/refractory B cell lymphomas. *Blood Cancer J*. (2021) 11:59. doi: 10.1038/s41408-021-00455-x

77. Wang N, Hu X, Cao W, Li C, Xiao Y, Cao Y, et al. Efficacy and safety of CAR19/22 T-cell cocktail therapy in patients with refractory/relapsed B-cell Malignancies. *Blood*. (2020) 135:17–27. doi: 10.1182/blood.2019000017

78. Cao Y, Xiao Y, Wang N, Wang G, Huang L, Hong Z, et al. CD19/CD22 chimeric antigen receptor T cell cocktail therapy following autologous transplantation in patients with relapsed/refractory aggressive B cell lymphomas. *Transplant Cell Ther*. (2021) 27:910.e1–.e11. doi: 10.1016/j.jtct.2021.08.012

79. Roddie C, Lekakis LJ, Marzolini MAV, Ramakrishnan A, Zhang Y, Hu Y, et al. Dual targeting of CD19 and CD22 with bicistronic CAR-T cells in patients with relapsed/refractory large B-cell lymphoma. *Blood*. (2023) 141:2470–82. doi: 10.1182/blood.2022018598

80. Amrolia PJ, Wynn R, Hough RE, Vora A, Bonney D, Veys P, et al. Phase I study of AUTO3, a bicistronic chimeric antigen receptor (CAR) T-cell therapy targeting CD19 and CD22, in pediatric patients with relapsed/refractory B-cell acute lymphoblastic leukemia (r/r B-ALL): amelia study. *Blood*. (2019) 134:2620. doi: 10.1182/blood-2019-123424

81. Abramson JS. A new CAR takes a test drive in DLBCL. *Blood*. (2023) 141:2410–1. doi: 10.1182/blood.2023020131

82. Qin H, Ramakrishna S, Nguyen S, Fountaine TJ, Ponduri A, Stetler-Stevenson M, et al. Preclinical development of bivalent chimeric antigen receptors targeting both CD19 and CD22. *Mol Ther - Oncolytics*. (2018) 11:127–37. doi: 10.1016/j.omto.2018.10.006

83. Spiegel JY, Patel S, Muffly L, Hossain NM, Oak J, Baird JH, et al. CAR T cells with dual targeting of CD19 and CD22 in adult patients with recurrent or refractory B cell Malignancies: a phase 1 trial. *Nat Med*. (2021) 27:1419–31. doi: 10.1038/s41591-021-01436-0

84. Velasco-Hernandez T, Zanetti SR, Roca-Ho H, Gutierrez-Aguera F, Petazzi P, Sánchez-Martínez D, et al. Efficient elimination of primary B-ALL cells in vitro and in *vivo* using a novel 4-1BB-based CAR targeting a membrane-distal CD22 epitope. *J Immunother Cancer*. (2020) 8:e000896. doi: 10.1136/jitc-2020-000896

85. Zanetti SR, Velasco-Hernandez T, Gutierrez-Agüera F, Díaz VM, Romecín PA, Roca-Ho H, et al. A novel and efficient tandem CD19- and CD22-directed CAR for B cell ALL. *Mol Ther*. (2022) 30:550–63. doi: 10.1016/j.ymthe.2021.08.033

86. Wei G, Zhang Y, Zhao H, Wang Y, Liu Y, Liang B, et al. CD19/CD22 dual-targeted CAR T-cell therapy for relapsed/refractory aggressive B-cell lymphoma: A safety and efficacy study. *Cancer Immunol Res*. (2021) 9:1061–70. doi: 10.1158/2326-6066.CIR-20-0675

87. Zhang Y, Li J, Lou X, Chen X, Yu Z, Kang L, et al. A prospective investigation of bispecific CD19/22 CAR T cell therapy in patients with relapsed or refractory B cell non-hodgkin lymphoma. *Front Oncol*. (2021) 11:664421. doi: 10.3389/fonc.2021.664421

88. Wang Y, Tong C, Dai H, Wu Z, Han X, Guo Y, et al. Low-dose decitabine priming endows CAR T cells with enhanced and persistent antitumor potential via epigenetic reprogramming. *Nat Commun*. (2021) 12:409. doi: 10.1038/s41467-020-20696-x

89. Qu C, Zou R, Wang P, Zhu Q, Kang L, Ping N, et al. Decitabine-primed tandem CD19/CD22 CAR-T therapy in relapsed/refractory diffuse large B-cell lymphoma patients. *Front Immunol*. (2022) 13:969660. doi: 10.3389/fimmu.2022.969660

90. Aranda-Orgilles B, Chion-Sotinel I, Skinner J, Grudman S, Mumford B, Dixon C, et al. Preclinical evidence of an allogeneic dual CD20xCD22 CAR to target a broad spectrum of patients with B-cell Malignancies. *Cancer Immunol Res*. (2023) 11:946–61. doi: 10.1158/2326-6066.CIR-22-0910

91. Poirot L, Philip B, Schiffer-Mannioui C, Le Clerre D, Chion-Sotinel I, Derniame S, et al. Multiplex genome-edited T-cell manufacturing platform for "Off-the-shelf" Adoptive T-cell immunotherapies. *Cancer Res*. (2015) 75:3853–64. doi: 10.1158/0008-5472.CAN-14-3321

92. Abramson JS, Ramakrishnan A, Pierola AA, Braunschweig I, Cartron G, Thieblemont C, et al. Preliminary results of nathali-01: A first-in-human phase I/IIa study of UCART20x22, a dual allogeneic CAR-T cell product targeting CD20 and CD22, in relapsed or refractory (R/R) non-hodgkin lymphoma (NHL). *Blood*. (2023) 142:2110. doi: 10.1182/blood-2023-186570

93. Smulski CR, Eibel H. BAFF and BAFF-receptor in B cell selection and survival. *Front Immunol*. (2018) 9:2285. doi: 10.3389/fimmu.2018.02285

94. Novak AJ, Grote DM, Stenson M, Ziesmer SC, Witzig TE, Habermann TM, et al. Expression of BLyS and its receptors in B-cell non-Hodgkin lymphoma: correlation with disease activity and patient outcome. *Blood*. (2004) 104:2247–53. doi: 10.1182/blood-2004-02-0762

95. Qin H, Dong Z, Wang X, Cheng WA, Wen F, Xue W, et al. CAR T cells targeting BAFF-R can overcome CD19 antigen loss in B cell Malignancies. *Sci Trans Med*. (2019) 11:eaaw9414. doi: 10.1126/scitranslmed.aaw9414

96. Wong DP, Roy NK, Zhang K, Anukanth A, Asthana A, Shirkey-Son NJ, et al. A BAFF ligand-based CAR-T cell targeting three receptors and multiple B cell cancers. *Nat Commun*. (2022) 13:217. doi: 10.1038/s41467-021-27853-w

97. Lemal R, Tournilhac O. State-of-the-art for CAR T-cell therapy for chronic lymphocytic leukemia in 2019. *J ImmunoTherapy Cancer*. (2019) 7:202. doi: 10.1186/s40425-019-0686-x

98. Hoffmann J-M, Schubert M-L, Wang L, Hückelhoven A, Sellner L, Stock S, et al. Differences in expansion potential of naive chimeric antigen receptor T cells from healthy donors and untreated chronic lymphocytic leukemia patients. *Front Immunol*. (2018) 8. doi: 10.3389/fimmu.2017.01956

99. Brudno JN, Somerville RPT, Shi V, Rose JJ, Halverson DC, Fowler DH, et al. Allogeneic T cells that express an anti-CD19 chimeric antigen receptor induce remissions of B-cell Malignancies that progress after allogeneic hematopoietic stem-cell transplantation without causing graft-versus-host disease. *J Clin Oncol*. (2016) 34:1112–21. doi: 10.1200/JCO.2015.64.5929

100. Todorovic Z, Todorovic D, Markovic V, Ladjevac N, Zdravkovic N, Djurdjevic P, et al. CAR T cell therapy for chronic lymphocytic leukemia: successes and shortcomings. *Curr Oncol*. (2022) 29:3647–57. doi: 10.3390/curroncol29050293

101. Barbanti MC, Appleby N, Kesavan M, Eyre TA. Cellular therapy in high-risk relapsed/refractory chronic lymphocytic leukemia and richter syndrome. *Front Oncol*. (2022) 12:888109. doi: 10.3389/fonc.2022.888109

102. Blackmon A, Danilov AV, Wang L, Pillai R, Babaei Mirshkarlo H, Rosen ST, et al. Richter's transformation after CD-19 directed CAR-T cells for relapsed/refractory chronic lymphocytic leukemia (CLL). *Blood*. (2021) 138:1430. doi: 10.1182/blood-2021-149815

103. Giordano Attianese GM, Marin V, Hoyos V, Savoldo B, Pizzitola I, Tettamanti S, et al. *In vitro* and in *vivo* model of a novel immunotherapy approach for chronic lymphocytic leukemia by anti-CD23 chimeric antigen receptor. *Blood*. (2011) 117:4736–45. doi: 10.1182/blood-2010-10-311845

104. Tettamanti S, Rotiroti MC, Giordano Attianese GMP, Arcangeli S, Zhang R, Banerjee P, et al. Lenalidomide enhances CD23.CAR T cell therapy in chronic lymphocytic leukemia. *Leuk Lymphoma*. (2022) 63:1566–79. doi: 10.1080/10428194.2022.2043299

105. Faitschuk E, Hombach AA, Frenzel LP, Wendtner C-M, Abken H. Chimeric antigen receptor T cells targeting Fc μ receptor selectively eliminate CLL cells while sparing healthy B cells. *Blood*. (2016) 128:1711–22. doi: 10.1182/blood-2016-01-692046

106. Kovalovsky D, Yoon JH, Cyr MG, Simon S, Voynova E, Rader C, et al. Siglec-6 is a target for chimeric antigen receptor T-cell treatment of chronic lymphocytic leukemia. *Leukemia*. (2021) 35:2581–91. doi: 10.1038/s41375-021-01188-3

107. Baskar S, Suschak JM, Samija I, Srinivasan R, Childs RW, Pavletic SZ, et al. A human monoclonal antibody drug and target discovery platform for B-cell chronic lymphocytic leukemia based on allogeneic hematopoietic stem cell transplantation and phage display. *Blood*. (2009) 114:4494–502. doi: 10.1182/blood-2009-05-222786

108. Rankin CT, Veri M-C, Gorlatov S, Tuaillon N, Burke S, Huang L, et al. CD32B, the human inhibitory Fc-γ receptor IIB, as a target for monoclonal antibody therapy of B-cell lymphoma. *Blood*. (2006) 108:2384–91. doi: 10.1182/blood-2006-05-020602

109. Lim SH, Vaughan AT, Ashton-Key M, Williams EL, Dixon SV, Chan HT, et al. Fc gamma receptor IIb on target B cells promotes rituximab internalization and reduces clinical efficacy. *Blood*. (2011) 118:2530–40. doi: 10.1182/blood-2011-01-330357

110. Wang G, Sun X, Zuo S, Li C, Niu Q, Xia Y, et al. Homogeneously high expression of CD32b makes it a potential target for CAR-T therapy for chronic lymphocytic leukemia. *J Hematol Oncol*. (2021) 14:149. doi: 10.1186/s13045-021-01160-9

111. Farley CR, Morris AB, Tariq M, Bennion KB, Potdar S, KudChadkar R, et al. FcγRIIB is a T cell checkpoint in antitumor immunity. *JCI Insight*. (2021) 6:e135623. doi: 10.1172/jci.insight.135623

112. Flieswasser T, Van den Eynde A, Van Audenaerde J, De Waele J, Lardon F, Riether C, et al. The CD70-CD27 axis in oncology: the new kids on the block. *J Exp Clin Cancer Res*. (2022) 41:12. doi: 10.1186/s13046-021-02215-y

113. Sauer T, Parikh K, Sharma S, Omer B, Sedloev D, Chen Q, et al. CD70-specific CAR T cells have potent activity against acute myeloid leukemia without HSC toxicity. *Blood*. (2021) 138:318–30. doi: 10.1182/blood.2020008221

114. Cheng J, Zhao Y, Hu H, Tang L, Zeng Y, Deng X, et al. Revealing the impact of CD70 expression on the manufacture and functions of CAR-70 T-cells based on single-cell transcriptomics. *Cancer Immunol Immunother*. (2023) 72:3163–74. doi: 10.1007/s00262-023-03475-7

115. Tu S, Zhou L, Huang R, Zhou X, Yang J, Li M, et al. Efficacy and safety of chimeric antigen receptor T cells therapy strategy with dual targeting of CD19 and CD70 to treat relapsed/refractory diffuse large B-cell lymphoma. *Blood*. (2023) 142:3492. doi: 10.1182/blood-2023-186888

116. Tu S, Huang R, Zhou X, He Y, Liang Z, Wang L, et al. Combination of CD19 and CD70 specific chimeric antigen receptor T cells achieves long-term disease-free survival in relapsed and refractory primary central nervous system diffuse large B cell lymphoma. *Blood*. (2018) 132:5389. doi: 10.1182/blood-2018-99-117694

117. Choi E, Chang J-W, Krueger J, Lahr WS, Pomeroy E, Walsh M, et al. Engineering CD70-directed CAR-NK cells for the treatment of hematological and solid Malignancies. *Blood*. (2021) 138:1691. doi: 10.1182/blood-2021-148649

118. Nix MA, Mandal K, Geng H, Paranjape N, Lin YT, Rivera JM, et al. Surface proteomics reveals CD72 as a target for *in vitro*-evolved nanobody-based CAR-T cells in KMT2A/MLL1-rearranged B-ALL. *Cancer Discovery*. (2021) 11:2032–49. doi: 10.1158/2159-8290.CD-20-0242

119. Temple WC, Nix MA, Naik A, Izgutdina A, Huang BJ, Wicaksono G, et al. Framework humanization optimizes potency of anti-CD72 nanobody CAR-T cells for B-cell Malignancies. *J Immunother Cancer*. (2023) 11:e006985. doi: 10.1136/jitc-2023-006985

120. Izgutdina A, Temple WC, Nix MA, Geng H, Ramos E, Naik A, et al. Affinity matured CD72 CAR-T improves efficacy versus low antigen density B-cell non-hodgkin lymphoma models. *Blood*. (2023) 142:2068. doi: 10.1182/blood-2023-182873

121. Polson AG, Yu S-F, Elkins K, Zheng B, Clark S, Ingle GS, et al. Antibody-drug conjugates targeted to CD79 for the treatment of non-Hodgkin lymphoma. *Blood*. (2007) 110:616–23. doi: 10.1182/blood-2007-01-066704

122. Chu F, Cao J, Liu J, Yang H, Davis TJ, S-q K, et al. Chimeric antigen receptor T cells to target CD79b in B-cell lymphomas. *J ImmunoTherapy Cancer*. (2023) 11: e007515. doi: 10.1136/jitc-2023-007515

123. Jiang D, Tian X, Bian X, Zhu T, Qin H, Zhang R, et al. T cells redirected against Igβ for the immunotherapy of B cell lymphoma. *Leukemia*. (2020) 34:821–30. doi: 10.1038/s41375-019-0607-5

124. Locke FL, Miklos DB, Tees M, Bees T, Li A, Truppel-Hartmann A, et al. CRC-403: A Phase 1/2 Study of bbT369, a Dual CD79a and CD20 Targeting CAR T Cell Drug Product with a Gene Edit, in Relapsed and/or Refractory B Cell Non-Hodgkin's Lymphoma (NHL). *Blood*. (2022) 140:12716–7. doi: 10.1182/blood-2022-162604

125. Ormhøj M, Scarfò I, Cabral ML, Bailey SR, Lorrey SJ, Bouffard AA, et al. Chimeric antigen receptor T cells targeting CD79b show efficacy in lymphoma with or without cotargeting CD19. *Clin Cancer Res*. (2019) 25:7046–57. doi: 10.1158/1078-0432.CCR-19-1337

126. Leung I, Templeton ML, Lo Y, Rajan A, Stull SM, Garrison SM, et al. Compromised antigen binding and signaling interfere with bispecific CD19 and CD79a chimeric antigen receptor function. *Blood Advances*. (2023) 7:2718–30. doi: 10.1182/bloodadvances.2022008559

127. Jahn L, Hombrink P, Hassan C, Kester MGD, van der Steen DM, Hagedoorn RS, et al. Therapeutic targeting of the BCR-associated protein CD79b in a TCR-based approach is hampered by aberrant expression of CD79b. *Blood*. (2015) 125:949–58. doi: 10.1182/blood-2014-07-587840

128. Lee BK, Wan Y, Chin ZL, Deng L, Deng M, Leung TM, et al. Developing ROR1 targeting CAR-T cells against solid tumors in preclinical studies. *Cancers (Basel)*. (2022) 14:3618. doi: 10.3390/cancers14153618

129. Peng H, Nerreter T, Mestermann K, Wachter J, Chang J, Hudecek M, et al. ROR1-targeting switchable CAR-T cells for cancer therapy. *Oncogene*. (2022) 41:4104–14. doi: 10.1038/s41388-022-02416-5

130. Pinilla Ibarz J, Lankford A, Sabzevari H, Shah BD, Soliman H, Chavez JC, et al. A phase1/1b dose escalation/dose expansion study of prgn-3007 ultracar-T cells in patients with advanced hematologic and solid tumor Malignancies. *Blood*. (2022) 140:7486–7. doi: 10.1182/blood-2022-168932

131. Wang ML, Frigault MJ, Yazji S, Katz Y, Robinson J, Breitmeyer JB, et al. Trial-in-progress: A phase 1/2 multi-center study of onct-808, a ROR1-specific CAR T, in adult patients with relapsed/refractory aggressive B cell lymphoma. *Blood*. (2023) 142:4857. doi: 10.1182/blood-2023-180989

132. Chan T, Scott SP, Du M, Bolinger C, Poortman C, Shepard L, et al. Preclinical evaluation of prgn-3007, a non-viral, multigenic, autologous ROR1 ultracar-T ® Therapy with novel mechanism of intrinsic PD-1 blockade for treatment of hematological and solid cancers. *Blood*. (2021) 138:1694. doi: 10.1182/blood-2021-149203

133. Jain MD, Locke FL. Seeing the light: CAR T-cell targeting of lambda-restricted B-cell lymphomas. *Clin Cancer Res*. (2021) 27:5736–8. doi: 10.1158/1078-0432.CCR-21-1450

134. Vera J, Savoldo B, Vigouroux S, Biagi E, Pule M, Rossig C, et al. T lymphocytes redirected against the κ light chain of human immunoglobulin efficiently kill mature B lymphocyte-derived Malignant cells. *Blood*. (2006) 108:3890–7. doi: 10.1182/blood-2006-04-017061

135. Ramos CA, Savoldo B, Torrano V, Ballard B, Zhang H, Dakhova O, et al. Clinical responses with T lymphocytes targeting Malignancy-associated κ light chains. *J Clin Invest*. (2016) 126:2588–96. doi: 10.1172/JCI86000

136. Ranganathan R, Shou P, Ahn S, Sun C, West J, Savoldo B, et al. CAR T cells targeting human immunoglobulin light chains eradicate mature B-cell Malignancies while sparing a subset of normal B cells. *Clin Cancer Res*. (2021) 27:5951–60. doi: 10.1158/1078-0432.CCR-20-2754

137. Martens AWJ, Rietveld JM, de Boer R, Peters FS, Ngo A, van Mil L, et al. Redirecting T-cell activity with anti-BCMA/anti-CD3 bispecific antibodies in chronic lymphocytic leukemia and other B-cell lymphomas. *Cancer Res Commun*. (2022) 2:330–41. doi: 10.1158/2767-9764.CRC-22-0083

138. Zhang H, Gao L, Liu L, Wang J, Wang S, Gao L, et al. A bcma and CD19 bispecific CAR-T for relapsed and refractory multiple myeloma. *Blood*. (2019) 134:3147. doi: 10.1182/blood-2019-131056

139. Roex G, Campillo-Davo D, Flumens D, Shaw PAG, Krekelbergh L, De Reu H, et al. Two for one: targeting BCMA and CD19 in B-cell Malignancies with off-the-shelf dual-CAR NK-92 cells. *J Trans Med*. (2022) 20:124. doi: 10.1186/s12967-022-03326-6

140. Zhou Z, Han Y, Pan H-B, Sang C-J, Shi D-L, Feng C, et al. Tri-specific CD19xCD20xCD22 VHH CAR-T cells (LCAR-AIO) eradicate antigen-heterogeneous B cell tumors, enhance expansion, and prolong persistence in preclinical *in vivo* models. *Blood*. (2021) 138:1700. doi: 10.1182/blood-2021-150650

141. Schneider D, Xiong Y, Wu D, Hu P, Alabanza L, Steimle B, et al. Trispecific CD19-CD20-CD22–targeting duoCAR-T cells eliminate antigen-heterogeneous B cell tumors in preclinical models. *Sci Trans Med*. (2021) 13:eabc6401. doi: 10.1126/scitranslmed.abc6401

142. Vasu S, Alinari L, Szuminski N, Schneider D, Denlinger N, Chan WK, et al. A phase I clinical trial of point-of-care manufactured fresh anti-CD19/20/22 chimeric antigen receptor T cells for treatment of relapsed or refractory lymphoid Malignancies (Non-hodgkin lymphoma, acute lymphoblastic leukemia, chronic lymphocytic leukemia, B prolymphocytic leukemia). *Blood*. (2022) 140:7474–5. doi: 10.1182/blood-2022-167340

143. Tamma R, Ingravallo G, Gaudio F, d'Amati A, Masciopinto P, Bellitti E, et al. The tumor microenvironment in classic hodgkin's lymphoma in responder and no-responder patients to first line ABVD therapy. *Cancers (Basel)*. (2023) 15:2803. doi: 10.3390/cancers15102803

144. Katsin M, Dormeshkin D, Meleshko A, Migas A, Dubovik S, Konoplya N. CAR-T cell therapy for classical hodgkin lymphoma. *HemaSphere*. (2023) 7:e971. doi: 10.1097/HS9.0000000000000971

145. Schwarting R, Gerdes J, Durkop H, Falini B, Pileri S, Stein H. BER-H2: a new anti-Ki-1 (CD30) monoclonal antibody directed at a formol- resistant epitope. *Blood*. (1989) 74:1678–89. doi: 10.1182/blood.V74.5.1678.1678

146. Li Z, Guo W, Bai O. Mechanism of action and therapeutic targeting of CD30 molecule in lymphomas. *Front Oncol*. (2023) 13. doi: 10.3389/fonc.2023.1301437

147. Ramos CA, Ballard B, Zhang H, Dakhova O, Gee AP, Mei Z, et al. Clinical and immunological responses after CD30-specific chimeric antigen receptor-redirected lymphocytes. *J Clin Invest*. (2017) 127:3462–71. doi: 10.1172/JCI94306

148. Hombach A, Heuser C, Sircar R, Tillmann T, Diehl V, Pohl C, et al. An anti-CD30 chimeric receptor that mediates CD3-zeta-independent T-cell activation against Hodgkin's lymphoma cells in the presence of soluble CD30. *Cancer Res*. (1998) 58:1116–9.

149. Ramos CA, Grover NS, Beaven AW, Lulla PD, Wu M-F, Ivanova A, et al. Anti-CD30 CAR-T cell therapy in relapsed and refractory hodgkin lymphoma. *J Clin Oncol*. (2020) 38:3794–804. doi: 10.1200/JCO.20.01342

150. Ahmed S, Flinn IW, Mei M, Riedell PA, Armand P, Grover NS, et al. Updated results and correlative analysis: autologous CD30.CAR-T-cell therapy in patients with relapsed or refractory classical hodgkin lymphoma (CHARIOT trial). *Blood*. (2022) 140:7496–7. doi: 10.1182/blood-2022-158869

151. Wang C-M, Wu Z-Q, Wang Y, Guo Y-L, Dai H-R, Wang X-H, et al. Autologous T cells expressing CD30 chimeric antigen receptors for relapsed or refractory hodgkin lymphoma: an open-label phase I trial. *Clin Cancer Res*. (2017) 23:1156–66. doi: 10.1158/1078-0432.CCR-16-1365

152. Wang D, Zeng C, Xu B, Xu JH, Wang J, Jiang LJ, et al. Anti-CD30 chimeric antigen receptor T cell therapy for relapsed/refractory CD30(+) lymphoma patients. *Blood Cancer J*. (2020) 10:8. doi: 10.1038/s41408-020-0274-9

153. Grover NS, Savoldo B. Challenges of driving CD30-directed CAR-T cells to the clinic. *BMC Cancer*. (2019) 19:203. doi: 10.1186/s12885-019-5415-9

154. Marques-Piubelli ML, Kim DH, Medeiros LJ, Lu W, Khan K, Gomez-Bolanos LI, et al. CD30 expression is frequently decreased in relapsed classic Hodgkin lymphoma after anti-CD30 CAR T-cell therapy. *Histopathology*. (2023) 83:143–8. doi: 10.1111/his.14910

155. Caballero Gonzalez ACC, Escribà-García L, Montserrat R, Escudero-López E, Pujol-Fernández P, Ujaldón-Miró C, et al. Phase 1 clinical trial of memory-enriched academic HSP-CAR30 for the treatment of relapsed/refractory hodgkin lymphoma and CD30+ T-cell lymphoma: clinical and biological studies. *Blood*. (2022) 140:405–7. doi: 10.1182/blood-2022-167504

156. Di Stasi A, De Angelis B, Rooney CM, Zhang L, Mahendravada A, Foster AE, et al. T lymphocytes coexpressing CCR4 and a chimeric antigen receptor targeting CD30 have improved homing and antitumor activity in a Hodgkin tumor model. *Blood*. (2009) 113:6392–402. doi: 10.1182/blood-2009-03-209650

157. Zhang S, Gu C, Huang L, Wu H, Shi J, Zhang Z, et al. The third-generation anti-CD30 CAR T-cells specifically homing to the tumor and mediating powerful antitumor activity. *Sci Rep*. (2022) 12:10488. doi: 10.1038/s41598-022-14523-0

158. Grover NS, Ivanova A, Moore DT, Cheng CJA, Babinec C, West J, et al. CD30-directed CAR-T cells co-expressing CCR4 in relapsed/refractory hodgkin lymphoma and CD30+ Cutaneous T cell lymphoma. *Blood*. (2021) 138:742. doi: 10.1182/blood-2021-148102

159. Voorhees TJ, Beaven AW, Dittus C, Hucks GE, Morrison JK, Cheng CJA, et al. Clinical activity of anti-PD-1 therapy following CD30 CAR-T cell therapy in relapsed hodgkin lymphoma. *Blood*. (2022) 140:12723–4. doi: 10.1182/blood-2022-156660

160. Sang W, Wang X, Geng H, Li T, Li D, Zhang B, et al. Anti-PD-1 therapy enhances the efficacy of CD30-directed chimeric antigen receptor T cell therapy in patients with relapsed/refractory CD30+ Lymphoma. *Front Immunol*. (2022) 13. doi: 10.3389/fimmu.2022.858021

161. Zhang P, Yang X, Cao Y, Wang J, Zhou M, Chen L, et al. Autologous stem cell transplantation in tandem with Anti-CD30 CAR T-cell infusion in relapsed/refractory CD30+ lymphoma. *Exp Hematol Oncol*. (2022) 11:72. doi: 10.1186/s40164-022-00323-9

162. Brudno JN, Natrakul DA, Karrs JX, Patel N, Maass-Moreno R, Ahlman MA, et al. Transient responses and significant toxicities of anti-CD30 CAR T cells for CD30 + lymphomas: results of a phase I trial. *Blood Adv*. (2023) 8(3):802–14. doi: 10.1182/bloodadvances.2023011470

163. Svoboda J, Rheingold SR, Gill SI, Grupp SA, Lacey SF, Kulikovskaya I, et al. Nonviral RNA chimeric antigen receptor–modified T cells in patients with Hodgkin lymphoma. *Blood*. (2018) 132:1022–6. doi: 10.1182/blood-2018-03-837609

164. Xue Y, Lai X, Li R, Ge C, Zeng B, Li Z, et al. CD19 and CD30 CAR T-cell immunotherapy for high-risk classical hodgkin's lymphoma. *Front Oncol*. (2020) 10:607362. doi: 10.3389/fonc.2020.607362

165. Tzankov A, Krugmann J, Fend F, Fischhofer M, Greil R, Dirnhofer S. Prognostic significance of CD20 expression in classical hodgkin lymphoma: A clinicopathological study of 119 cases1. *Clin Cancer Res*. (2003) 9:1381–6.

166. Zhao X, Ma Y, Bian H, Liu Z. CD20 expression is closely associated with Epstein–Barr virus infection and an inferior survival in nodular sclerosis classical Hodgkin lymphoma. *Front Oncol*. (2022) 12. doi: 10.3389/fonc.2022.993768

167. Rehwald U, Schulz H, Reiser M, Sieber M, Staak JO, Morschhauser F, et al. Treatment of relapsed CD20+ Hodgkin lymphoma with the monoclonal antibody rituximab is effective and well tolerated: results of a phase 2 trial of the German Hodgkin Lymphoma Study Group. *Blood*. (2003) 101:420–4. doi: 10.1182/blood.V101.2.420

168. El Achi H, Dupont E, Paul S, Khoury JD. CD123 as a biomarker in hematolymphoid Malignancies: principles of detection and targeted therapies. *Cancers (Basel)*. (2020) 12:3087. doi: 10.3390/cancers12113087

169. Ruella M, Klichinsky M, Kenderian SS, Shestova O, Ziober A, Kraft DO, et al. Overcoming the immunosuppressive tumor microenvironment of hodgkin lymphoma using chimeric antigen receptor T cells. *Cancer Discovery*. (2017) 7:1154–67. doi: 10.1158/2159-8290.CD-16-0850

170. Gower M, Tikhonova AN. Avoiding fratricide: a T-ALL order. *Blood*. (2022) 140:3–4. doi: 10.1182/blood.2022016772

171. Maciocia PM, Wawrzyniecka PA, Maciocia NC, Burley A, Karpanasamy T, Devereaux S, et al. Anti-CCR9 chimeric antigen receptor T cells for T-cell acute lymphoblastic leukemia. *Blood*. (2022) 140:25–37. doi: 10.1182/blood.2021013648

172. Scarfò I, Ormhøj M, Frigault MJ, Castano AP, Lorrey S, Bouffard AA, et al. Anti-CD37 chimeric antigen receptor T cells are active against B- and T-cell lymphomas. *Blood*. (2018) 132:1495–506. doi: 10.1182/blood-2018-04-842708

173. Luo L, Zhou X, Zhou L, Liang Z, Yang J, Tu S, et al. Current state of CAR-T therapy for T-cell Malignancies. *Ther Adv Hematol*. (2022) 13:20406207221143025. doi: 10.1177/20406207221143025

174. Morris KE, Bowman J, Lee J-A, Tyner JW, Palazzolo M, Druker BJ, et al. CAR-NK specific targeting of clonal tcrvβ Chain to treat T-cell non-hodgkin's lymphoma. *Blood*. (2023) 142:6841. doi: 10.1182/blood-2023-189477

175. Dimitri A, Herbst F, Fraietta JA. Engineering the next-generation of CAR T-cells with CRISPR-Cas9 gene editing. *Mol Cancer*. (2022) 21:78. doi: 10.1186/s12943-022-01559-z

176. Hill LC, Rouce RH, Smith TS, Boriskie B, Srinivasan M, Thakkar SG, et al. Enhanced anti-tumor activity of CD5 CAR T cells manufactured with tyrosine kinase inhibitors in patients with relapsed/refractory T-ALL. *J Clin Oncol*. (2023) 41:7002. doi: 10.1200/JCO.2023.41.16_suppl.7002

177. Watanabe N, Mo F, Zheng R, Ma R, Bray VC, van Leeuwen DG, et al. Feasibility and preclinical efficacy of CD7-unedited CD7 CAR T cells for T cell Malignancies. *Mol Ther*. (2023) 31:24–34. doi: 10.1016/j.ymthe.2022.09.003

178. Li H, Song W, Wu J, Shi Z, Gao Y, Li J, et al. CAR-T cells targeting CD38 and LMP1 exhibit robust antitumor activity against NK/T cell lymphoma. *BMC Med*. (2023) 21:330. doi: 10.1186/s12916-023-03040-0

179. Mamonkin M, Rouce RH, Tashiro H, Brenner MK. A T-cell-directed chimeric antigen receptor for the selective treatment of T-cell Malignancies. *Blood*. (2015) 126:983–92. doi: 10.1182/blood-2015-02-629527

180. Mamonkin M, Mukherjee M, Srinivasan M, Sharma S, Gomes-Silva D, Mo F, et al. Reversible transgene expression reduces fratricide and permits 4-1BB

costimulation of CAR T cells directed to T-cell Malignancies. *Cancer Immunol Res*. (2018) 6:47–58. doi: 10.1158/2326-6066.CIR-17-0126

181. Hill LC, Rouce RH, Wu M, Wang T, Ma R, Zhang H, et al. Anti-tumor efficacy and safety of unedited autologous CD5.CAR T cells in relapsed/refractory mature T-cell lymphomas. *Blood*. (2023) 143(13):1231–41. doi: 10.1182/blood.2023022204

182. Pan J, Tan Y, Shan L, Deng B, Ling Z, Song W, et al. Phase I study of donor-derived CD5 CAR T cells in patients with relapsed or refractory T-cell acute lymphoblastic leukemia. *J Clin Oncol*. (2022) 40:7028. doi: 10.1200/JCO.2022.40.16_suppl.7028

183. Chen KH, Wada M, Pinz KG, Liu H, Lin KW, Jares A, et al. Preclinical targeting of aggressive T-cell Malignancies using anti-CD5 chimeric antigen receptor. *Leukemia*. (2017) 31:2151–60. doi: 10.1038/leu.2017.8

184. Xu Y, Liu Q, Zhong M, Wang Z, Chen Z, Zhang Y, et al. 2B4 costimulatory domain enhancing cytotoxic ability of anti-CD5 chimeric antigen receptor engineered natural killer cells against T cell Malignancies. *J Hematol Oncol*. (2019) 12:49. doi: 10.1186/s13045-019-0732-7

185. Cooper ML, Choi J, Staser K, Ritchey JK, Devenport JM, Eckardt K, et al. An "off-the-shelf" fratricide-resistant CAR-T for the treatment of T cell hematologic Malignancies. *Leukemia*. (2018) 32:1970–83. doi: 10.1038/s41375-018-0065-5

186. Ghobadi A, Aldoss I, Maude S, Wayne AS, Bhojwani D, Bajel A, et al. P356: Phase 1/2 dose-escalation study of anti-CD7 allogenic CAR-T cell in relapsed or refractory(R/R) T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma(T-ALL/LBL). *Hemasphere*. (2023) 7:e1789302. doi: 10.1097/01.HS9.0000968336.17893.02

187. Pan J, Tan Y, Wang G, Deng B, Ling Z, Song W, et al. Donor-derived CD7 chimeric antigen receptor T cells for T-cell acute lymphoblastic leukemia: first-in-human, phase I trial. *J Clin Oncol*. (2021) 39:3340–51. doi: 10.1200/JCO.21.00389

188. Zhang Y, Li C, Du M, Jiang H, Luo W, Tang L, et al. Allogenic and autologous anti-CD7 CAR-T cell therapies in relapsed or refractory T-cell Malignancies. *Blood Cancer J*. (2023) 13:61. doi: 10.1038/s41408-023-00822-w

189. Li Z, An N, Yang K, Meng F, Xu T, Peng X, et al. Donor CD7 chimeric antigen receptor T cell bridging to allogeneic hematopoietic stem cell transplantation for T cell hematologic Malignancy. *Transplant Cell Ther*. (2023) 29:167–73. doi: 10.1016/j.jtct.2022.11.013

190. Lu P, Liu Y, Yang J, Zhang X, Yang X, Wang H, et al. Naturally selected CD7 CAR-T therapy without genetic manipulations for T-ALL/LBL: first-in-human phase 1 clinical trial. *Blood*. (2022) 140:321–34. doi: 10.1182/blood.2021014498

191. You F, Wang Y, Jiang L, Zhu X, Chen D, Yuan L, et al. A novel CD7 chimeric antigen receptor-modified NK-92MI cell line targeting T-cell acute lymphoblastic leukemia. *Am J Cancer Res*. (2019) 9:64–78.

192. Zhu X-Y, Liu X, Wang X-B, Wang A-Y, Wang M, Liu N-N, et al. [Killing effect of A CD7 chimeric antigen receptor-modified NK-92MI cell line on CD7-positive hematological Malignant cells]. *Zhongguo Shi Yan Xue Ye Xue Za Zhi*. (2020) 28:1367–75. doi: 10.19746/j.cnki.issn.1009-2137.2020.04.049

193. Dai Z, Mu W, Zhao Y, Cheng J, Lin H, Ouyang K, et al. T cells expressing CD5/CD7 bispecific chimeric antigen receptors with fully human heavy-chain-only domains mitigate tumor antigen escape. *Signal Transduction Targeted Ther*. (2022) 7:85. doi: 10.1038/s41392-022-00898-z

194. Binder C, Cvetkovski F, Sellberg F, Berg S, Paternina Visbal H, Sachs DH, et al. CD2 immunobiology. *Front Immunol*. (2020) 11:1090. doi: 10.3389/fimmu.2020.01090

195. Angelos MG, Patel RP, Chiang Y-H, Xie W, Pajarillo R, Shaw CE, et al. Fratricide-resistant anti-CD2 chimeric antigen receptor T-cells with endogenous CD2 knockout are highly effective against T-cell neoplasms. *Blood*. (2023) 142:885. doi: 10.1182/blood-2023-181282

196. Xiang J, Devenport J, Carter AJ, Staser K, Rettig MP, Kim MY, et al. An "Off-the-shelf" CD2 universal CAR-T therapy combined with a long-acting IL-7 for T-cell Malignancies. *Blood*. (2023) 142:764. doi: 10.1182/blood-2023-181087

197. Geng JJ, Tang J, Yang XM, Chen R, Zhang Y, Zhang K, et al. Targeting CD147 for T to NK lineage reprogramming and tumor therapy. *EBioMedicine*. (2017) 20:98–108. doi: 10.1016/j.ebiom.2017.05.022

198. Zhang R-Y, Wei D, Liu Z-K, Yong Y-L, Wei W, Zhang Z-Y, et al. Doxycycline inducible chimeric antigen receptor T cells targeting CD147 for hepatocellular carcinoma therapy. *Front Cell Dev Biol*. (2019) 7. doi: 10.3389/fcell.2019.00233

199. Chen XH, Chen R, Shi MY, Tian RF, Zhang H, Xin ZQ, et al. Chimeric antigen receptor T cells targeting CD147 for non-small cell lung cancer therapy. *Transl Oncol*. (2022) 16:101309. doi: 10.1016/j.tranon.2021.101309

200. Tseng H-c, Xiong W, Badeti S, Yang Y, Ma M, Liu T, et al. Efficacy of anti-CD147 chimeric antigen receptors targeting hepatocellular carcinoma. *Nat Commun*. (2020) 11:4810. doi: 10.1038/s41467-020-18444-2

201. Zheng NS, Zhao XY, Wei D, Miao JL, Liu ZK, Yong YL, et al. CD147-specific chimeric antigen receptor T cells effectively inhibit T cell acute lymphoblastic leukemia. *Cancer Lett*. (2022) 542:215762. doi: 10.1016/j.canlet.2022.215762

202. Zhang C, Palashati H, Rong Z, Lin N, Shen L, Liu Y, et al. Pre-depletion of TRBC1+ T cells promotes the therapeutic efficacy of anti-TRBC1 CAR-T for T-cell Malignancies. *Mol Cancer*. (2020) 19:162. doi: 10.1186/s12943-020-01282-7

203. Cwynarski K, Iacoboni G, Tholouli E, Menne TF, Irvine DA, Balasubramaniam N, et al. First in human study of AUTO4, a TRBC1-targeting CAR T-cell therapy in relapsed/refractory TRBC1-positive peripheral T-cell lymphoma. *Blood*. (2022) 140:10316–7. doi: 10.1182/blood-2022-165971

204. Maciocia PM, Wawrzyniecka PA, Philip B, Ricciardelli I, Akarca AU, Onuoha SC, et al. Targeting the T cell receptor β-chain constant region for immunotherapy of T cell Malignancies. *Nat Med*. (2017) 23:1416–23. doi: 10.1038/nm.4444

205. Cwynarski K, Iacoboni G, Tholouili E, Menne T, Irvine D, Balasubramaniam N, et al. First in human study of auto4, a TRBC1-tragetting CART T cell therapy in relapsed/refractory TRBC1-positive peripheral T-cell lymphoma. *Hematological Oncol*. (2023) 41:80–1. doi: 10.1002/hon.3163_44

206. Li F, Zhang H, Wang W, Yang P, Huang Y, Zhang J, et al. T cell receptor β-chain-targeting chimeric antigen receptor T cells against T cell Malignancies. *Nat Commun*. (2022) 13:4334. doi: 10.1038/s41467-022-32092-8

207. Safarzadeh Kozani P, Safarzadeh Kozani P, Rahbarizadeh F. CAR-T cell therapy in T-cell Malignancies: Is success a low-hanging fruit? *Stem Cell Res Ther*. (2021) 12:527. doi: 10.3389/fimmu.2021.765097

208. Rasaiyaah J, Georgiadis C, Preece R, Mock U, Qasim W. TCRαβ/CD3 disruption enables CD3-specific antileukemic T cell immunotherapy. *JCI Insight*. (2018) 3:e99442. doi: 10.1172/jci.insight.99442

209. Chen KH, Wada M, Firor AE, Pinz KG, Jares A, Liu H, et al. Novel anti-CD3 chimeric antigen receptor targeting of aggressive T cell Malignancies. *Oncotarget*. (2016) 7:56219–32. doi: 10.18632/oncotarget.11019

210. Qian H, Gay FPH, Pang JWL, Lee Y, Ang J, Tan HC, et al. Development of anti-CD3 chimeric antigen receptor (CAR)-T cells for allogeneic cell therapy of peripheral T-cell lymphoma (PTCL). *Blood*. (2022) 140:4510–1. doi: 10.1182/blood-2022-162222

211. Zhang H, Feng J, Zhang W, Chen Q, Cao Y, Pinz K, et al. First-in-human CD4 CAR clinical trial on peripheral T-cell lymphoma. *Blood*. (2019) 134:2881. doi: 10.1182/blood-2019-122789

212. Pinz KG, Yakaboski E, Jares A, Liu H, Firor AE, Chen KH, et al. Targeting T-cell Malignancies using anti-CD4 CAR NK-92 cells. *Oncotarget*. (2017) 8:112783–96. doi: 10.18632/oncotarget.22626

213. Horwitz S, O'Connor OA, Pro B, Trümper L, Iyer S, Advani R, et al. The ECHELON-2 Trial: 5-year results of a randomized, phase III study of brentuximab vedotin with chemotherapy for CD30-positive peripheral T-cell lymphoma. *Ann Oncol*. (2022) 33:288–98. doi: 10.1016/j.annonc.2021.12.002

214. Voorhees TJ, Ghosh N, Grover N, Block J, Cheng C, Morrison K, et al. Long-term remission in multiply relapsed enteropathy-associated T-cell lymphoma following CD30 CAR T-cell therapy. *Blood Advances*. (2020) 4:5925–8. doi: 10.1182/bloodadvances.2020003218

215. Frazao A, Rethacker L, Messaoudene M, Avril M-F, Toubert A, Dulphy N, et al. NKG2D/NKG2-ligand pathway offers new opportunities in cancer treatment. *Front Immunol*. (2019) 10. doi: 10.3389/fimmu.2019.00661

216. Spear P, Barber A, Rynda-Apple A, Sentman CL. NKG2D CAR T-cell therapy inhibits the growth of NKG2D ligand heterogeneous tumors. *Immunol Cell Biol*. (2013) 91:435–40. doi: 10.1038/icb.2013.17

217. Curio S, Jonsson G, Marinović S. A summary of current NKG2D-based CAR clinical trials. *Immunother Adv*. (2021) 1:ltab018. doi: 10.1093/immadv/ltab018

218. Perera LP, Zhang M, Nakagawa M, Petrus MN, Maeda M, Kadin ME, et al. Chimeric antigen receptor modified T cells that target chemokine receptor CCR4 as a therapeutic modality for T-cell Malignancies. *Am J Hematol*. (2017) 92:892–901. doi: 10.1002/ajh.24794

219. Watanabe K, Gomez AM, Kuramitsu S, Siurala M, Da T, Agarwal S, et al. Identifying highly active anti-CCR4 CAR T cells for the treatment of T-cell lymphoma. *Blood Adv*. (2023) 7:3416–30. doi: 10.1182/bloodadvances.2022008327

220. Bobrowicz M, Kusowska A, Krawczyk M, Slusarczyk A, Barankiewicz J, Domagala J, et al. CD20 expression regulates CD37 levels in B-cell lymphoma – implications for immunotherapies. *bioRxiv*. (2023). doi: 10.1101/2023.12.06.570441

221. Khopanlert W, Prompat N, Saetang J, Maneechai K, Okuno S, Terakura S, et al. Impact of glycine-serine linker on target antigen binding and subsequent CD37CAR-T performance. *Blood*. (2023) 142:3623. doi: 10.1182/blood-2023-173354

222. Frigault MJ, Chen Y-B, Gallagher KME, Horick NK, El-Jawahri A, Scarfò I, et al. Phase 1 study of CD37-directed CAR T cells in patients with relapsed or refractory CD37+ Hematologic Malignancies. *Blood*. (2021) 138:653. doi: 10.1182/blood-2021-146236

223. Imai K, Takeuchi Y, Terakura S, Okuno S, Adachi Y, Osaki M, et al. Dual CAR-T cells targeting CD19 and CD37 are effective in target antigen loss B-cell tumor models. *Mol Cancer Ther*. (2023) 23(3):OF1–OF13. doi: 10.1158/1535-7163.MCT-23-0408

224. Golubovskaya V, Zhou H, Li F, Valentine M, Sun J, Berahovich R, et al. Novel CD37, humanized CD37 and bi-specific humanized CD37-CD19 CAR-T cells specifically target lymphoma. *Cancers*. (2021) 13:981. doi: 10.3390/cancers13050981

225. Glisovic-Aplenc T, Diorio C, Chukinas JA, Veliz K, Shestova O, Shen F, et al. CD38 as a pan-hematologic target for chimeric antigen receptor T cells. *Blood Advances*. (2023) 7:4418–30. doi: 10.1182/bloodadvances.2022007059

226. Isabelle C, McConnell K, Boles AE, Brammer JE, Berge R, Portocarrero C, et al. Therapeutic potential and role of CD38 in cutaneous T-cell lymphoma pathogenesis. *Blood*. (2022) 140:9216–8. doi: 10.1182/blood-2022-170550

227. Johnson WT, Isabelle C, Vogel AN, Brammer JE, Boles AE, McConnell K, et al. Efficient targeting of CD38 in mature T-cell neoplasms with daratumumab and allogeneic NK cells. *Blood*. (2021) 138:2408. doi: 10.1182/blood-2021-154234

228. Wang X, Yu X, Li W, Neeli P, Liu M, Li L, et al. Expanding anti-CD38 immunotherapy for lymphoid Malignancies. *J Exp Clin Cancer Res*. (2022) 41:210. doi: 10.1186/s13046-022-02421-2

229. Troy EC, Sezgin Y, Pereira MDS, Caporale J, Behbehani GK, Lyberger J, et al. CD38 KO/CD38-CAR human primary natural killer cells enhance cytotoxicity against CD38-expressing primary lymphoma, leukemia, and myeloma. *Blood*. (2023) 142:3443. doi: 10.1182/blood-2023-174742

230. Isabelle C, Boles A, McConnell K, Keller R, Cheslow L, Xu J, et al. Pathobiology and targeting of CD38 in cutaneous T-cell lymphoma. *Blood*. (2023) 142:1650. doi: 10.1182/blood-2023-189556

231. Gurney M, Stikvoort A, Nolan E, Kirkham-McCarthy L, Khoruzhenko S, Shivakumar R, et al. CD38 knockout natural killer cells expressing an affinity optimized CD38 chimeric antigen receptor successfully target acute myeloid leukemia with reduced effector cell fratricide. *Haematologica*. (2022) 107:437–45. doi: 10.3324/haematol.2020.271908

232. Ambinder RF. Epstein-barr virus and hodgkin lymphoma. *Hematology*. (2007) 2007:204–9. doi: 10.1182/asheducation-2007.1.204

233. Donzel M, Bonjour M, Combes J-D, Broussais F, Sesques P, Traverse-Glehen A, et al. Lymphomas associated with Epstein-Barr virus infection in 2020: Results from a large, unselected case series in France. *eClinicalMedicine*. (2022) 54:101674. doi: 10.1016/j.eclinm.2022.101674

234. Zhang X, Zhang R, Ren C, Xu Y, Wu S, Meng C, et al. Epstein Barr virus-positive B-cell lymphoma is highly vulnerable to MDM2 inhibitors in vivo. *Blood Adv*. (2022) 6:891–901. doi: 10.1182/bloodadvances.2021006156

235. Murray PG, Young LS. An etiological role for the Epstein-Barr virus in the pathogenesis of classical Hodgkin lymphoma. *Blood*. (2019) 134:591–6. doi: 10.1182/blood.2019000568

236. Singh DR, Nelson SE, Pawelski AS, Kansra AS, Fogarty SA, Bristol JA, et al. Epstein–Barr virus LMP1 protein promotes proliferation and inhibits differentiation of epithelial cells via activation of YAP and TAZ. *Proc Natl Acad Sci*. (2023) 120: e2219755120. doi: 10.1073/pnas.2219755120

237. Ho C, Ruella M, Levine BL, Svoboda J. Adoptive T-cell therapy for Hodgkin lymphoma. *Blood Adv*. (2021) 5:4291–302. doi: 10.1182/bloodadvances.2021005304

238. Tang X, Zhou Y, Li W, Tang Q, Chen R, Zhu J, et al. T cells expressing a LMP1-specific chimeric antigen receptor mediate antitumor effects against LMP1-positive nasopharyngeal carcinoma cells in *vitro* and in vivo. *J BioMed Res*. (2014) 28:468–75. doi: 10.7555/JBR.28.20140066

239. Harada S, Ando M, Ando J, Ishii M, Yamaguchi T, Yamazaki S, et al. Dual-antigen targeted iPSC-derived chimeric antigen receptor-T cell therapy for refractory lymphoma. *Mol Ther*. (2022) 30:534–49. doi: 10.1016/j.ymthe.2021.10.006

240. Slabik C, Kalbarczyk M, Danisch S, Zeidler R, Klawonn F, Volk V, et al. CAR-T cells targeting epstein-barr virus gp350 validated in a humanized mouse model of EBV infection and lymphoproliferative disease. *Mol Ther - Oncolytics*. (2020) 18:504–24. doi: 10.1016/j.omto.2020.08.005

241. Zhang AQ, Hostetler A, Chen LE, Mukkamala V, Abraham W, Padilla LT, et al. Universal redirection of CAR T cells against solid tumors via membrane-inserted ligands for the CAR. *Nat Biomed Engineering*. (2023) 7:1113–28. doi: 10.1038/s41551-023-01048-8

242. Aalipour A, Le Boeuf F, Tang M, Murty S, Simonetta F, Lozano AX, et al. Viral delivery of CAR targets to solid tumors enables effective cell therapy. *Mol Ther - Oncolytics*. (2020) 17:232–40. doi: 10.1016/j.omto.2020.03.018

243. Lai J, Mardiana S, House IG, Sek K, Henderson MA, Giuffrida L, et al. Adoptive cellular therapy with T cells expressing the dendritic cell growth factor Flt3L drives epitope spreading and antitumor immunity. *Nat Immunol*. (2020) 21:914–26. doi: 10.1038/s41590-020-0676-7

244. D'Aloia MM, Caratelli S, Palumbo C, Battella S, Arriga R, Lauro D, et al. T lymphocytes engineered to express a CD16-chimeric antigen receptor redirect T-cell immune responses against immunoglobulin G–opsonized target cells. *Cytotherapy*. (2016) 18:278–90. doi: 10.1016/j.jcyt.2015.10.014

245. Caratelli S, Sconocchia T, Arriga R, Coppola A, Lanzilli G, Lauro D, et al. FCγ Chimeric receptor-engineered T cells: methodology, advantages, limitations, and clinical relevance. *Front Immunol*. (2017) 8. doi: 10.3389/fimmu.2017.00457

246. Zahavi D, Weiner L. Monoclonal antibodies in cancer therapy. *Antibodies (Basel)*. (2020) 9:34. doi: 10.3390/antib9030034

247. Munoz J, Flinn IW, Cohen JB, Sachs J, Exter B, Ranger A, et al. Results from a phase 1 study of ACTR707 in combination with rituximab in patients with relapsed or refractory CD20(+) B cell lymphoma. *Transplant Cell Ther*. (2023). doi: 10.1016/j.jtct.2023.10.014

248. Daei Sorkhabi A, Mohamed Khosroshahi L, Sarkesh A, Mardi A, Aghebati-Maleki A, Aghebati-Maleki L, et al. The current landscape of CAR T-cell therapy for solid tumors: Mechanisms, research progress, challenges, and counterstrategies. *Front Immunol*. (2023) 14. doi: 10.3389/fimmu.2023.1113882

249. George P, Dasyam N, Giunti G, Mester B, Bauer E, Andrews B, et al. Third-generation anti-CD19 chimeric antigen receptor T-cells incorporating a TLR2 domain for relapsed or refractory B-cell lymphoma: a phase I clinical trial protocol (ENABLE). *BMJ Open*. (2020) 10:e034629. doi: 10.1136/bmjopen-2019-034629

250. Svoboda J, Gerson JN, Landsburg DJ, Chong EA, Barta SK, Dwivedy Nasta S, et al. Interleukin-18 secreting autologous anti-CD19 CAR T-cells (huCART19-IL18) in patients with non-hodgkin lymphomas relapsed or refractory to prior CAR T-cell therapy. *Blood*. (2022) 140:4612–4. doi: 10.1182/blood-2022-162393

251. Adachi K, Kano Y, Nagai T, Okuyama N, Sakoda Y, Tamada K. IL-7 and CCL19 expression in CAR-T cells improves immune cell infiltration and CAR-T cell survival in the tumor. *Nat Biotechnol*. (2018) 36:346–51. doi: 10.1038/nbt.4086

252. Perales M-A, Ahmed S, Dahiya S, Riedell PA, McGuirk JP, Oluwole OO, et al. A phase 2/3, randomized, double blind, placebo-controlled, multicenter study of NKTR-255 vs placebo following CD-19 directed CAR-T therapy in patients with relapsed/refractory large B-cell lymphoma. *Blood*. (2022) 140:7488–90. doi: 10.1182/blood-2022-162511

253. Wang X, Diamond DJ, Forman SJ, Nakamura R. Development of CMV-CD19 bi-specific CAR T cells with post-infusion in *vivo* boost using an anti-CMV vaccine. *Int J Hematol*. (2021) 114:544–53. doi: 10.1007/s12185-021-03215-6

254. Rataj F, Kraus FBT, Chaloupka M, Grassmann S, Heise C, Cadilha BL, et al. PD1-CD28 fusion protein enables CD4+ T cell help for adoptive T cell therapy in models of pancreatic cancer and non-hodgkin lymphoma. *Front Immunol*. (2018) 9:1955. doi: 10.3389/fimmu.2018.01955

255. Prosser ME, Brown CE, Shami AF, Forman SJ, Jensen MC. Tumor PD-L1 co-stimulates primary human CD8(+) cytotoxic T cells modified to express a PD1:CD28 chimeric receptor. *Mol Immunol*. (2012) 51:263–72. doi: 10.1016/j.molimm.2012.03.023

256. Ankri C, Shamalov K, Horovitz-Fried M, Mauer S, Cohen CJ. Human T cells engineered to express a programmed death 1/28 costimulatory retargeting molecule display enhanced antitumor activity. *J Immunol*. (2013) 191:4121–9. doi: 10.4049/jimmunol.1203085

257. Juillerat A, Tkach D, Busser BW, Temburni S, Valton J, Duclert A, et al. Modulation of chimeric antigen receptor surface expression by a small molecule switch. *BMC Biotechnol*. (2019) 19:44. doi: 10.1186/s12896-019-0537-3

258. Dwivedi A, Karulkar A, Ghosh S, Srinivasan S, Kumbhar BV, Jaiswal AK, et al. Robust antitumor activity and low cytokine production by novel humanized anti-CD19 CAR T cells. *Mol Cancer Ther*. (2021) 20:846–58. doi: 10.1158/1535-7163.MCT-20-0476

259. Karulkar A, Jain H, Shah S, Khan A, Jaiswal A, Firfiray A, et al. Making anti-CD19 CAR-T cell therapy accessible and affordable: first-in-human phase I clinical trial experience from India. *Blood*. (2022) 140:4610–1. doi: 10.1182/blood-2022-168928

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 10 May 2024
DOI 10.3389/fonc.2024.1398902

OPEN ACCESS

EDITED BY
Saad Zafar Usmani,
Levine Cancer Institute, United States

REVIEWED BY
Sanjay De Mel,
National University Cancer Institute, Singapore
Francesca Cottini,
The Ohio State University, United States

*CORRESPONDENCE
Sridevi Rajeeve
rajeeves@mskcc.org

RECEIVED 11 March 2024
ACCEPTED 24 April 2024
PUBLISHED 10 May 2024

CITATION
Miller K, Hashmi H and Rajeeve S (2024) Beyond BCMA: the next wave of CAR T cell therapy in multiple myeloma.
Front. Oncol. 14:1398902.
doi: 10.3389/fonc.2024.1398902

Beyond BCMA: the next wave of CAR T cell therapy in multiple myeloma

Kevin Miller, Hamza Hashmi and Sridevi Rajeeve*

Myeloma Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, United States

Chimeric antigen receptor (CAR) T cell therapy has transformed the treatment landscape of relapsed/refractory multiple myeloma. The current Food and Drug Administration approved CAR T cell therapies idecabtagene vicleucel and ciltacabtagene autoleucel both target B cell maturation antigen (BCMA), which is expressed on the surface of malignant plasma cells. Despite deep initial responses in most patients, relapse after anti-BCMA CAR T cell therapy is common. Investigations of acquired resistance to anti-BCMA CAR T cell therapy are underway. Meanwhile, other viable antigenic targets are being pursued, including G protein-coupled receptor class C group 5 member D (GPRC5D), signaling lymphocytic activation molecule family member 7 (SLAMF7), and CD38, among others. CAR T cells targeting these antigens, alone or in combination with anti-BCMA approaches, appear to be highly promising as they move from preclinical studies to early phase clinical trials. This review summarizes the current data with novel CAR T cell targets beyond BCMA that have the potential to enter the treatment landscape in the near future.

KEYWORDS

multiple myeloma, CAR T cell therapy, chimeric antigen receptor, non-BCMA, immunotherapy

Introduction

The treatment landscape of multiple myeloma has vastly changed over the past two decades with the introduction of novel classes of drugs which significantly improved survival outcomes. More recently, the emergence of immunotherapies including chimeric antigen receptor (CAR) T cell therapy further expanded the myeloma treatment armamentarium (1–7). CAR T cells are cellular therapy products derived from the *ex vivo* genetic modification of T cells with a CAR construct. CARs are modular transgenes comprised of a target-binding domain that recognizes cell surface molecules in a major histocompatibility complex-independent fashion, a hinge or spacer domain, a transmembrane domain, co-stimulatory domain(s) such as CD28 or 4-1BB, and a CD3ζ signaling domain (8). After *ex vivo* expansion, CAR T cells are infused into lymphodepleted patients. Upon target engagement, CARs activate T cells, causing them to destroy tumor

cells bearing their cognate antigen. To date, CAR T cell therapy has been clinically effective in several hematologic malignancies leading to durable responses in a subset of treatment refractory patients (9). However, apart from logistical and financial challenges associated with creating and administering these autologous therapeutics, other limitations include unique toxicities such as cytokine release syndrome (CRS) and immune effector cell associated neurotoxicity syndrome (ICANS), which can cause significant morbidity and in some cases be fatal (10). In addition, there are significant risks of infection and persistent cytopenias after CAR T cell therapy (11–13). Thus, administering CAR T cell therapy is a cost- and labor-intensive endeavor that requires significant multi-disciplinary expertise (14). Despite these limitations, the promise of living drugs that can expand *in vivo*, eliminate tumor cells, and potentially persist for years – the fruits of decades of research – has generated immense enthusiasm.

There are currently two Food and Drug Administration (FDA) approved CAR T cell therapies for the treatment of relapsed/refractory (R/R) myeloma: Idecabtagene vicleucel (ide-cel) and ciltacabtagene autoleucel (cilta-cel), which were first approved in March 2021 and February 2022, respectively, for use in patients treated with at least 4 prior lines of therapy (4, 15). Both ide-cel and cilta-cel target B cell maturation antigen (BCMA), which is a tumor necrosis factor superfamily receptor expressed almost exclusively on human plasma cells that has a functional role in myeloma tumorigenesis (16–18). Ide-cel was the first FDA approved CAR T cell in myeloma and bears a murine single chain variable fragment (scFv) anti-BCMA target-binding domain as well as a 4-1BB co-stimulatory domain. Initial FDA approval was based on the phase 2 KarMMa study reported by Munshi et al., where in 128 infused patients with R/R myeloma, ide-cel demonstrated an overall response rate (ORR) of 73%, with a complete response (CR) rate of 33%, measurable residual disease (MRD)-negative rate of 26% (from here, defined as less than 1 in 10^{-5} nucleated cells), and median progression-free survival (PFS) of 8.8 months (15). The second FDA approved CAR T cell therapy is cilta-cel, which has a target-binding domain comprised of two camelid heavy-chain only anti-BCMA fragments and includes a 4-1BB co-stimulatory domain. Initial approval was supported by the phase 1/2 CARTITUDE-1 trial, where in 97 infused patients, the response rate was 98% with a CR rate of 83%, MRD-negative rate of 92% (among evaluable patients) and remarkable median PFS of 34.9 months (4, 19, 20).

In April 2024, the FDA revised the label of both ide-cel and cilta-cel to include patients treated with 1-2 prior lines of therapy based on results of two randomized phase 3 trials: KarMMa-3 and CARTITUDE-4, respectively. KarMMa-3 compared ide-cel with standard regimens in patients with R/R myeloma, and demonstrated significant improvements in response rate (71% vs. 42%), MRD-negative rate (20% vs. 1%) and PFS (1-year PFS, 55% vs. 30%) (6). CARTITUDE-4 compared cilta-cel to standard regimens in R/R myeloma, demonstrating an improved response rate (84.6% vs. 67.3%), MRD-negative rate (60.6% vs. 15.6%) and PFS (1-year PFS, 75.9% vs. 48.6%) (7). While the trial designs, patient populations, and prior therapies/refractoriness were slightly different, both showed that anti-BCMA CAR T-cell therapy improved depth and duration of remission compared to standard salvage therapies. These trials established anti-BCMA CAR T cell therapy as superior for patients with daratumumab-refractory disease. However, it remains unclear whether anti-BCMA CAR T cells are better than existing salvage regimens for patients who are daratumumab-naïve or daratumumab-exposed (but not refractory), especially for those with standard-risk disease biology. This question can be addressed by in-depth analysis of the CARTITUDE-4 trial as well as from real-world evidence on the use of anti-BCMA CAR T cells in earlier lines of therapy. Taken together, clearly ide-cel and cilta-cel have revolutionized the treatment paradigm for patients with R/R myeloma. There are also several other anti-BCMA CAR T cell products currently in various stages of clinical development, including several allogeneic products, as well as combination trials such as with an oral γ-secretase inhibitor to increase BCMA surface antigen density (21–36).

Despite remarkable efficacy in R/R myeloma, it is increasingly evident that most patients with deep responses after anti-BCMA CAR T cell therapy subsequently relapse (15, 19). Notably, in the phase 2 trial with ide-cel, almost all evaluable patients (96%) retained BCMA expression by immunohistochemical (IHC) analysis at the time of relapse, suggesting complete loss of BCMA is rare (15). However, there is emerging data that even if BCMA expression is maintained, decreased antigen density may contribute to resistance (37). Interestingly, in the infrequent cases of complete loss of BCMA, acquired biallelic *BCMA* deletions and/or truncating mutations have been described (38–40). In a slightly different context – i.e. relapse after exposure to anti-BCMA bispecific T cell engagers (TCE) – Lee et al. described several patients who developed non-truncating mutations in the *BCMA* extracellular domain that functionally abrogated drug binding despite retained surface protein expression (40). Whether this mechanism contributes to relapses solely after CAR T cell therapy requires further investigation, but was not identified in the aforementioned study. Taking target loss out of the equation, several other factors could contribute to both primary resistance and relapse after anti-BCMA CAR T cell therapy. In fact, the growing experience from patients treated with anti-CD19 CAR T cell therapy for B cell lymphomas has identified the importance of CAR T cell immunophenotypic characteristics, including propensity toward immune exhaustion and complex signaling cross-talk within the tumor microenvironment as major determinants of therapeutic response (41–50). The contribution of these mechanisms toward resistance to anti-BCMA CAR T cell therapy is an area of active investigation (51–55). For example, using single-cell techniques, Freeman et al. recently presented that durable anti-BCMA CAR T cell therapy responders had a lower baseline CD4+ and CD8+ T cell exhaustion signature relative to non-responders (53). Another group presented data showing that anti-BCMA CAR T cells with a predominantly terminally differentiated phenotype and exhaustion signature were associated with poor response, compared with central or effector memory CAR T cells, which were associated with durable response (51). Finally, Ledergor et al. recently showed that patients whose CAR T cells had a CD8+ effector memory cell phenotype had more frequent durable

responses, whereas increased exhausted CD4+ CAR T cells were associated with early relapse (55). As further studies are published in this in the coming years, the hope is that immunologic features associated with poor response may be further clarified and potentially mitigated with novel techniques.

Myeloma has well characterized genomic instability, intratumoral heterogeneity, and immune-evasive properties (56–59). Thus, while it is aspirational to hope that anti-BCMA CAR T cells, perhaps with next-generation constructs, will produce long-lasting remissions for patients, pursuing other targets beyond BCMA is likely necessary to overcome complex resistance mechanisms (60). What is more, a rare but noteworthy toxicity of anti-BCMA CAR T cell therapy is treatment-associated parkinsonism, which is poorly understood, but has generated a measure of justifiable apprehension (61, 62). In this review, we aim to summarize several emerging CAR T cell therapies with novel non-BCMA targets in myeloma, focusing on targets with more extensive preclinical rationale and clinical trial data (see Figure 1). Notably, the scope of this review does not include other novel anti-BCMA CAR T cells in earlier stages of investigation, nor advancements in other immunotherapeutic modalities such as bispecific TCE (63–66).

GPRC5D

G protein-coupled receptor class C group 5 member D (GPRC5D) was initially identified as a transcribed mRNA in malignant plasma cells over a decade ago, but it was not until 2019 that GPRC5D was shown to be expressed on the cell surface (67, 68). Human GPRC5D expression otherwise appeared to be limited to hair follicles and skin, which made it a potentially promising immunotherapeutic target. In their seminal paper, Smith et al. developed and characterized multiple anti-GPRC5D CAR T cell constructs with *in vitro* and *in vivo* activity and no significant cross-reactivity to other tissues (including hair and skin) in mice and non-human primates (68). Consequently, these and

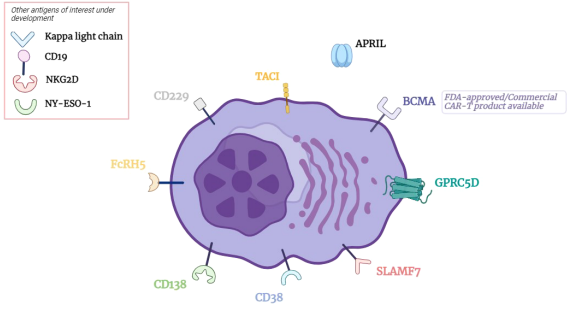


FIGURE 1
CAR T cell Therapy Targets in Multiple Myeloma. BCMA, B cell maturation antigen; GPRC5D, G protein coupled receptor class C group 5 member D; SLAMF7, signaling lymphocytic activation molecule family member 7; CD, cluster of differentiation; FcRH5, Fc receptor homolog 5; TACI, transmembrane activator and CAML interactor; APRIL, A proliferation inducing ligand.

other anti-GPRC5D CAR T cells were rapidly translated into early phase clinical trials. The first phase 1 study reported in 2022 by Mailankody et al. described 17 heavily pre-treated patients infused with MCARH109, which contains a humanized anti-GPRC5D scFv target-binding domain and a 4-1BB co-stimulatory domain (69). CAR T cell doses ranged from 25×10^6 to 450×10^6 cells. Many of the toxicities were akin to prior experience with other FDA approved CAR T cell therapies including CRS (overall, 88%; grade ≥3, 6%), ICANS (overall, 6%; grade ≥3, 6%), infections (overall, 18%; grade ≥3, 12%), and cytopenias (grade ≥3, 94%). Of note, nail loss was common (65%), although this reversed without intervention in most. Rash was uncommon but reported (grade 1, 18%), as was dysgeusia (grade 1, 12%). An important discovery was the evolution of a persistent cerebellar syndrome in two patients (12%) who both received the highest dose level (450×10^6 cells), characterized by visual fixation problems, appendicular and truncal ataxia, gait abnormalities and dysarthria. Neural imaging in both patients did not reveal any focal lesions. However, the authors noted that analysis of the Allen Brain Atlas revealed there is focal expression of GPRC5D in the inferior olivary nucleus in the human brainstem, which they hypothesized may account for this unique toxicity, although further research is required (70). In terms of efficacy, MCARH109 had an ORR of 71%, with a 35% CR rate, MRD-negative rate of 47%, and a median duration of response (DOR) of 7.8 months.

Several other groups have now reported phase 1 trial results with other anti-GPRC5D CAR T cell products (see Table 1 for direct comparisons among reported non-BCMA targeted CAR T cell trials). Bal et al. presented results of 70 patients treated with BMS-986393 (CC-95266), which reportedly has a similar construct to MCARH109: ORR was 86%, with 38% CR; toxicities were similar to MCARH109 including two patients with a cerebellar syndrome (71). In China, Xia et al. reported results of 33 patients treated with an anti-GPRC5D CAR T cell construct with a humanized single scFv target-binding domain and a 4-1BB co-stimulatory domain (72). Patients in the study were randomized to receive all-trans retinoic acid (ATRA) in the peri-CAR T cell infusion period based on a preclinical study that posited ATRA may affect GPRC5D expression (79). In the entire cohort, the ORR was 91% with a 64% CR rate, although there was no significant difference in response noted in the ATRA exposed patients. Toxicities were comparable to prior experience with anti-GPRC5D CAR T cells, including CRS (overall, 76%; grade ≥3, 0%), ICANS (overall, 6%; grade ≥3, 3%), nail changes (27%) and skin toxicity, including palm/sole desquamation (3%). Notably, no cerebellar syndrome was reported. One patient died of cerebral hemorrhage in the setting of severe thrombocytopenia. Another group from China reported the results of ten patients treated with OriCAR-017, which is a dual epitope camelid heavy-chain only anti-GPRC5D construct (73). In the study, the ORR was 100% with a 60% CR rate. Again, toxicities were comparable to prior experience with anti-GPRC5D CAR T cells, although here with no reported ICANS or cerebellar syndrome. Finally, Li et al. reported on seven patients infused with an anti-GPRC5D CAR T cell product, with an ORR of 86%, with 43% CR, and similar toxicities to prior reports (74). Several

TABLE 1 Results from Clinical Trials with Non-BCMA CAR T cell Therapies.

Target	Product name	N	High-risk cytogenetics*†	Extra-medullary disease*	Prior lines of therapy	Prior anti-BCMA*	CRS (grade ≥3)*	ICANS (grade ≥3)*	ORR (CR)*	Sponsor	Country	Ref.
GPRC5D	MCARH109	17	76	47	6	59	88 (6)	6 (6)	71 (35)	Memorial Sloan Kettering Cancer Center	USA	(69)
GPRC5D	BMS-986393	70	46	43	–	46	84 (4)	11 (3)	86 (38)	Bristol Myers Squibb	USA	(71)
GPRC5D	–	33	39	33	4	27	76 (0)	6 (3)	91 (64)	Xuzhou Medical University	China	(72)
GPRC5D	OriCAR-017	10	60	40	6	50	90 (0)	0	100 (60)	Zhejiang University	China	(73)
GPRC5D	–	7	–	–	–	43	86 (0)	0	86 (43)	920th Hospital of Joint Logistics Support Force	China	(74)
SLAMF7/ BCMA	–	16	–	38	4	13	38 (6)	0	81 (38)	Union Hospital, Tongji Medical College	China	(75)
CD38/ BCMA	–	23	–	39	4	0	87 (17)	0	87 (52)	Union Hospital, Tongji Medical College	China	(76)
CD38/ BCMA	–	16	69	50	3	0	75 (31)	–	88 (81)	Jingzhou Central Hospital	China	(77)
CD38/ BCMA	–	22	86	14	–	0	73 (27)	14 (0)	91 (55)	Tianjin First Central Hospital	China	(78)

*Reported as percentage of total patients in clinical trial.
†High risk cytogenetics defined as del(17p), t(4;14), t(14;16) and/or 1q amplification.

10.3389/fonc.2024.1398902

other anti-GPRC5D CAR T cell therapy trials are registered (see Table 2 for a summary of registered non-BCMA CAR T cell therapy trials without published results).

Mechanisms of resistance to anti-GPRC5D CAR T cell therapy are beginning to be dissected. Unlike relapse after anti-BCMA CAR T cells, where complete BCMA loss is rare, four of six patients who had an initial response then relapsed in the study by Mailankody et al. demonstrated complete loss of GPRC5D expression (69). In one of these patients, biallelic deletions encompassing the *GPRC5D* loci was uncovered (80). Two recent publications characterized relapses after anti-GPRC5D bispecific TCE (40, 81). Both highlighted complex subclonal *GPRC5D* deletions and mutations that precipitated loss of GPRC5D expression in several patients. Fascinatingly, Derrien et al. also described a patient with acquired loss of chromatin accessibility in the promoter of *GPRC5D*, as well as more distant enhancer regions, indicating epigenetic silencing (81). Although it is not known if these mechanisms are involved in relapse after anti-GPRC5D CAR T cell therapy, these studies highlight the complex nature of myeloma tumor biology and requirement of layered treatment strategies to thwart resistance.

TABLE 2 Single-Target Non-BCMA CAR T cell Therapy Clinical Trials.

Target	ClinicalTrials.gov ID	Sponsor	Country
GPRC5D	NCT04674813	Bristol Myers Squibb	USA
GPRC5D	NCT05739188	920th Hospital of Joint Logistics Support Force of PLA of China	China
GPRC5D	NCT05219721	Tongji Hospital	China
GPRC5D	NCT05759793	Nanjing IASO Biotechnology Company	China
GPRC5D	NCT05749133	Institute of Hematology & Blood Diseases Hospital	China
GPRC5D	NCT05838131	Shanghai Changzheng Hospital	China
SLAMF7	NCT03958656	National Cancer Institute	USA
SLAMF7	NCT03710421	City of Hope Medical Center	USA
SLAMF7	NCT04499339	Wuerzburg University Hospital	Europe
SLAMF7	NCT04541368	Zhejiang University	China
CD38	NCT05442580	University of Pennsylvania	USA
CD38	NCT03464916	Sorrento Therapeutics	USA
CD138	NCT03672318	University of North Carolina – Chapel Hill	USA
BCMA/TACI (APRIL)	NCT04657861	Zhejiang University	China
BCMA/TACI (TriPRIL)	NCT05020444	Massachusetts General Hospital	USA
CD70	NCT04662294	Zhejiang University	China

One obvious solution would be to concurrently or sequentially target BCMA, which appears to have heterogeneity of expression independent from GPRC5D (68). In fact, Smith et al. showed in a myeloma xenograft model of relapse mediated by BCMA loss, anti-GPRC5D CAR T cells effectively eradicated residual tumor (68). Pointing toward future approaches, a preclinical study showed that a bicistronic construct (distinct anti-BCMA and anti-GPRC5D CARs transduced via a single vector) was an optimal method of dual-antigen targeting (82). Several dual-antigen targeting concepts are being clinically tested (see Table 3 for a summary of registered combination CAR T cell therapy trials). One study is testing pooled anti-GPRC5D and anti-BCMA CAR T cells, i.e. with MCARH109 and MCARH125, respectively (NCT05431608). There are also several trials with dual-targeted anti-GPRC5D and anti-BCMA constructs (United States: NCT06153251; China: NCT05509530, NCT05998928, NCT05325801). Trials are also underway with other anti-GPRC5D CAR T cell therapeutic combinations, including with an anti-BCMA bispecific TCE, as well as with cereblon E3 ligase modulatory drugs (NCT06121843). Preclinical approaches to improve the efficacy of anti-GPRC5D CAR T cell therapy are under investigation, such as with lysophosphatidic acid receptor modulation (83). There are also other novel approaches including anti-GPRC5D allogeneic CAR T cells and CAR natural killer (NK) cells in the pipeline (84, 85). Taken together, the encouraging safety and efficacy data from early phase anti-GPRC5D CAR T trials have propelled GPRC5D forward as the next promising therapeutic target moving closer to regulatory approval in myeloma.

SLAMF7

The glycoprotein cell surface receptor signaling lymphocytic activation molecule family member 7 (SLAMF7, also called CS1 or CD319) is highly expressed on malignant plasma cells. SLAMF7 is functionally important for plasma cell survival, and its discovery prompted development of the anti-SLAMF7 antibody elotuzumab, which is FDA-approved for administration in combination with lenalidomide and dexamethasone for the treatment of R/R myeloma (86–88).

Several groups developed anti-SLAMF7 CAR T cells and tested them in preclinical models (89–92). Gogishvili et al. developed an anti-SLAMF7 CAR T cell construct using the target-binding domain from elotuzumab paired with a CD28 co-stimulatory domain (90). These anti-SLAMF7 CAR T cells effectively killed myeloma cells in patient samples and a murine xenograft model. Of note, unlike BCMA or GPRC5D, SLAMF7 is expressed on other immune cell types including T cells, B cells, NK cells, monocytes, and dendritic cells. As such, the capacity for fratricide both of anti-SLAMF7 CAR T cells and other immune cells was investigated. Unsurprisingly, at the end of manufacturing, the anti-SLAMF7 CAR T cells were SLAMF7-negative/low, likely due to fratricide of SLAMF7-high cells. In co-culture with autologous immune cells,

TABLE 3 Combination CAR T cell Therapy Clinical Trials.

Target (s)	Co-targeting strategy	Consolidation Therapy	ClinicalTrials.gov ID	Sponsor	Country
BCMA	–	Lenalidomide	NCT03070327	Memorial Sloan Kettering Cancer Center	USA
BCMA	–	γ-secretase inhibitor (JSMD194)	NCT03502577	Fred Hutchinson Cancer Center	USA
BCMA	–	Belantamab Mafodotin	NCT05117008	Medical College of Wisconsin	USA
BCMA	–	Cevostamab (anti-FcRH5 TCE)	NCT05801939	University of Pennsylvania	USA
BCMA/ CD38	Dual-targeted construct		NCT03767751	Chinese PLA General Hospital	China
GPRC5D	–	Alnuctamab (anti-BCMA TCE), mezigdomide, or iberdomide	NCT06121843	Juno Therapeutics	USA
GPRC5D/ BCMA	Pooled CAR T cells	–	NCT05431608	Memorial Sloan Kettering Cancer Center	USA
GPRC5D/ BCMA	Dual-targeted construct	–	NCT06153251	Juno Therapeutics	USA
GPRC5D/ BCMA	Dual-targeted construct	–	NCT05998928	Wuhan Union Hospital	China
GPRC5D/ BCMA	Dual-targeted construct	–	NCT05325801	Zhejiang University	China
GPRC5D/ BCMA	Dual-targeted construct	–	NCT05509530	Xuzhou Medical University	China
GPRC5D/ BCMA	Dual-targeted construct	–	NCT06068400	Guangzhou Bio-gene Technology Company	China
SLAMF7/ BCMA	Dual-targeted construct	–	NCT05950113	UCLA Jonsson Comprehensive Cancer Center	USA

anti-SLAMF7 CAR T cells also induced fratricide of SLAMF7-high unmodified T cells, but spared SLAMF7-negative/low T cells, and only caused partial B cell depletion. Importantly, SLAMF7 negative/low unmodified T cells retained the ability to respond to viral antigens in their study.

Another group generated anti-SLAMF7 CAR T cells with a slightly different target-binding epitope (distal V2 domain), and a third-generation combination CD28 and 4-1BB co-stimulatory domain, which effectively killed malignant plasma cells (92). Interestingly, these CAR T cells were predominantly CD4+, suggesting that CD8+ CAR T cells fell victim to fratricide during *ex vivo* production. As such, they used CRISPR/Cas9 technology to delete *SLAMF7* and create fratricide resistant anti-SLAMF7 CAR T cells. Despite yielding a more balanced CD4:CD8 T cell profile, SLAMF7-deficient CAR T cells were not significantly more effective in their murine xenograft models. Taking these studies together, while the consequences of fratricide of both anti-SLAMF7 CAR T cells and other immune cells require further investigation, it appears that anti-SLAMF7 CAR T cells could be a promising therapeutic strategy for the treatment of myeloma.

Clinical trials are now assessing anti-SLAMF7 CAR T cell therapy in patients. Based on a preclinical study with a dual-targeted single-stalk CAR with anti-BCMA and anti-SLAMF7 domains, a phase 1/2 trial was initiated and the results with 16 infused patients were recently reported (75, 93). Toxicities included CRS (overall, 38%; grade ≥3, 6%), with no reported ICANS, but significant cytopenias (overall, 100%; grade ≥3, 100%), and infections (overall, 38%; grade ≥3, 31%). In terms of efficacy, the ORR was 81%, with 38% CR, and 1-year DOR was 56%. Other groups have developed different CAR constructs co-targeting BCMA and SLAMF7, and one is being clinically tested (NCT0595011) (94, 95). An allogeneic fratricide resistant anti-SLAMF7 CAR T showed promising preclinical results, but the phase 1 trial (NCT04142619) was terminated, in part due to a fatal cardiac arrest event necessitating a FDA hold, and no results are published (96). Several trials are registered with CAR T cells targeting SLAMF7 alone (United States: NCT03958656, NCT03710421; Europe: NCT04499339; China: NCT04541368).

CD38

The discovery that the ectoenzyme CD38 was highly expressed on the cell surface plasma cells led to one of the most significant therapeutic advancements in myeloma of the past decade – the advent of anti-CD38 targeting antibodies (97, 98). The first FDA approved anti-CD38 antibody daratumumab transformed the treatment of relapsed disease and more recently has moved into the front-line treatment setting (99, 100). Besides plasma cells, CD38 is expressed on hematopoietic progenitor cells, as well as T cell

subsets, NK cells, myeloid-derived suppressor cells, and regulatory B cells. Unsurprisingly, exposure to daratumumab has been shown to perturb some of these cell populations (101, 102). Nevertheless, the lack of significant organ toxicity, including paucity of cytopenias, after anti-CD38 antibody exposure underpins its value as a therapeutic target, with elevated risk of infections being the primary adverse effect associated with treatment.

A number of anti-CD38 CAR T cells have been generated and evaluated in preclinical studies (103–107). Several groups noted that expanded anti-CD38 CAR T cells were CD38-negative, likely secondary to fratricide of CD38-positive cells (103, 105). Even so, these CAR T cells effectively killed myeloma cells in model systems, in line with prior knockout mouse studies suggesting that CD38 is generally dispensable for T cell function (108). Interestingly, anti-CD38 CAR T cells generated by Glisovic-Aplenc et al. did not undergo fratricide, likely due to a protective effect mediated by the CAR construct itself (107). Importantly, although anti-CD38 CAR T cells reduce CD34+ CD38+ hematopoietic progenitor cells *in vitro* and *in vivo*, several groups noted anti-CD38 CAR T cells do not have a significant effect on downstream hematopoietic lineages, suggesting the CD34+ CD38-low/negative compartment is sufficient to recapitulate hematopoiesis (105, 107). Obviously, as these therapies move into human testing, close observations of the effect of anti-CD38 CAR T cell exposure on the number and function of immunologic and hematopoietic cells in patients is required.

Several Chinese groups have now reported results of phase 1 trials with anti-CD38 CAR T cell therapy, albeit in combination with anti-BCMA therapy (76–78). Mei et al. designed a dual-targeted single-stalk CAR with anti-CD38 and anti-BCMA domains and a 4-1BB co-stimulatory domain (76). Because of concerns that CD38 targeting would cause significant hematopoietic toxicity, they selected a scFv with reduced binding affinity to CD38 relative to the anti-BCMA domain. These CAR T cells were infused in 23 patients and outcomes were reported. Most common toxicities included CRS (overall, 87%; grade ≥3, 17%), with no reported ICANS, although many patients had significant cytopenias (overall, 96%; grade ≥3, 87%), which persisted longer than a month in a considerable proportion. Two patients died: one from infection, one from cerebral hemorrhage. The reported ORR was 87%, with 52% CR, and 1-year DOR of 76%. Another group performed a trial with a similar dual-targeted construct (77). In 16 infused patients, toxicities were similar to the prior report with CRS and cytopenias; notably, one patient who had a CR died of infection in the setting of prolonged CRS and persistent cytopenias secondary to hemophagocytic lymphohistiocytosis. The reported ORR was 88%, with 81% CR, and several of the responses lasted over a year, although DOR was not formally reported. Finally, a third group reported the results with a pooled infusion of separate anti-CD38 and anti-BCMA CAR T cells (78). In 22 infused patients, toxicities were consistent with prior reports, although notably two patients died of refractory CRS. The ORR was 91%, with 55% CR. Another trial in China concurrently targeting CD38 and BCMA is registered (NCT03767751).

Taken together, the major caveat in interpreting these trials is that the observed toxicity and responses attributable to the anti-CD38 component of therapy is unclear, given all were combined with anti-BCMA constructs. Moreover, many patients were early in their treatment course, all were naïve to anti-BCMA therapeutics, many had no prior daratumumab exposure, and a subset were even naïve to standard up-front therapies including immunomodulatory drugs, making comparison to other trials dubious. Early phase clinical trials targeting anti-CD38 alone in the appropriate clinical context with correlative studies are necessary. In fact, several trials are currently registered in the United States with anti-CD38 CAR T cells (NCT03464916, NCT05442580). There are other novel approaches in the pre-clinical pipeline targeting CD38 as well, including anti-CD38 CAR NK cells (109–111).

CD138

The transmembrane proteoglycan CD138 (also called Syndecan-1) is expressed on terminally differentiated B cells and plays a key role in plasma cell survival (112). A priori, its utility as a therapeutic target is theoretically limited by CD138 expression on other cell types including subsets of epithelial and endothelial cells (113). With these potential pitfalls in mind, anti-CD138 CAR T cells have been preclinically developed and characterized by several groups (114, 115). Notably, the anti-CD138 CAR T cells described by Sun et al. did not lyse endothelial or epithelial cell lines in co-culture experiments (114). A clinical trial with five patients treated with anti-CD138 CAR T cells conducted in China was reported in 2016 (115). Although no objective responses were observed, there was no standard reporting of toxicities, limiting the interpretation of this study. Another clinical trial with anti-CD138 CAR T cells is enrolling in the United States (NCT03672318). Preclinical studies to optimize anti-CD138 CAR T cell therapy are also underway. For example, a recent publication detailed the design and optimization of a dual-split CAR construct with anti-CD38 and anti-CD138 domains, whereby CAR T cell activation occurred only in the presence of both antigens (116). These dual-targeted CAR T cells were effective at eliminating malignant plasma cells, but importantly spared other cell types including hematopoietic precursors.

FcRH5

Fc receptor-homolog 5 (FcRH5) is a surface antigen that is highly expressed on plasma cells and has emerged as an exciting target for immunotherapy in myeloma (117). FcRH5 expression in human tissues otherwise seems to be limited to select B cell subsets. Interestingly, the corresponding gene *FCRL5* is located on chromosome 1q, thus FcRH5 is highly expressed in patients with amplification of 1q21, a poor-risk marker in myeloma (118, 119). Cevostamab is an anti-FcRH5 bispecific TCE currently in early phase clinical trials that has shown promising responses with limited toxicity in patients (120). Preclinically, an anti-FcRH5 CAR T cell was recently developed and shown to eliminate myeloma cells *in vitro* and *in vivo*, including in a model of BCMA antigen loss (119). The authors also developed a dual-targeted single-stalk anti-BCMA and anti-FcRH5 CAR T construct that appeared promising. Although no anti-FcRH5 CAR T cell clinical trials are registered presently, it seems likely to be pursued.

CD229

The SLAM family receptor CD229 (also called Ly-9), like SLAMF7, is highly expressed on plasma cells (121). Anti-CD229 CAR T cells were recently described and shown to be highly effective at eradicating myeloma cells in preclinical models (122). Importantly, CD229 expression is otherwise limited to T cells and to a lesser extent B cells, but, unlike SLAMF7, is not appreciably expressed on NK cells, monocytes, or dendritic cells. Thus, similar to the aforementioned reports with anti-SLAMF7 CAR T cells, fratricide of unmodified lymphocytes emerged as a potential downside of anti-CD229 CAR T cell exposure (90, 122). To address this potential issue, Vander Mause et al. recently published a novel approach whereby the affinity of the anti-CD229 target-binding domain was slightly reduced, which, in conjunction with modifying the CAR construct to over-express the transcription factor c-Jun, generated CAR T cells with a favorable immunophenotype that targeted myeloma cells but spared healthy unmodified lymphocytes (123). To date, there are no clinical trials registered with anti-CD229 CAR T cells.

APRIL

A Proliferation-Inducing Ligand (APRIL) is an endogenous ligand of BCMA. APRIL also binds to Transmembrane Activator and CAML Interactor (TACI), another tumor necrosis factor superfamily receptor that, similar to BCMA, is expressed almost exclusively on plasma cells (124, 125). To take advantage of its ability to bind both BCMA and TACI with high affinity, CAR T cells with a target-binding domain derived from APRIL itself were developed by several groups (125, 126). APRIL-based CAR T cells effectively killed myeloma cells in preclinical experiments, including models of BCMA antigen loss (125, 126). However, in a phase 1 clinical trial with monomeric APRIL-based CAR T cells, the response rate was 46%, which was disappointing compared to other contemporaneous anti-BCMA CAR T cell trials (127). Subsequent studies revealed that APRIL-based CAR T cells exhibited inadequate T cell activation, secreted lower than expected levels of cytokines and thus had poor *in vivo* expansion, perhaps explaining the tepid results. Schmidts et al. devised trimeric APRIL-based CAR T cells with the goal of more closely approximating the natural APRIL ligand conformation (125). These "TriPRIL" CAR T cells had enhanced functionality compared to monomeric APRIL-based CAR T cells and are being clinically tested (NCT05020444). The same group also recently designed a more traditional scFv-based dual-targeted anti-TACI and anti-BCMA CAR T construct that was highly effective in preclinical models, including when expression of either antigen was lost (128).

Other targets

Clinical trials with non-BCMA targeted immune effector cell therapies that have not shown remarkable efficacy to date include the following:

- κ light chain: Taking advantage of the κ light chain-restricted nature of a considerable proportion of B cell tumors, a phase 1 trial with anti-κ light chain CAR T cells was published, although there were no responses demonstrated in myeloma patients (129).
- NKG2D: Given a wide range of tumors including myeloma cells upregulate NKG2D ligands on the cell surface, several trials with anti-NKG2D ligand CAR T cells were reported, although no responses were observed (130, 131).
- CD19: The use of anti-CD19 CAR T cell therapies given immediately after autologous hematopoietic cell transplantation (AHCT) has been explored, with the idea that CD19 may be expressed on a tumor propagating myeloma stem cell compartment that could be putatively eradicated (132–134). Results with this approach were mixed, and it is difficult to isolate the effect of the anti-CD19 CAR T cells *per se*.
- NY-ESO-1: A trial with anti-NY-ESO-1 TCR-engineered T cells given after autologous HCT was reported, with similar caveats to anti-CD19 CAR T cell therapy trials, although correlative studies suggested potential biologic activity associated with response (135, 136).

Furthermore, a number of preclinical studies have defined other CAR T cell targets in myeloma, including integrin β_7, CD44 splice isoform variant 6, CD56, CD70, Lewis Y antigen, and leukocyte immunoglobulin-like receptor subfamily B member 4 (137–142).

Discussion

The early experience with ide-cel and cilta-cel underpinned the potency of anti-BCMA CAR T cell therapy in multiple myeloma, and triggered a rapid evolution in the treatment of the disease (15, 19). In April 2024, the FDA granted regulatory approval for use of ide-cel and cilta-cel in the early relapsed setting (1-2 lines of prior therapy), and consequently patients are increasingly being treated with anti-BCMA CAR T cell therapy earlier in their disease course. Furthermore, upcoming randomized phase 3 clinical trials are evaluating anti-BCMA CAR T cells as a component of front-line therapy for newly diagnosed myeloma. For example, cilta-cel is being tested as consolidation in transplant-ineligible patients (CARTITUDE-5, NCT04923893). Cilta-cel is also being compared with AHCT as consolidation after induction for transplant-eligible patients (CARTITUDE-6, NCT05257083). In addition, ide-cel is being tested as a consolidation therapy for patients with a sub-optimal response after AHCT (KarMMa-9, NCT06045806). While depth and duration of response are key endpoints for these clinical trials, a focus on treatment-related toxicities including long-term neurologic toxicities as well as secondary malignancies should be prioritized.

The ideal sequencing of anti-BCMA CAR T cell therapy with other novel immunotherapies is not known. For example, in patients exposed to anti-BCMA bispecific TCE, Lee et al. described acquired mutations in the *BCMA* extracellular domain

that abrogated drug binding (40). However, to date there are no validated assays to test for these mutations in the clinic. At minimum, BCMA expression should be tested by immunohistochemistry prior to anti-BCMA CAR T cell therapy in patients exposed to prior BCMA-directed treatments. Overall, the challenge of relapse after treatment with anti-BCMA therapies is becoming increasingly relevant and urgently requires innovative approaches.

Novel non-BCMA targeted CAR T cell therapies are a potential solution to combat baseline intratumoral antigen heterogeneity, acquired antigen loss and immune exhaustion (8, 60, 143, 144). For example, CAR T cells targeting GPRC5D are furthest in the developmental pipeline, and to date have shown promising safety and efficacy, including in patients who relapsed after prior anti-BCMA therapies (69, 71). The optimal sequencing of anti-BCMA and anti-GPRC5D directed treatments is not known. Early data suggests that surface expression as well as acquired mutations in BCMA and GPRC5D appear to be the consequence of selective pressures from specific therapies and therefore largely independent of each other (40). Unlike *BCMA*, it appears *GPRC5D* may be genetically lost more frequently, so testing for expression by immunohistochemistry prior to anti-GPRC5D CAR T cell therapy is prudent, especially if patients have prior exposure to anti-GPRC5D bispecific TCE (40, 69, 80, 81). A number of other promising targets of interest were highlighted in the review and in the early stages of clinical evaluation.

There are many pressing questions as CAR T cell therapies in myeloma are refined. Understanding the co-expression patterns of multiple antigens such as BCMA, GPRC5D and FcRH5 will be relevant as combination therapies, e.g., dual-targeted CAR T cells, are tested in the clinic (145). Given that antigen density on tumor cells is a crucial factor mediating CAR T cell resistance, the effect of tumor mutations such as *KRAS* and *TP53* on surface antigen expression could be informative (37, 146, 147). Moreover, as a potential combination therapy with anti-BCMA CAR T cells, it is noteworthy that γ-secretase inhibition may affect expression of other antigens as well (23, 148). Several recent presentations at the American Society of Hematology 2023 meeting highlighted ongoing work to decipher the immunologic landscape of response and relapse after anti-BCMA CAR T cells (51–54). These analyses build on the growing understanding of optimal CAR T cell immune characteristics and will be required for CAR T cell therapies targeting other antigens. Further, as trials investigate using bispecific TCE to amplify responses after CAR T cell therapy, studies deciphering how TCE impact the immunologic milieu are necessary (149, 150). Much work remains to fine-tune the CAR T cell approach in cancer more broadly as well (8, 151, 152). Perhaps the advent of combinatorial logic-gated CAR T cells will further open the search for other relevant targets (152, 153). Although not discussed in this review, alternative immune effector cell therapies such as CAR NK cells or CAR macrophages may have certain advantageous properties and could make an impact as well (154–157).

In summary, the future of CAR T cell therapy in myeloma is bright as constructs aimed at targets beyond BCMA enter the treatment landscape. Rigorously designed CAR T cell clinical trials with accompanying correlative studies are necessary to generate hypotheses that can be investigated in translational laboratories, particularly to elucidate mechanisms of relapse and optimal sequencing of therapies aimed at different targets. Moreover, ongoing work to interrogate the safety profile of these CAR T cell therapies is vital, as both anti-BCMA and anti-GPRC5D CAR T cells have raised concerns about rare but serious long-term neurologic toxicities which are poorly understood (61, 69). Future practical considerations of rechallenging with CAR T cell therapy, albeit against different antigens, include the unknown cumulative clinical consequences of repeated exposure to cytotoxic lymphodepleting chemotherapy, as well as potential diminishing immunologic fitness after multiple lines of prior therapy affecting the manufacturing quality of CAR T cell products. Finally, the financial toxicities associated with sequential administration of highly costly cellular therapeutics is uncharted outside of clinical trial settings and will likely be a major challenge. With all these caveats in mind, the rapid pace of innovation suggests highly active CAR T cell therapies leading to more durable remissions for patients with myeloma could be on the horizon.

Author contributions

KM: Writing – original draft, Writing – review & editing. HH: Writing – original draft, Writing – review & editing. SR: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

We would like to thank Drs. Saad Usmani, Nilanjan Ghosh and Peter Voorhees for the invitation to write this review.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Ali SA, Shi V, Maric I, Wang M, Stroncek DF, Rose JJ, et al. T cells expressing an anti–B-cell maturation antigen chimeric antigen receptor cause remissions of multiple myeloma. *Blood*. (2016) 128:1688–700. doi: 10.1182/blood-2016-04-711903

2. Cohen AD, Garfall AL, Stadtmauer EA, Melenhorst JJ, Lacey SF, Lancaster E, et al. B cell maturation antigen–specific CAR T cells are clinically active in multiple myeloma. *J Clin Invest*. (2019) 129:2210–21. doi: 10.1172/JCI126397

3. Raje N, Berdeja J, Lin Y, Siegel D, Jagannath S, Madduri D, et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. *New Engl J Med*. (2019) 380:1726–37. doi: 10.1056/NEJMoa1817226

4. 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) 398:314–24. doi: 10.1016/S0140-6736(21)00933-8

5. Boussi LS, Avigan ZM, Rosenblatt J. Immunotherapy for the treatment of multiple myeloma. *Front Immunol*. (2022) 13:1027385. doi: 10.3389/fimmu.2022.1027385

6. 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. *New Engl J Med*. (2023) 388:1002–14. doi: 10.1056/NEJMoa2213614

7. San-Miguel J, Dhakal B, Yong K, Spencer A, Anguille S, Mateos M-V, et al. Cilta-cel or standard care in lenalidomide-refractory multiple myeloma. *New Engl J Med*. (2023) 389:335–47. doi: 10.1056/NEJMoa2303379

8. Labanieh L, Mackall CL. CAR immune cells: design principles, resistance and the next generation. *Nature*. (2023) 614:635–48. doi: 10.1038/s41586-023-05707-3

9. Gill S, Maus MV, Porter DL. Chimeric antigen receptor T cell therapy: 25years in the making. *Blood Rev*. (2016) 30:157–67. doi: 10.1016/j.blre.2015.10.003

10. Morris EC, Neelapu SS, Giavridis T, Sadelain M. Cytokine release syndrome and associated neurotoxicity in cancer immunotherapy. *Nat Rev Immunol*. (2022) 22:85–96. doi: 10.1038/s41577-021-00547-6

11. Hill JA, Li D, Hay KA, Green ML, Cherian S, Chen X, et al. Infectious complications of CD19-targeted chimeric antigen receptor–modified T-cell immunotherapy. *Blood*. (2018) 131:121–30. doi: 10.1182/blood-2017-07-793760

12. Little JS, Tandon M, Hong JS, Nadeem O, Sperling AS, Raje N, et al. Respiratory infections predominate after day 100 following B-cell maturation antigen-directed CAR T-cell therapy. *Blood Adv*. (2023) 7:5485–95. doi: 10.1182/bloodadvances.2023010524

13. Jain T, Olson TS, Locke FL. How I treat cytopenias after CAR T-cell therapy. *Blood*. (2023) 141:2460–9. doi: 10.1182/blood.2022017415

14. Cliff ERS, Kelkar AH, Russler-Germain DA, Tessema FA, Raymakers AJN, Feldman WB, et al. High cost of chimeric antigen receptor T-cells: challenges and solutions. *Am Soc Clin Oncol Educ Book*. (2023):e397912. doi: 10.1200/EDBK_397912

15. Munshi NC, Anderson LD, Shah N, Madduri D, Berdeja J, Lonial S, et al. Idecabtagene vicleucel in relapsed and refractory multiple myeloma. *New Engl J Med*. (2021) 384:705–16. doi: 10.1056/NEJMoa2024850

16. O'Connor BP, Raman VS, Erickson LD, Cook WJ, Weaver LK, Ahonen C, et al. BCMA is essential for the survival of long-lived bone marrow plasma cells. *J Exp Med*. (2004) 199:91–8. doi: 10.1084/jem.20031330

17. Tai Y-T, Acharya C, An G, Moschetta M, Zhong MY, Feng X, et al. APRIL and BCMA promote human multiple myeloma growth and immunosuppression in the bone marrow microenvironment. *Blood J Am Soc Hematol*. (2016) 127:3225–36. doi: 10.1182/blood-2016-01-691162

18. Carpenter RO, Evbuomwan MO, Pittaluga S, Rose JJ, Raffeld M, Yang S, et al. B-cell maturation antigen is a promising target for adoptive T-cell therapy of multiple myeloma. *Clin Cancer Res*. (2013) 19:2048–60. doi: 10.1158/1078-0432.CCR-12-2422

19. 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) 41:1265–74. doi: 10.1200/JCO.22.00842

20. Lin Y, Martin TG, Usmani SZ, Berdeja JG, Jakubowiak AJ, Agha ME, et al. CARTITUDE-1 final results: Phase 1b/2 study of ciltacabtagene autoleucel in heavily pretreated patients with relapsed/refractory multiple myeloma. *J Clin Oncol*. (2023) 41:8009–. doi: 10.1200/JCO.2023.41.16_suppl.8009

21. 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) 29:422–9. doi: 10.1038/s41591-022-02182-7

22. Dholaria B, Kocoglu MH, Kin A, Asch AS, Ramakrishnan A, Bachier C, et al. Early safety results of P-BCMA-ALLO1, a fully allogeneic chimeric antigen receptor T-cell (CAR-T), in patients with relapsed/refractory multiple myeloma (RRMM). *Blood*. (2023) 142:3479. doi: 10.1182/blood-2023-182430

23. Cowan AJ, Pont MJ, Sather BD, Turtle CJ, Till BG, Libby EN 3rd, et al. γ-Secretase inhibitor in combination with BCMA chimeric antigen receptor T-cell immunotherapy for individuals with relapsed or refractory multiple myeloma: a phase 1, first-in-human trial. *Lancet Oncol*. (2023) 24:811–22. doi: 10.1016/S1470-2045(23)00246-2

24. Frigault MJ, Bishop MR, Rosenblatt J, O'Donnell EK, Raje N, Cook D, et al. Phase 1 study of CART-ddBCMA for the treatment of subjects with relapsed and refractory multiple myeloma. *Blood Adv*. (2023) 7:768–77. doi: 10.1182/bloodadvances.2022007210

25. Zhao WH, Wang BY, Chen LJ, Fu WJ, Xu J, Liu J, et al. Four-year follow-up of LCAR-B38M in relapsed or refractory multiple myeloma: a phase 1, single-arm, open-label, multicenter study in China (LEGEND-2). *J Hematol Oncol*. (2022) 15:86. doi: 10.1186/s13045-022-01301-8

26. Asherie N, Kfir-Erenfeld S, Avni B, Assayag M, Dubnikov T, Zalcman N, et al. Development and manufacture of novel locally produced anti-BCMA CAR T cells for the treatment of relapsed/refractory multiple myeloma: results from a phase I clinical trial. *Haematologica*. (2023) 108:1827–39. doi: 10.3324/haematol.2022.281628

27. Qu X, An G, Sui W, Wang T, Zhang X, Yang J, et al. Phase 1 study of C-CAR088, a novel humanized anti-BCMA CAR T-cell therapy in relapsed/refractory multiple myeloma. *J Immunother Cancer*. (2022) 10. doi: 10.1136/jitc-2022-005145

28. Colonna L, Navarro G, Devries T, Beckett V, Amsberry A, Radhakrishnan A, et al. Orvacabtagene autoleucel (orva-cel; JCARH125): A fully human BCMA-targeted second-generation CAR T cell product characterized by a predominant central memory phenotype with high in vitro and *in vivo* proliferative potential and sustained *in vivo* persistence. *Blood*. (2020) 136:11–2. doi: 10.1182/blood-2020-136748

29. Sperling AS, Derman BA, Nikiforow S, Im S-Y, Ikegawa S, Prabhala RH, et al. Updated phase I study results of PHE885, a T-Charge manufactured BCMA-directed CAR-T cell therapy, for patients (pts) with r/r multiple myeloma (RRMM). *J Clin Oncol*. (2023) 41:8004–. doi: 10.1200/JCO.2023.41.16_suppl.8004

30. Fu C, Chen W, Cai Z, Yan L, Wang H, Shang J, et al. Three-year follow-up on efficacy and safety results from phase 1 lummicar study 1 of zevorcabtagene autoleucel in chinese patients with relapsed or refractory multiple myeloma. *Blood*. (2023) 142:4845. doi: 10.1182/blood-2023-184373

31. Oliver-Caldés A, González-Calle V, Cabañas V, Español-Rego M, Rodríguez-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) 24:913–24. doi: 10.1016/S1470-2045(23)00222-X

32. Costa LJ, Kumar SK, Atrash S, Liedtke M, Kaur G, Derman BA, et al. Results from the first phase 1 clinical study of the B-cell Maturation Antigen (BCMA) nex T Chimeric Antigen Receptor (CAR) T cell therapy CC-98633/BMS-986354 in patients (pts) with relapsed/refractory multiple myeloma (RRMM). *Blood*. (2022) 140:1360–2. doi: 10.1182/blood-2022-160038

33. Li C, Wang D, Fang B, Song Y, Huang H, Li J, et al. Updated results of fumanba-1: A phase 1b/2 study of a novel fully human B-cell maturation antigen-specific CAR T cells (CT103A) in patients with relapsed and/or refractory multiple myeloma. *Blood*. (2022) 140:7435–6. doi: 10.1182/blood-2022-166465

34. Brudno JN, Maric I, Hartman SD, Rose JJ, Wang M, Lam N, et al. T cells genetically modified to express an anti–B-cell maturation antigen chimeric antigen receptor cause remissions of poor-prognosis relapsed multiple myeloma. *J Clin Oncol*. (2018) 36:2267. doi: 10.1200/JCO.2018.77.8084

35. Cohen AD, Garfall AL, Stadtmauer EA, Melenhorst JJ, Lacey SF, Lancaster E, et al. B cell maturation antigen-specific CAR T cells are clinically active in multiple myeloma. *J Clin Invest*. (2019) 129:2210–21. doi: 10.1172/JCI126397

36. Xu Y, Zhang X, Xin D, Zhang J, Wang L, Fan Y, et al. CD27-armored BCMA-CAR T cell (CBG-002) therapy for relapsed and refractory multiple myeloma: A phase I clinical trial. *Blood*. (2023) 142:3468–. doi: 10.1182/blood-2023-174364

37. Perica K, Nataraj S, Sjojstrand ML, Nagel K, Pavkovic E, Payson E, et al. Low target antigen expression mediates resistance to BCMA CAR T cell therapy. *Blood*. (2023) 142:2124–. doi: 10.1182/blood-2023-178954

38. Samur MK, Fulciniti M, Aktas Samur A, Bazarbachi AH, Tai Y-T, Prabhala R, et al. Biallelic loss of BCMA as a resistance mechanism to CAR T cell therapy in a patient with multiple myeloma. *Nat Commun*. (2021) 12:868. doi: 10.1038/s41467-021-21177-5

39. Da Vià MC, Dietrich O, Truger M, Arampatzi P, Duell J, Heidemeier A, et al. Homozygous BCMA gene deletion in response to anti-BCMA CAR T cells in a patient with multiple myeloma. *Nat Med*. (2021) 27:616–9. doi: 10.1038/s41591-021-01245-5

40. Lee H, Ahn S, Maity R, Leblay N, Ziccheddu B, Truger M, et al. Mechanisms of antigen escape from BCMA- or GPRC5D-targeted immunotherapies in multiple myeloma. *Nat Med*. (2023) 29:2295–306. doi: 10.1038/s41591-023-02491-5

41. 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) 558:307–12. doi: 10.1038/s41586-018-0178-z

42. 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) 24:563–71. doi: 10.1038/s41591-018-0010-1

43. 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) 26:1878–87. doi: 10.1038/s41591-020-1061-7

44. Boulch M, Cazaux M, Loe-Mie Y, Thibaut R, Corre B, Lemaître F, et al. A cross-talk between CAR T cell subsets and the tumor microenvironment is essential for sustained cytotoxic activity. *Sci Immunol*. (2021) 6:eabd4344. doi: 10.1126/sciimmunol.abd4344

45. 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) 137:2621–33. doi: 10.1182/blood.2020007445

46. 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) 28:1860–71. doi: 10.1038/s41591-022-01960-7

47. 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) 28:1848–59. doi: 10.1038/s41591-022-01959-0

48. Scholler N, Perbost R, Locke FL, Jain MD, Turcan S, Danan C, et al. Tumor immune contexture is a determinant of anti-CD19 CAR T cell efficacy in large B cell lymphoma. *Nat Med*. (2022) 28:1872–82. doi: 10.1038/s41591-022-01916-x

49. 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) 602:503–9. doi: 10.1038/s41586-021-04390-6

50. 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 lymphoma. *Cancer Discovery*. (2022) 12:1886–903. doi: 10.1158/2159-8290.CD-21-1586

51. Rodriguez-Madoz JR, Jordana-Urriza L, Serrano G, Oliver-Caldes A, Calleja-Cervantes ME, San-Martin P, et al. Sequential scmultiomics of *in vivo* CAR-T cells allows characterization of transcriptional differences between patients, and identifies IL10 as a potential mechanism of resistance to CAR-T cells in MM. *Blood*. (2023) 142:3433. doi: 10.1182/blood-2023-182887

52. Merz M, Fischer L, Sia J, Born P, Weiss R, Grieb N, et al. Single cell multi-omic dissection of response and resistance to chimeric antigen receptor T cells against BCMA in relapsed multiple myeloma. *Blood*. (2023) 142:453. doi: 10.1182/blood-2023-187371

53. Freeman CLL, Zhao X, Meads MB, Noble J, Alugubelli RR, Renatino-Canevarolo R, et al. Single Cell RNA Sequencing of Sequential Samples before and after BCMA-Directed CAR-T Reveal Features Associated with Non-Durable Response, Exhausted T-Cells and Decreased Expression of Genes Encoding Key Surface Targets in Particular in Patients with Extramedullary Disease. *Blood*. (2023) 142:3304. doi: 10.1182/blood-2023-191013

54. Vieira dos Santos J, Melnekoff D, Aleman A, Bhalla S, Mouhieddine TH, Van Oekelen O, et al. Multimodal single-cell transcriptomic and proteomic correlatives of patients outcomes following anti-BCMA cellular therapy with ciltacabtagene autoleucel (Cilta-cel) in relapsed multiple myeloma. *Blood*. (2023) 142:93–. doi: 10.1182/blood-2023-186395

55. Ledergor G, Fan Z, Wu K, McCarthy E, Hyrenius-Wittsten A, Starzinski A, et al. CD4+ CAR-T cell exhaustion associated with early relapse of multiple myeloma after BCMA CAR-T cell therapy. *Blood Adv*. (2024). doi: 10.1182/bloodadvances.2023012416

56. Bianchi G, Munshi NC. Pathogenesis beyond the cancer clone(s) in multiple myeloma. *Blood*. (2015) 125:3049–58. doi: 10.1182/blood-2014-11-568881

57. Rasche L, Schinke C, Maura F, Bauer MA, Ashby C, Deshpande S, et al. The spatio-temporal evolution of multiple myeloma from baseline to relapse-refractory states. *Nat Commun*. (2022) 13:4517. doi: 10.1038/s41467-022-32145-y

58. Maura F, Bolli N, Angelopoulos N, Dawson KJ, Leongamornlert D, Martincorena I, et al. Genomic landscape and chronological reconstruction of driver events in multiple myeloma. *Nat Commun*. (2019) 10:3835. doi: 10.1038/s41467-019-11680-1

59. Nakamura K, Smyth MJ, Martinet L. Cancer immunoediting and immune dysregulation in multiple myeloma. *Blood*. (2020) 136:2731–40. doi: 10.1182/blood.2020006540

60. van de Donk N, Themeli M, Usmani SZ. Determinants of response and mechanisms of resistance of CAR T-cell therapy in multiple myeloma. *Blood Cancer Discovery*. (2021) 2:302–18. doi: 10.1158/2643-3230.BCD-20-0227

61. Karschnia P, Miller KC, Yee AJ, Rejeski K, Johnson PC, Raje N, et al. Neurologic toxicities following adoptive immunotherapy with BCMA-directed CAR T cells. *Blood*. (2023) 142:1243–8. doi: 10.1182/blood.2023020571

62. Van Oekelen O, Aleman A, Upadhyaya B, Schnakenberg S, Madduri D, Gavane S, et al. Neurocognitive and hypokinetic movement disorder with features of parkinsonism after BCMA-targeting CAR-T cell therapy. *Nat Med*. (2021) 27:2099–103. doi: 10.1038/s41591-021-01564-7

63. Holstein SA, Grant SJ, Wildes TM. Chimeric antigen receptor T-cell and bispecific antibody therapy in multiple myeloma: moving into the future. *J Clin Oncol*. (2023) 41:4416–29. doi: 10.1200/JCO.23.00512

64. Scheller L, Tebuka E, Rambau PF, Einsele H, Hudecek M, Prommersberger SR, et al. BCMA CAR-T cells in multiple myeloma-ready for take-off? *Leuk Lymphoma*. (2023) 65(2):143–57. doi: 10.1080/10428194.2023.2276676

65. Rodriguez-Otero P, San-Miguel JF. Cellular therapy for multiple myeloma: what's now and what's next. *Hematol Am Soc Hematol Educ Program*. (2022) 2022:180–9. doi: 10.1182/hematology.2022000396

66. van de Donk N, O'Neill C, de Ruijter MEM, Verkleij CPM, Zweegman S. T-cell redirecting bispecific and trispecific antibodies in multiple myeloma beyond BCMA. *Curr Opin Oncol*. (2023) 35:601–11. doi: 10.1097/CCO.0000000000000983

67. Atamaniuk J, Gleiss A, Porpaczy E, Kainz B, Grunt TW, Raderer M, et al. Overexpression of G protein-coupled receptor 5D in the bone marrow is associated with poor prognosis in patients with multiple myeloma. *Eur J Clin Invest*. (2012) 42:953–60. doi: 10.1111/j.1365-2362.2012.02679.x

68. Smith EL, Harrington K, Staehr M, Masakayan R, Jones J, Long TJ, et al. GPRC5D is a target for the immunotherapy of multiple myeloma with rationally designed CAR T cells. *Sci Transl Med*. (2019) 11. doi: 10.1126/scitranslmed.aau7746

69. Mailankody S, Devlin SM, Landa J, Nath K, Diamonte C, Carstens EJ, et al. GPRC5D-targeted CAR T cells for myeloma. *N Engl J Med*. (2022) 387:1196–206. doi: 10.1056/NEJMoa2209900

70. Hawrylycz MJ, Lein ES, Guillozet-Bongaarts AL, Shen EH, Ng L, Miller JA, et al. An anatomically comprehensive atlas of the adult human brain transcriptome. *Nature*. (2012) 489:391–9. doi: 10.1038/nature11405

71. Bal S, Htut M, Nadeem O, Anderson LD, Koçoğlu H, Gregory T, et al. BMS-986393 (CC-95266), a G protein-coupled receptor class C group 5 member D (GPRC5D)-targeted chimeric antigen receptor (CAR) T-cell therapy for relapsed/refractory multiple myeloma (RRMM): updated results from a phase 1 study. *Blood*. (2023) 142:219. doi: 10.1182/blood-2023-181857

72. Xia J, Li H, Yan Z, Zhou D, Wang Y, Qi Y, et al. Anti-G protein-coupled receptor, class C group 5 member D chimeric antigen receptor T cells in patients with relapsed or refractory multiple myeloma: A single-arm, phase II Trial. *J Clin Oncol*. (2023) 41:2583–93. doi: 10.1200/JCO.22.01824

73. Zhang M, Wei G, Zhou L, Zhou J, Chen S, Zhang W, et al. GPRC5D CAR T cells (OriCAR-017) in patients with relapsed or refractory multiple myeloma (POLARIS): a first-in-human, single-centre, single-arm, phase 1 trial. *Lancet Haematol*. (2023) 10: e107–e16. doi: 10.1016/S2352-3026(22)00372-6

74. Li S, Yuan Z, Liu L, Li Y, Luo L, Chen Y, et al. Safety and efficacy of GPRC5D CAR T cell therapy in relapsed/refractory multiple myeloma patients. *Blood*. (2023) 142:3472. doi: 10.1182/blood-2023-179147

75. Li C, Xu J, Luo W, Liao D, Xie W, Wei Q, et al. Bispecific CS1-BCMA CAR-T cells are clinically active in relapsed or refractory multiple myeloma. *Leukemia*. (2023) 38(1):149–59. doi: 10.1038/s41375-023-02065-x

76. Mei H, Li C, Jiang H, Zhao X, Huang Z, Jin D, et al. A bispecific CAR-T cell therapy targeting BCMA and CD38 in relapsed or refractory multiple myeloma. *J Hematol Oncol*. (2021) 14:161. doi: 10.1186/s13045-021-01170-7

77. Tang Y, Yin H, Zhao X, Jin D, Liang Y, Xiong T, et al. High efficacy and safety of CD38 and BCMA bispecific CAR-T in relapsed or refractory multiple myeloma. *J Exp Clin Cancer Res*. (2022) 41:2. doi: 10.1186/s13046-021-02214-z

78. Zhang H, Liu M, Xiao X, Lv H, Jiang Y, Li X, et al. A combination of humanized anti-BCMA and murine anti-CD38 CAR-T cell therapy in patients with relapsed or refractory multiple myeloma. *Leuk Lymphoma*. (2022) 63:1418–27. doi: 10.1080/10428194.2022.2030476

79. Bräuner-Osborne H, Jensen AA, Sheppard PO, Brodin B, Krogsgaard-Larsen P, O'Hara P. Cloning and characterization of a human orphan family C G-protein coupled receptor GPRC5D1GenBank accession Nos. for GPRC5C: AF207989, for Gprc5d: AF218809 and for GPRC5D: AF209923.1. *Biochim Biophys Acta (BBA) - Gene Structure Expression*. (2001) 1518:237–48. doi: 10.1016/S0167-4781(01)00197-X

80. Mi X, Penson A, Abdel-Wahab O, Mailankody S. Genetic basis of relapse after GPRC5D-targeted CAR T cells. *New Engl J Med*. (2023) 389:1435–7. doi: 10.1056/NEJMc2308544

81. Derrien J, Gastineau S, Frigout A, Giordano N, Cherkaoui M, Gaborit V, et al. Acquired resistance to a GPRC5D-directed T-cell engager in multiple myeloma is mediated by genetic or epigenetic target inactivation. *Nat Cancer*. (2023) 4:1536–43. doi: 10.1038/s43018-023-00625-9

82. Fernández de Larrea C, Staehr M, Lopez AV, Ng KY, Chen Y, Godfrey WD, et al. Defining an optimal dual-targeted CAR T-cell therapy approach simultaneously targeting BCMA and GPRC5D to prevent BCMA escape–driven relapse in multiple myeloma. *Blood Cancer Discovery*. (2020) 1:146–54. doi: 10.1158/2643-3230.BCD-20-0020

83. Zhou X, Yang Y, Hu X, Luo T, Liu S, Zhang C, et al. Identification of LPAR1/LPAR5 as novel GPCR partners of GPRC5D for the efficient CAR-T therapy of multiple myeloma. *Blood*. (2023) 142:4679. doi: 10.1182/blood-2023-186150

84. Kinder J, Estrada CA, Granger B, O'Rourke A, Liang O, Lampano A, et al. Development of a novel, allogeneic GPRC5D-directed CAR for treatment of multiple myeloma patients. *Blood*. (2023) 142:3290. doi: 10.1182/blood-2023-189665

85. Fu J, Jiang L, Zhu Z, Yan Y, Wu G, Wei M, et al. Efficacy of human iPSC-derived CAR-NK cells targeting multiple myeloma cells. *Blood*. (2023) 142:4802. doi: 10.1182/blood-2023-181613

86. Tai YT, Dillon M, Song W, Leiba M, Li XF, Burger P, et al. Anti-CS1 humanized monoclonal antibody HuLuc63 inhibits myeloma cell adhesion and induces antibody-dependent cellular cytotoxicity in the bone marrow milieu. *Blood*. (2008) 112:1329–37. doi: 10.1182/blood-2007-08-107292

87. Hsi ED, Steinle R, Balasa B, Szmania S, Draksharapu A, Shum BP, et al. CS1, a potential new therapeutic antibody target for the treatment of multiple myeloma. *Clin Cancer Res*. (2008) 14:2775–84. doi: 10.1158/1078-0432.CCR-07-4246

88. Lonial S, Dimopoulos M, Palumbo A, White D, Grosicki S, Spicka I, et al. Elotuzumab therapy for relapsed or refractory multiple myeloma. *N Engl J Med*. (2015) 373:621–31. doi: 10.1056/NEJMoa1505654

89. Chu J, He S, Deng Y, Zhang J, Peng Y, Hughes T, et al. Genetic modification of T cells redirected toward CS1 enhances eradication of myeloma cells. *Clin Cancer Res*. (2014) 20:3989–4000. doi: 10.1158/1078-0432.CCR-13-2510

90. Gogishvili T, Danhof S, Prommersberger S, Rydzek J, Schreder M, Brede C, et al. SLAMF7-CAR T cells eliminate myeloma and confer selective fratricide of SLAMF7+ normal lymphocytes. *Blood*. (2017) 130:2838–47. doi: 10.1182/blood-2017-04-778423

91. Amatya C, Pegues MA, Lam N, Vanasse D, Geldres C, Choi S, et al. Development of CAR T cells expressing a suicide gene plus a chimeric antigen receptor targeting signaling lymphocytic-activation molecule F7. *Mol Ther*. (2021) 29:702–17. doi: 10.1016/j.ymthe.2020.10.008

92. O'Neal J, Ritchey JK, Cooper ML, Niswonger J, Sofia González L, Street E, et al. CS1 CAR-T targeting the distal domain of CS1 (SLAMF7) shows efficacy in high tumor burden myeloma model despite fratricide of CD8+CS1 expressing CAR-T cells. *Leukemia*. (2022) 36:1625–34. doi: 10.1038/s41375-022-01559-4

93. Golubovskaya V, Zhou H, Li F, Berahovich R, Sun J, Valentine M, et al. Novel CS1 CAR-T cells and bispecific CS1-BCMA CAR-T cells effectively target multiple myeloma. *Biomedicines*. (2021) 9:1422. doi: 10.3390/biomedicines9101422

94. Zah E, Nam E, Bhuvan V, Tran U, Ji BY, Gosliner SB, et al. Systematically optimized BCMA/CS1 bispecific CAR-T cells robustly control heterogeneous multiple myeloma. *Nat Commun*. (2020) 11:2283. doi: 10.1038/s41467-020-16160-5

95. Chen KH, Wada M, Pinz KG, Liu H, Shuai X, Chen X, et al. A compound chimeric antigen receptor strategy for targeting multiple myeloma. *Leukemia*. (2018) 32:402–12. doi: 10.1038/leu.2017.302

96. Korst CLBM, Bruins WSC, Cosovic M, Verkleij CPM, Twickler I, Le Clerre D, et al. Preclinical activity of allogeneic CS1-specific CAR T-cells (UCARTCS1) in multiple myeloma. *Blood*. (2022) 140:4215–6. doi: 10.1182/blood-2022-157950

97. Lin P, Owens R, Tricot G, Wilson CS. Flow cytometric immunophenotypic analysis of 306 cases of multiple myeloma. *Am J Clin Pathol*. (2004) 121:482–8. doi: 10.1309/74R4TB90BUWH27JX

98. Deaglio S, Vaisitti T, Billington R, Bergui L, Omede' P, Genazzani AA, et al. CD38/CD19: a lipid raft–dependent signaling complex in human B cells. *Blood*. (2007) 109:5390–8. doi: 10.1182/blood-2006-12-061812

99. Lokhorst HM, Plesner T, Laubach JP, Nahi H, Gimsing P, Hansson M, et al. Targeting CD38 with daratumumab monotherapy in multiple myeloma. *New Engl J Med*. (2015) 373:1207–19. doi: 10.1056/NEJMoa1506348

100. Sonneveld P, Dimopoulos MA, Boccadoro M, Quach H, Ho PJ, Beksac M, et al. Daratumumab, bortezomib, lenalidomide, and dexamethasone for multiple myeloma. *New Engl J Med*. (2023) 390(4):301–313. doi: 10.1056/NEJMoa2312054

101. Krejcik J, Casneuf T, Nijhof IS, Verbist B, Bald J, Plesner T, et al. Daratumumab depletes CD38+ immune regulatory cells, promotes T-cell expansion, and skews T-cell repertoire in multiple myeloma. *Blood*. (2016) 128:384–94. doi: 10.1182/blood-2015-12-687749

102. Casneuf T, Xu XS, Adams HC 3rd, Axel AE, Chiu C, Khan I, et al. Effects of daratumumab on natural killer cells and impact on clinical outcomes in relapsed or refractory multiple myeloma. *Blood Adv*. (2017) 1:2105–14. doi: 10.1182/bloodadvances.2017006866

103. Mihara K, Yanagihara K, Takigahira M, Imai C, Kitanaka A, Takihara Y, et al. Activated T–cell-mediated immunotherapy with a chimeric receptor against CD38 in B-cell non-hodgkin lymphoma. *J Immunotherapy*. (2009) 32:737–43. doi: 10.1097/CJI.0b013e3181adaff1

104. Mihara K, Bhattacharyya J, Kitanaka A, Yanagihara K, Kubo T, Takei Y, et al. T-cell immunotherapy with a chimeric receptor against CD38 is effective in eliminating myeloma cells. *Leukemia*. (2012) 26:365–7. doi: 10.1038/leu.2011.205

105. Drent E, Groen RW, Noort WA, Themeli M, Lammerts van Bueren JJ, Parren PW, et al. Pre-clinical evaluation of CD38 chimeric antigen receptor engineered T cells for the treatment of multiple myeloma. *Haematologica*. (2016) 101:616–25. doi: 10.3324/haematol.2015.137620

106. An N, Hou YN, Zhang QX, Li T, Zhang QL, Fang C, et al. Anti-multiple myeloma activity of nanobody-based anti-CD38 chimeric antigen receptor T cells. *Mol Pharm*. (2018) 15:4577–88. doi: 10.1021/acs.molpharmaceut.8b00584

107. Glisovic-Aplenc T, Diorio C, Chukinas JA, Veliz K, Shestova O, Shen F, et al. CD38 as a pan-hematologic target for chimeric antigen receptor T cells. *Blood Adv*. (2023) 7:4418–30. doi: 10.1182/bloodadvances.2022007059

108. Partida-Sánchez S, Cockayne DA, Monard S, Jacobson EL, Oppenheimer N, Garvy B, et al. Cyclic ADP-ribose production by CD38 regulates intracellular calcium release, extracellular calcium influx and chemotaxis in neutrophils and is required for bacterial clearance in vivo. *Nat Med*. (2001) 7:1209–16. doi: 10.1038/nm1101-1209

109. Troy EC, Sezgin Y, Pereira MDS, Caporale J, Behbehani GK, Lyberger J, et al. CD38 KO/CD38-CAR human primary natural killer cells enhance cytotoxicity against CD38-expressing primary lymphoma, leukemia, and myeloma. *Blood*. (2023) 142:3443. doi: 10.1182/blood-2023-174742

110. Karvouni M, Vidal-Manrique M, Susek KH, Hussain A, Gilljam M, Zhang Y, et al. Challenges in αCD38-chimeric antigen receptor (CAR)-expressing natural killer (NK) cell-based immunotherapy in multiple myeloma: Harnessing the CD38dim phenotype of cytokine-stimulated NK cells as a strategy to prevent fratricide. *Cytotherapy*. (2023) 25:763–72. doi: 10.1016/j.jcyt.2023.03.006

111. Edri A, Ben-Haim N, Hailu A, Brycman N, Berhani-Zipori O, Rifman J, et al. Nicotinamide-expanded allogeneic natural killer cells with CD38 deletion, expressing an enhanced CD38 chimeric antigen receptor, target multiple myeloma cells. *Int J Mol Sci*. (2023) 24. doi: 10.3390/ijms242417231

112. McCarron MJ, Park PW, Fooksman DR. CD138 mediates selection of mature plasma cells by regulating their survival. *Blood*. (2017) 129:2749–59. doi: 10.1182/blood-2017-01-761643

113. Palaiologou M, Delladetsima I, Tiniakos D. CD138 (syndecan-1) expression in health and disease. *Histol Histopathol*. (2014) 29(2):177–89. doi: 10.14670/HH-29.177

114. Sun C, Mahendravada A, Ballard B, Kale B, Ramos C, West J, et al. Safety and efficacy of targeting CD138 with a chimeric antigen receptor for the treatment of multiple myeloma. *Oncotarget*. (2019) 10:2369–83. doi: 10.18632/oncotarget.v10i24

115. Guo B, Chen M, Han Q, Hui F, Dai H, Zhang W, et al. CD138-directed adoptive immunotherapy of chimeric antigen receptor (CAR)-modified T cells for multiple myeloma. *J Cell Immunotherapy*. (2016) 2:28–35. doi: 10.1016/j.jocit.2014.11.001

116. van der Schans JJ, Wang Z, van Arkel J, van Schaik T, Katsarou A, Ruiter R, et al. Specific Targeting of Multiple Myeloma by Dual Split-signaling Chimeric Antigen Receptor T cells Directed against CD38 and CD138. *Clin Cancer Res*. (2023) 29:4219–29. doi: 10.1158/1078-0432.CCR-23-0132

117. Li J, Stagg NJ, Johnston J, Harris MJ, Menzies SA, DiCara D, et al. Membrane-proximal epitope facilitates efficient T cell synapse formation by anti-fcRH5/CD3 and is a requirement for myeloma cell killing. *Cancer Cell*. (2017) 31:383–95. doi: 10.1016/j.ccell.2017.02.001

118. Schmidt TM, Fonseca R, Usmani SZ. Chromosome 1q21 abnormalities in multiple myeloma. *Blood Cancer J*. (2021) 11:83. doi: 10.1038/s41408-021-00474-8

119. Jiang D, Huang H, Qin H, Tang K, Shi X, Zhu T, et al. Chimeric antigen receptor T cells targeting FcRH5 provide robust tumour-specific responses in murine xenograft models of multiple myeloma. *Nat Commun*. (2023) 14:3642. doi: 10.1038/s41467-023-39395-4

120. Trudel S, Cohen AD, Krishnan AY, Fonseca R, Spencer A, Berdeja JG, et al. Cevostamab monotherapy continues to show clinically meaningful activity and manageable safety in patients with heavily pre-treated relapsed/refractory multiple myeloma (RRMM): updated results from an ongoing phase I study. *Blood*. (2021) 138:157. doi: 10.1182/blood-2021-147983

121. Atanackovic D, Panse J, Hildebrandt Y, Jadczak A, Kobold S, Cao Y, et al. Surface molecule CD229 as a novel target for the diagnosis and treatment of multiple myeloma. *Haematologica*. (2011) 96:1512–20. doi: 10.3324/haematol.2010.036814

122. Radhakrishnan SV, Luetkens T, Scherer SD, Davis P, Vander Mause ER, Olson ML, et al. CD229 CAR T cells eliminate multiple myeloma and tumor propagating cells without fratricide. *Nat Commun*. (2020) 11:798. doi: 10.1038/s41467-020-14619-z

123. Vander Mause ER, Baker JM, Dietze KA, Radhakrishnan SV, Iraguha T, Omili D, et al. Systematic single amino acid affinity tuning of CD229 CAR T cells retains efficacy against multiple myeloma and eliminates on-target off-tumor toxicity. *Sci Transl Med*. (2023) 15:eadd7900. doi: 10.1126/scitranslmed.add7900

124. Novak AJ, Darce JR, Arendt BK, Harder B, Henderson K, Kindsvogel W, et al. Expression of BCMA, TACI, and BAFF-R in multiple myeloma: a mechanism for growth and survival. *Blood*. (2004) 103:689–94. doi: 10.1182/blood-2003-06-2043

125. Schmidts A, Ormhøj M, Choi BD, Taylor AO, Bouffard AA, Scarfò I, et al. Rational design of a trimeric APRIL-based CAR-binding domain enables efficient targeting of multiple myeloma. *Blood Adv*. (2019) 3:3248–60. doi: 10.1182/bloodadvances.2019000703

126. Lee L, Draper B, Chaplin N, Philip B, Chin M, Galas-Filipowicz D, et al. An APRIL-based chimeric antigen receptor for dual targeting of BCMA and TACI in multiple myeloma. *Blood*. (2018) 131:746–58. doi: 10.1182/blood-2017-05-781351

127. Lee L, Lim WC, Galas-Filipowicz D, Fung K, Taylor J, Patel D, et al. Limited efficacy of APRIL CAR in patients with multiple myeloma indicate challenges in the use of natural ligands for CAR T-cell therapy. *J ImmunoTherapy Cancer*. (2023) 11:e006699. doi: 10.1136/jitc-2023-006699

128. Larson RC, Kann MC, Graham C, Mount CW, Castano AP, Lee W-H, et al. Anti-TACI single and dual-targeting CAR T cells overcome BCMA antigen loss in multiple myeloma. *Nat Commun*. (2023) 14:7509. doi: 10.1038/s41467-023-43416-7

129. Ramos CA, Savoldo B, Torrano V, Ballard B, Zhang H, Dakhova O, et al. Clinical responses with T lymphocytes targeting Malignancy-associated κ light chains. *J Clin Invest*. (2016) 126:2588–96. doi: 10.1172/JCI86000

130. Baumeister SH, Murad J, Werner L, Daley H, Trebeden-Negre H, Gicobi JK, et al. Phase I trial of autologous CAR T cells targeting NKG2D ligands in patients with AML/MDS and multiple myeloma. *Cancer Immunol Res*. (2019) 7:100–12. doi: 10.1158/2326-6066.CIR-18-0307

131. Sallman DA, Kerre T, Havelange V, Poiré X, Lewalle P, Wang ES, et al. CYAD-01, an autologous NKG2D-based CAR T-cell therapy, in relapsed or refractory acute myeloid leukaemia and myelodysplastic syndromes or multiple myeloma (THINK): haematological cohorts of the dose escalation segment of a phase 1 trial. *Lancet Haematol*. (2023) 10:e191–202. doi: 10.1016/S2352-3026(22)00378-7

132. Matsui W, Wang Q, Barber JP, Brennan S, Smith BD, Borrello I, et al. Clonogenic multiple myeloma progenitors, stem cell properties, and drug resistance. *Cancer Res*. (2008) 68:190–7. doi: 10.1158/0008-5472.CAN-07-3096

133. Hajek R, Okubote SA, Svachova H. Myeloma stem cell concepts, heterogeneity and plasticity of multiple myeloma. *Br J Haematol*. (2013) 163:551–64. doi: 10.1111/bjh.12563

134. Garfall AL, Stadtmauer EA, Hwang WT, Lacey SF, Melenhorst JJ, Krevvata M, et al. Anti-CD19 CAR T cells with high-dose melphalan and autologous stem cell transplantation for refractory multiple myeloma. *JCI Insight*. (2018) 3. doi: 10.1172/jci.insight.120505

135. Stadtmauer EA, Faitg TH, Lowther DE, Badros AZ, Chagin K, Dengel K, et al. Long-term safety and activity of NY-ESO-1 SPEAR T cells after autologous stem cell transplant for myeloma. *Blood Adv*. (2019) 3:2022–34. doi: 10.1182/bloodadvances.2019000194

136. Rapoport AP, Stadtmauer EA, Binder-Scholl GK, Goloubeva O, Vogl DT, Lacey SF, et al. NY-ESO-1-specific TCR-engineered T cells mediate sustained antigen-specific antitumor effects in myeloma. *Nat Med*. (2015) 21:914–21. doi: 10.1038/nm.3910

137. Hosen N, Matsunaga Y, Hasegawa K, Matsuno H, Nakamura Y, Makita M, et al. The activated conformation of integrin β7 is a novel multiple myeloma–specific target for CAR T cell therapy. *Nat Med*. (2017) 23:1436–43. doi: 10.1038/nm.4431

138. Casucci M, Nicolis di Robilant B, Falcone L, Camisa B, Norelli M, Genovese P, et al. CD44v6-targeted T cells mediate potent antitumor effects against acute myeloid leukemia and multiple myeloma. *Blood*. (2013) 122:3461–72. doi: 10.1182/blood-2013-04-493361

139. Benjamin R, Condomines M, Gunset G, Sadelain M. CD56 targeted chimeric antigen receptors for immunotherapy of multiple myeloma. *Cancer Res*. (2012) 72:3499–. doi: 10.1158/1538-7445.AM2012-3499

140. Shaffer DR, Savoldo B, Yi Z, Chow KK, Kakarla S, Spencer DM, et al. T cells redirected against CD70 for the immunotherapy of CD70-positive Malignancies. *Blood*. (2011) 117:4304–14. doi: 10.1182/blood-2010-04-278218

141. Peinert S, Prince HM, Guru PM, Kershaw MH, Smyth MJ, Trapani JA, et al. Gene-modified T cells as immunotherapy for multiple myeloma and acute myeloid leukemia expressing the Lewis Y antigen. *Gene Ther*. (2010) 17:678–86. doi: 10.1038/gt.2010.21

142. Di Meo F, John S, Zhang C, Freeman CLL, Perna F. Chimeric antigen receptor T cells targeting LILRB4, an immunoreceptor mediating T-cell suppression, are potently effective in multiple myeloma. *Blood*. (2023) 142:4804. doi: 10.1182/blood-2023-182022

143. Banerjee R, Lee SS, Cowan AJ. Innovation in BCMA CAR-T therapy: Building beyond the Model T. *Front Oncol*. (2022) 12:1070353. doi: 10.3389/fonc.2022.1070353

144. Anderson LD Jr., Dhakal B, Jain T, Oluwole OO, Shah GL, Sidana S, et al. Chimeric antigen receptor T cell therapy for myeloma: where are we now and what is needed to move chimeric antigen receptor T cells forward to earlier lines of therapy? Expert panel opinion from the american society for transplantation and cellular therapy. *Transplant Cell Ther*. (2023) 30(1):17–37. doi: 10.1016/j.jtct.2023.10.022

145. Dekhtiarenko I, Lelios I, Jacob W, Schneider M, Weisser M, Carlo-Stella C, et al. Co-expression of GPRC5D, fcRH5 and BCMA suggests that targeting more than one cell surface marker may be a viable strategy in relapsed/refractory multiple myeloma (RRMM): biomarker results from the phase I study of forimtamig, a GPRC5DxCD3 bispecific antibody. *Blood*. (2023) 142:1948. doi: 10.1182/blood-2023-177669

146. Majzner RG, Rietberg SP, Sotillo E, Dong R, Vachharajani VT, Labanieh L, et al. Tuning the antigen density requirement for CAR T-cell activity. *Cancer Discovery*. (2020) 10:702–23. doi: 10.1158/2159-8290.CD-19-0945

147. Munawar U, Eiring P, Vogt C, Haertle L, Han S, Nerreter S, et al. Secondary genetic events impact the expression of key immunotargets on the surface of multiple myeloma cells. *Blood*. (2023) 142:451. doi: 10.1182/blood-2023-180638

148. Coffey D, Landgren O, Cowan AJ, Ataca Atilla P, Simon S, Pont M, et al. Impact of gamma-secretase inhibition on the multiple myeloma immune microenvironment. *Blood*. (2023) 142:4683. doi: 10.1182/blood-2023-186980

149. Friedrich MJ, Neri P, Kehl N, Michel J, Steiger S, Kilian M, et al. The pre-existing T cell landscape determines the response to bispecific T cell engagers in multiple myeloma patients. *Cancer Cell*. (2023) 41:711–25.e6. doi: 10.1016/j.ccell.2023.02.008

150. Cohen AD, Hwang W-T, Susanibar-Adaniya S, Vogl DT, Garfall AL, Waxman A, et al. Sequential T-cell engagement for myeloma ("STEM") trial: A phase 2 study of cevostamab consolidation following BCMA CAR T cell therapy. *Blood*. (2023) 142:3389. doi: 10.1182/blood-2023-187409

151. Larson RC, Maus MV. Recent advances and discoveries in the mechanisms and functions of CAR T cells. *Nat Rev Cancer*. (2021) 21:145–61. doi: 10.1038/s41568-020-00323-z

152. Allen GM, Lim WA. Rethinking cancer targeting strategies in the era of smart cell therapeutics. *Nat Rev Cancer*. (2022) 22:693–702. doi: 10.1038/s41568-022-00505-x

153. Dannenfelser R, Allen GM, VanderSluis B, Koegel AK, Levinson S, Stark SR, et al. Discriminatory power of combinatorial antigen recognition in cancer T cell therapies. *Cell Syst*. (2020) 11:215–28.e5. doi: 10.1016/j.cels.2020.08.002

154. Daher M, Rezvani K. Outlook for new CAR-based therapies with a focus on CAR NK cells: what lies beyond CAR-engineered T cells in the race against cancer. *Cancer Discovery*. (2021) 11:45–58. doi: 10.1158/2159-8290.CD-20-0556

155. O'Neal J, Cooper ML, Ritchey JK, Gladney S, Niswonger J, González LS, et al. Anti-myeloma efficacy of CAR-iNKT is enhanced with a long-acting IL-7, rhIL-7-hyFc. *Blood Adv*. (2023) 7:6009–22. doi: 10.1182/bloodadvances.2023010032

156. Lin P, Reyes Silva FC, Lin P, Gilbert AL, Acharya S, Nunez Cortes AK, et al. CD70 CAR NK cells in the treatment of multiple myeloma. *Blood*. (2023) 142:3463. doi: 10.1182/blood-2023-190612

157. Klichinsky M, Ruella M, Shestova O, Lu XM, Best A, Zeeman M, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat Biotechnol*. (2020) 38:947–53. doi: 10.1038/s41587-020-0462-y

frontiers | Frontiers in Oncology

TYPE Mini Review
PUBLISHED 21 May 2024
DOI 10.3389/fonc.2024.1396490

OPEN ACCESS

EDITED BY
Alice Di Rocco,
Sapienza University of Rome, Italy

REVIEWED BY
Jacopo Mariotti,
Humanitas Research Hospital, Italy

*CORRESPONDENCE
Manisha Bhutani
manisha.bhutani@atriumhealth.org

RECEIVED 05 March 2024
ACCEPTED 07 May 2024
PUBLISHED 21 May 2024

CITATION
Ferreri CJ and Bhutani M (2024) Mechanisms and management of CAR T toxicity.
Front. Oncol. 14:1396490.
doi: 10.3389/fonc.2024.1396490

Mechanisms and management of CAR T toxicity

Christopher J. Ferreri and Manisha Bhutani*

Department of Hematologic Oncology and Blood Disorders, Levine Cancer Institute, Atrium Health Wake Forest University School of Medicine, Charlotte, NC, United States

Chimeric antigen receptor (CAR) T cell therapies have dramatically improved treatment outcomes for patients with relapsed or refractory B-cell acute lymphoblastic leukemia, large B-cell lymphoma, follicular lymphoma, mantle cell lymphoma, and multiple myeloma. Despite unprecedented efficacy, treatment with CAR T cell therapies can cause a multitude of adverse effects which require monitoring and management at specialized centers and contribute to morbidity and non-relapse mortality. Such toxicities include cytokine release syndrome, immune effector cell-associated neurotoxicity syndrome, neurotoxicity distinct from ICANS, immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome, and immune effector cell-associated hematotoxicity that can lead to prolonged cytopenias and infectious complications. This review will discuss the current understanding of the underlying pathophysiologic mechanisms and provide guidelines for the grading and management of such toxicities.

KEYWORDS

chimeric antigen receptor (CAR T), cytokine release syndrome (CRS), ICANS - immune effector cell-associated neurotoxicity syndrome, HLH - hemophagocytic lymphohistiocytosis, tocilizumab (IL-6 inhibitor), large B cell lymphoma, multiple myeloma

Introduction

Chimeric antigen receptors (CAR) are engineered recombinant receptors consisting of an extracellular target antigen-binding domain, hinge region, transmembrane domain, and intracellular signaling domain that can be transduced into immune effector cells such as T cells. This allows the engineered T cells to bind the target antigen in a major histocompatibility complex (MHC)-independent fashion, followed by robust activation and expansion of the CAR T cell population which promotes tumor elimination. (1) The anti-CD19 CAR T cell therapies tisagenlecleucel (tisa-cel) and axicabtagene autoleucel (axi-cel) were the first to receive regulatory approval in 2017 after demonstrating unprecedented efficacy for the treatment of relapsed/refractory pediatric B-cell acute lymphoblastic leukemia (B-ALL) and large B-cell lymphoma (LBCL). (2–4) Subsequently, lisocabtagene maraleucel (liso-cel) received approval for relapsed/refractory LBCL, followed by both liso-cel and axi-cel becoming indicated for second-

line use in LBCL based on positive phase 3 trial data. (5–7) Anti-CD19 CAR-T has additionally received regulatory approval for relapsed/refractory follicular lymphoma, adult B-ALL, and relapsed/refractory mantle cell lymphoma. (8–10) Idecabtagene vicleucel (ide-cel) and ciltacabtagene autoleucel (cilta-cel) are both B-cell maturation antigen (BCMA)-targeted CAR T cell therapies approved for the treatment of relapsed/refractory multiple myeloma after four or more prior lines of therapy (11, 12).

Despite remarkable efficacy noted with these therapies, supraphysiologic T cell activation, expansion, and systemic hyperinflammatory response mediated by cytokine production can result in potentially severe and life-threatening complications. Prototypical toxicities include cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). Beyond CRS and ICANS, the systemic inflammatory response triggered by CAR-T can culminate in less frequent but potentially fatal immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS). Cytopenias after CAR T cell therapy, recently termed immune effector cell-associated hematotoxicity (ICAHT), can lead to significant transfusion dependence and predispose patients to bleeding and infectious sequelae. Neurologic complications distinct from ICANS such as delayed movement and neurocognitive treatment–emergent adverse events (MNTs) are also associated with cellular therapy. (13) Post-infusion monitoring for these toxicities at specialized centers and the interventions required for toxicity management contribute significantly to the total cost of care. (14, 15) With the expected expanded indications for CAR T cell therapy in hematologic malignancies, as well as potential applications in solid tumors and autoimmune disease, optimizing prevention and treatment of the associated toxicities will become increasingly important to establish frameworks for institutions and health care providers to ultimately improve patient outcomes (16, 17).

Cytokine release syndrome (CRS): clinical manifestations and mechanisms

The American Society for Transplantation and Cellular Therapy (ASTCT) consensus grading criteria defines cytokine release syndrome as "a supraphysiologic response following any immune therapy that results in the activation or engagement of endogenous or infused T cells and/or other immune effector cells. Symptoms can be progressive, must include fever at the onset, and may include hypotension, capillary leak (hypoxia) and end organ dysfunction." (18)While fever is the first symptom of CRS and typically occurs within the first 14 days after infusion, clinical manifestations can range across a spectrum of severity. In less severe forms, self-limited cases may consist of fever, myalgias, fatigue, and headache. With escalating severity, CRS can lead to increased vascular permeability and capillary leak causing hypotension, pulmonary edema, or acute lung injury, requiring ICU level care. (13, 19–21) Sustained inflammation with CRS has been associated with cardiovascular toxicities of elevated serum troponin reflective of myocardial injury, decreased left ventricular ejection fraction, and cardiac arrhythmias. (22) Electrolyte derangements, liver function test abnormalities, and renal insufficiency potentially requiring temporary hemodialysis support have also been associated with CRS. (13) Table 1 includes a summary of the incidence rate and median time to onset of CRS observed in the respective registrational clinical trial for each of the commercially available anti-CD19 and anti-BCMA CAR T cell therapies.

Inflammatory markers and cytokines that have been implicated in CRS include tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-ɣ), interleukin-1 (IL-1), IL-2, soluble IL2Rα, IL-4, IL-6,

TABLE 1 Incidence of immune effector cell-associated toxicities seen on registrational trials for approved CAR T cell therapies.

CAR T	Indication	CRS	Neurotoxicity	Cytopenias	Infection
Tisa-cel NCT02435849 (2)	R/R B-ALL for age < 25	77% 47% G ≥ 3 Median onset 3 days	40% 13% G ≥ 3	24% with cytopenia G ≥ 3 not resolved by day 28	43% 24% G ≥ 3
Tisa-cel JULIET (3)	R/R LBCL ≥ 2 prior LOT	58% 22% G ≥ 3 Median onset 3 days	21% 12% G ≥ 3	32% with cytopenia G ≥ 3 not resolved by day 28	34% 20% G ≥ 3
Axi-cel ZUMA-1 (4)	R/R LBCL ≥ 2 prior LOT	93% 13% G ≥ 3 Median onset 2 days	64% 28% G ≥ 3	78% G ≥ 3 neutropenia 38% G ≥ 3 TCP 43% G ≥ 3 anemia	Not reported

(Continued)

TABLE 1 Continued

CAR T	Indication	CRS	Neurotoxicity	Cytopenias	Infection
Axi-cel ZUMA-7 (7)	Primary refractory LBCL or relapse within 12 months of 1st line therapy	92% 6% G ≥ 3 Median onset 3 days	60% 21% G ≥ 3	29% with G ≥ 3 cytopenia not resolved by day 30	41% 14% G ≥ 3
Axi-cel ZUMA-5 (8)	R/R follicular lymphoma	78% 6% G ≥ 3 Median onset 4 days	56% 15% G ≥ 3	33% with G ≥ 3 cytopenia not resolved by day 30	18% G ≥ 3
Liso-cel TRANSCEND NHL 001 (5)	R/R LBCL ≥ 2 prior LOT	42% 2% G ≥ 3 Median onset 5 days	30% 10% G ≥ 3	37% with G ≥ 3 cytopenia not resolved by day 28	12% G ≥ 3
Liso-cel TRANSFORM (6)	Primary refractory LBCL, relapse within 12 months of 1st line therapy, relapse and not eligible for HSCT	49% 1% G ≥ 3 Median onset 5 days	11% 4% G ≥ 3	43% with G ≥ 3 cytopenia not resolved by day 35	15% G ≥ 3
Brexu-cel ZUMA-3 (10)	Adult B-ALL	89% 24% G ≥ 3 Median onset 5 days	60% 26% G ≥ 3	36% with G ≥ 3 cytopenia not resolved by day 30	25% G ≥ 3
Brexu-cel ZUMA-2 (9)	R/R mantle cell lymphoma	91% 15% G ≥ 3 Median onset 2 days	63% 31% G ≥ 3	26% with G ≥ 3 cytopenia not resolved by day 90	32% G ≥ 3
Ide-cel KarMMa (11)	RRMM with ≥ 4 prior LOT	84% 5% G ≥ 3 Median onset 1 day	18% 3% G ≥ 3	52% with G ≥ 3 neutropenia and 62% with G ≥ 3 TCP not resolved by day 28	22% G ≥ 3
Cilta-cel CARTITUDE-1 (12)	RRMM with ≥ 4 prior LOT	95% 5% G ≥ 3 Median onset 7 days	17% ICANS 2% G ≥ 3 12% other neurotoxicities, 9% G ≥ 3	30% with G ≥ 3 neutropenia and 41% with G ≥ 3 TCP not resolved by day 30	20% G ≥ 3

CRS, cytokine release syndrome; R/R, relapsed/refractory; B-ALL, B-cell acute lymphoblastic leukemia; G, grade; LBCL, large B-cell lymphoma; LOT, lines of therapy; IEC-HS), immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome; TCP, thrombocytopenia; HSCT, hematopoietic stem cell transplantation; RRMM, relapsed/refractory multiple myeloma.

IL-8, IL-10, ferritin, C-reactive protein (CRP), granulocyte/macrophage colony stimulating factor (GM-CSF), macrophage inflammatory protein-1α (MIP-1α), monocyte chemoattractant protein-1 (MCP-1), granzyme B, and soluble gp130. (23) The term "cytokine release syndrome" was first used in the early 1990s after systemic reactions associated with elevations in TNF-α and IFN-ɣ were noted after immunosuppressive treatment with the anti-T-cell antibody muromonab-CD3 (OKT3), which was tempered with glucocorticoids. Subsequently, CRS has been associated with various immunotherapies including monoclonal antibodies, antibody-drug conjugates, immune checkpoint inhibitors, bispecific T cell redirecting antibodies, and CAR T cell therapies. (24) Similar elevations in serum cytokines were observed in the first clinical studies of CAR T cell therapies for chronic lymphocytic leukemia (CLL) and B-ALL (19, 25, 26).

Activation of the vascular endothelium has also been implicated in the pathophysiology of CRS. RNA *in-situ* hybridization studies from a patient who died due to CRS after treatment with anti-CD19 CAR T cell therapy noted that both interstitial cells and vascular endothelium lining cells expressed IL-6, which was markedly elevated. (27) Patients with grade ≥2 CRS after anti CD19 CAR T infusion had increased prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen, D-dimer, factor VIII, von Willebrand factor (vWF), and decreased platelet count and antithrombin levels compared to those with lower grade or no CRS, suggesting that endothelial activation was predictive for

greater CRS severity and development of disseminated intravascular coagulopathy (DIC). (28) A multivariable analysis of 133 adult patients treated with anti-CD19 CAR T noted that biomarkers of endothelial activation including angiopoietin-2 and vWF were increased during severe CRS, and also relatively increased prior to lymphodepletion chemotherapy in patients who subsequently developed CRS. Severe thrombocytopenia prior to lymphodepletion was predictive for development of CRS, and it was postulated that these patients were more susceptible to endothelial activation due to platelets being a primary source of the endothelial stabilizing cytokine angiopoietin-1 (29).

While retrospective analysis of serum cytokines in relation to CRS severity have been informative in understanding the underlying pathophysiology, the development of animal models of cytokine release syndrome has also been critical to further elucidating these mechanisms. Experiments from a murine model of B-ALL treated with anti-CD19 CAR T demonstrated that CAR T cells produced negligible levels of IL-6, but instead bystander proinflammatory monocytes and macrophages were responsible for IL-1 and IL-6 production. It was noted that IL-1 secretion preceded IL-6 production by several hours, and treatment with the IL-1 receptor inhibitor anakinra was equally effective as the IL-6 receptor inhibitor tocilizumab in treating CRS in this model. (30) Another murine model of CRS developing after anti-CD19 CAR T cell therapy for B-ALL also demonstrated that CRS severity was mediated by IL-6, IL-1, and nitric oxide production from recipient macrophages rather than directly from the CAR T cells themselves (31).

Though IL-1 and IL-6 secretion primarily occurs from bystander monocytes and macrophages in CRS, upstream IFN-ɣ secretion occurs from activated CAR T cells and hence IFN-ɣ production is used as a potency assay for CAR T cell effector function. IFN-ɣ activates innate immune cells such as macrophages to upregulate antigen-presentation pathways, which also leads to immune checkpoint expression. *In vitro* experiments utilizing xenograft models of hematologic malignancy and serum samples obtained from patients who developed clinically significant CRS demonstrated that IFN-ɣ inhibition and deletion resulted in decreased macrophage activation and proinflammatory cytokine production, as well as reduced immune checkpoint expression. Compared to inhibition of IL-1 and IL-6, IFN-ɣ blockade dampened downstream proinflammatory cytokine production to a greater extent. (32) In a simplified humanized murine model of CRS/neurotoxicity after treatment with anti-CD19 CAR T, treatment with the IFN-ɣ inhibitor emapalumab led to decreased inflammatory signaling and toxicity mitigation without impacting CAR T efficacy. (33) However, studies from a fully immunocompetent mouse model demonstrated that treatment with IFN-ɣ knockout CAR T cells resulted in similar levels of proinflammatory cytokine elevation and occurrence of clinical CRS in the treated mice compared to those treated with the wild-type CAR T. While possible that IFN-ɣ signaling plays a more significant role in humans compared to mice, these results suggest that *in vivo* IFN-ɣ signaling may be redundant or not directly causal for CRS development (34).

Lastly, an *in vitro* model of the macrophage-endothelial interface suggested that endothelial inflammation occurs after CAR T cell therapy independent of signaling from macrophages, which in turn amplifies macrophage-mediated inflammatory signaling. The transcription factor STAT3 was implicated in hyperinflammatory signaling driven by the activated endothelium, and inhibition of the JAK-STAT pathway with ruxolitinib in combination with dexamethasone resulted in the greatest reduction of pro-inflammatory cytokine secretion. (35) Further refinement of *in vivo* animal models will be pivotal for discovering the more precise molecular underpinnings of CRS development and severity.

Factors associated with CRS risk

Identifying factors associated with an increased risk for the development of severe CRS can help inform decisions about ensuing monitoring and management. Such factors may be intrinsic to the CAR product or related to patient and disease specific factors. The dose of infused CAR T cells has been shown to be one such intrinsic factor, with several studies noting that CRS was observed more commonly in patients treated with a higher cell dose. (29, 36). Analysis of clinical trials and real-world experience studies have suggested that treatment with CARs incorporating a CD28 costimulatory motif may pose a greater risk for incidence and severity of CRS compared to those with a 4–1BB costimulatory domain. (21, 36, 37) Several studies have suggested that CD4-postiive CAR T cells contribute to CRS development to a greater degree than CD8-positive CAR T cells, indicating that the CD4/CD8 ratio of the CAR product may be potentially be predictive for CRS (34, 38).

Regarding disease and patient specific factors, a higher disease burden prior to CAR T cell infusion (e.g., degree of bone marrow involvement prior to lymphodepletion) has generally been associated with increased severity of CRS. (21, 39–42) The underlying disease biology also impacts CRS risk, with patients having a more proliferative malignancy such as B-ALL or LBCL seemingly at risk for higher grade CRS compared to those with more indolent disease such as follicular lymphoma or multiple myeloma. (8, 11, 12, 43–45) The modified Endothelial Activation and Stress Index (m-EASIX) score consists of laboratory parameters that are readily available (lactate dehydrogenase [LDH in U/L] x C-reactive protein [CRP in mg/dL]/platelets [PLTs in 10^9 cells/L]). In a retrospective analysis of 118 patients receiving anti-CD19 CAR T cell therapy for the treatment of either B-ALL or LBCL, the m-EASIX score prior to infusion was associated with onset of any grade and severe CRS, and m-EASIX score on days +1 and +3 post-infusion were associated with onset of severe CRS. Furthermore, when analyzing individual variables (CRP, LDH, PLTs, creatinine), lower platelet counts and higher CRP were independently associated with the onset of severe CRS. Thus, the m-EASIX score can be used as a predictor of endothelial activation which may indicate increased risk for more severe CRS (46).

Grading and management of CRS

There were initially multiple heterogeneous definition and grading systems for CRS when CAR T cell therapies were first being studied in humans, such as the Common Terminology Criteria for Adverse Events (CTCAE), University of Pennsylvania, Memorial Sloan Kettering Cancer Center (MSKCC), and CAR-T cell therapy-associated toxicity (CARTOX) criteria. (41, 47–49) A multi-institutional effort to refine CRS grading, known as the Lee criteria, defined fever as a hallmark of CRS and included hypotension responsive to a low dose of one vasopressor as grade 2 CRS. Furthermore, the Lee criteria incorporated a treatment algorithm that corresponded with each grade of toxicity. (50) Given the differences among the published grading systems for CAR T-associated toxicity, an expert panel was convened to draft the ASTCT consensus grading criteria for CRS and neurologic toxicity associated with immune effector cells in 2018. (18) The ASTCT consensus criteria have since been applied as a uniform grading system for subsequent clinical trials and real-world analyses.

Given that symptoms of CRS are non-specific, the symptoms defining CRS must be attributed specifically to treatment with the immune effector cell therapy. In addition to having a reasonable temporal relation, typically with onset occurring within 14 days of therapy, other causes of fever, hypotension, or hypoxia such as sepsis should be excluded. While there is overlap between CRS and other immune effector cell-associated toxicities (ICANS, IEC-HS), these toxicities are excluded from the definition of CRS and are graded separately. Laboratory parameters of inflammation such as CRP and ferritin are often trended for patients receiving cell therapy; however, specific laboratory parameters are excluded from the definition and grading of CRS due to the non-specificity of these markers of inflammation, and because cytokine panels are not readily available at most institutions. For the purposes of defining CRS, fever is defined by the CTCAE version 5.0 criteria as a temperature ≥ 38.0 °C. The severity of CRS is defined by the degree of hypotension and hypoxia, as other specific organ toxicities generally occur in the setting of hypotension and hypoxia and do not influence treatment decisions with glucocorticoids or anti-cytokine therapy. While fever may resolve quickly after the initiation of anti-cytokine therapies, CRS is not considered resolved until all signs and symptoms leading to the original diagnosis of CRS have resolved unless they can be clearly attributed to an alternative etiology. The ASTCT CRS consensus grading system is summarized in Table 2 (18).

Acknowledging the relatively high incidence of CRS with most approved CAR T cell therapies, patients require close monitoring initially with frequent vital sign assessment, laboratory studies (complete blood count, complete metabolic panel, coagulation studies, inflammatory markers), and neurologic assessment. (13) Outpatient administration has been successfully demonstrated in both clinical trial and real-world settings; however, the majority of CAR T cell therapies administered to date have occurred inpatient with a defined period of monitoring for the development of the associated toxicities. (51–53) Management of CRS involves evaluating for infectious etiologies of fever, supportive care, and varying degrees of immunosuppressive therapy based on CRS severity. (13) Tocilizumab is a monoclonal antibody antagonist of the IL-6 receptor that has received regulatory approval for the treatment of CRS. (54) Each approved CAR T cell therapy is administered through a Risk Evaluation and Mitigation Strategy (REMS) program which mandates tocilizumab availability at the site of treatment in case it is needed for the management of CRS. Furthermore, the prescribing information for each CAR product includes management guidance for each grade of CRS (55–60).

As fever without hypotension or hypoxia defines grade 1 CRS, initial management consists of excluding infectious etiology with blood cultures, urine culture, and chest radiography. The underlying hematologic malignancy and lymphodepletion chemotherapy administered prior to CAR T often result in neutropenia concomitant with onset of fever, and thus neutropenic fever should be managed with broad spectrum antibiotics and granulocyte stimulating factor (G-CSF) per institutional guidelines. Grade 1 CRS can often be managed with

TABLE 2 The ASTCT consensus grading criteria for cytokine release syndrome (CRS).

CRS Parameter	Grade 1	Grade 2	Grade 3	Grade 4
Fever*	Temperature ≥ 38.0 °C at onset for all grades			
With				
Hypotension	None	Not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
And/or†				
Hypoxia	None	Requiring low-flow nasal cannula‡ or blow-by	Requiring high-flow nasal cannula‡, facemask, nonrebreather mask, or Venturi mask	Requiring positive pressure (e.g., CPAP, BiPAP, intubation and mechanical ventilation)

*Fever is defined as temperature ≥ 38.0 °C not attributable to another cause. In patients who have CRS then receive antipyretic or anti-cytokine therapy, fever is no longer required to grade subsequent CRS severity. In this case CRS grading is driven by hypotension and/or hypoxia.
†CRS grade is determined by the more severe event if both hypotension and hypoxia are present.
‡Low-flow nasal cannula is defined as oxygen delivered at ≤ 6 liters per minute. High-flow nasal cannula is defined as oxygen delivered at ≥ 6 liters per minute.
This table was adapted from the ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. (18)

supportive care alone, such as antipyretics, intravenous fluids, and symptomatic management; however, patients with recurrent or persistent fever may be managed with tocilizumab as per grade ≥ 2 CRS (61).

Management of grade 2 CRS includes intravenous fluids for hypotension and/or supplemental oxygenation as needed. Patients with grade ≥2 CRS should undergo more intensive monitoring with continuous pulse oximetry and cardiac telemetry. All patients with grade 2 CRS should receive tocilizumab 8 mg/kg IV (dose capped at 800 mg/dose). Tocilizumab may be given every eight hours for a maximum of four total doses. For patients with persistent borderline hypotension after reasonable intravenous fluid bolus administration and one to two doses of tocilizumab, the addition of glucocorticoid therapy with dexamethasone 10 mg IV (or equivalent) every 12 hours should be considered. If brisk improvement is not observed, vasopressor therapy should be initiated for grade 3 CRS and level of care should be escalated to an intensive care unit. The management of grade 3 CRS includes supportive care for hypotension and hypoxia with vasopressors and escalating supplemental oxygenation as necessary, and echocardiography should be performed to assess cardiac function. Tocilizumab and glucocorticoid should be administered concurrently, with tocilizumab dosing as per labeled use above and with glucocorticoid dose increased to dexamethasone 10 mg IV every 6 hours (or equivalent) followed by a rapid taper once symptoms are noted to improve. Refractory grade 3 or escalation to grade 4 CRS may necessitate further titration of glucocorticoid dose up to a maximum methylprednisolone dose of 1,000 mg IV every 12 hours plus consideration of alternative anti-cytokine or immunosuppressive therapies after tocilizumab has been administered for the maximum of four doses (61).

The management of CRS refractory to standard anti-inflammatory therapy with tocilizumab and glucocorticoids can pose significant challenges, with data often limited to case reports or retrospective series. It is first prudent to exclude active infection or progression of malignancy as a contributing factor. (62) As murine models of CAR T-associated toxicities have implied IL-1 as an important pathophysiologic mediator of both CRS and ICANS, the IL-1 receptor antagonist anakinra, which is approved for the treatment of rheumatoid arthritis and neonatal-onset multisystem inflammatory disease, has become increasingly used for the treatment of refractory toxicity. (30, 31, 62) While anakinra has demonstrated greater efficacy in the treatment of refractory ICANS, it has been shown to facilitate resolution of refractory CRS in case reports and retrospective studies (63, 64).

Siltuximab is a monoclonal antibody that binds directly to IL-6 preventing its interaction with IL-6 receptors, whereas tocilizumab is an inhibitor of the IL-6 receptor. Siltuximab has been demonstrated to be efficacious in the treatment of CRS both alone and in combination with tocilizumab. (5, 65, 66) The TNF-α receptor inhibitor etanercept, approved for various rheumatologic indications, has been shown to abate CRS in case reports of both anti-CD19 and anti-BCMA CAR T cell therapies. (26, 67) Emapalumab is a monoclonal antibody inhibitor of IFN-ɣ approved for the treatment of primary hemophagocytic lymphohistiocytosis (HLH). Preclinical studies have supported a potential role for its use in CAR T-associated toxicities, and emapalumab was given with success for a case of refractory grade 4 CRS with secondary HLH. (33, 68) Furthermore, in a case series of pediatric patients with B-ALL who had refractory CRS despite IL-6 inhibition, glucocorticoids, and IL-1 blockade, a single dose of emapalumab 1 mg/kg resulted in a profound decrease in inflammatory markers and significant clinical improvement in five of six patients treated (69).

The tyrosine kinase inhibitor dasatinib has been demonstrated to inhibit the lymphocyte-specific protein tyrosine kinase, which impairs downstream signaling in various CAR constructs leading to a cessation in cytokine production, cytolytic activity, and in decreased CAR T cell proliferation in both *in vitro* and *in vivo* models. In a murine CRS model, dasatinib was able to mitigate the effects of CRS and CAR-T cells were able to regain antitumor cytolytic activity after dasatinib discontinuation. (70, 71) In one published case of grade 3 CRS and grade 4 ICANS observed after anti-CD19 CAR T cell therapy for LBCL despite treatment with four doses of tocilizumab and high-dose glucocorticoids, treatment with dasatinib 100 mg daily for seven days resulted in substantial clinical improvement. While CAR T expansion was noted to decline markedly for this patient after dasatinib initiation without evidence of re-expansion after discontinuation, it did not appear to hamper efficacy as the patient was in an ongoing complete remission over two years from the time of CAR T cell infusion. (72)

Inflammatory cytokines such as IFN-ɣ, IL-6, IL-12, and TNF-α signal through Janus kinase 1 (JAK1), with activation of JAK leading to phosphorylation of signal transducer and activator of transcription proteins (STATs) which subsequently modulates gene expression. The selective JAK1 inhibitor itacitinib was able to effectively reduce CRS-related cytokines produced by anti-CD19 CAR T cells in a dose-dependent fashion in both *in vitro* and *in vivo* models without impacting CAR T proliferation or cytolytic function. (73) This preclinical rationale for JAK-STAT inhibition has been translated to the clinical setting with several case reports noting resolution of refractory CRS after treatment with the JAK-inhibitor ruxolitinib. (74–77) Lastly, a variety of different inducible safety switches to either reversibly or irreversibly impair CAR T cell function have been designed as a mechanism for potentially managing severe toxicities; however, they have yet to become significant in clinical practice. (78) Pharmacotherapy for the management of CRS is summarized in Table 3.

Because the management of CRS often requires pharmacologic intervention with anti-cytokine therapy or glucocorticoids, there has been theoretical concern that such anti-inflammatory therapy may hamper the efficacy of CAR T cell therapy. Preclinical studies did not suggest hindrance of antitumor activity with IL-1, IL-6 and IFN-ɣ inhibition respectively. (30, 33) A retrospective analysis regarding the timing of tocilizumab administration for patients treated with anti-BCMA CAR T cell therapy demonstrated that patients receiving tocilizumab earlier (<12 hours from CRS onset) experienced a shorter median duration of CRS without negative

TABLE 3 Pharmacotherapy for the treatment of cytokine release syndrome.

CRS Grade	Pharmacotherapy Recommendations
Grade 1	• Broad-spectrum antibiotics if concomitant neutropenia • Anti-pyretics (acetaminophen) • Consider Tocilizumab for persistent or refractory cases
Grade 2	• Intravenous fluids for hypotension and/or supplemental oxygen • Tocilizumab 8 mg/kg IV may be given every eight hours for a maximum of four total doses • Consider adjunctive dexamethasone 10 mg IV every 12 hours for persistent or refractory cases
Grade 3 or Grade 4	• Vasopressors for hypotension and/or supplemental oxygen • Tocilizumab 8 mg/kg IV may be given every eight hours for a maximum of four total doses • Adjunctive dexamethasone 10 mg IV every 6 hours (or equivalent), which can be escalated up to a dose of methylprednisolone 1,000 mg IV every 12 hours for refractory cases • Consider alternative anti-cytokine or immunosuppressive therapies for refractory cases after tocilizumab
Alternative Therapies for Refractory CRS	
Anakinra – IL-1 receptor antagonist, case reports and retrospective studies demonstrating efficacy (63, 64)	
Siltuximab – IL-6 inhibitor, demonstrated efficacy (5, 65, 66)	
Etanercept – TNF-α receptor inhibitor, demonstrated efficacy in case reports (26, 67)	
Emapalumab – IFN-ɣ inhibitor, demonstrated efficacy in case report (69)	
Dasatinib – tyrosine kinase inhibitor, demonstrated efficacy in case report (72)	
JAK-STAT inhibitors • Itacitinib has been shown to reduce levels of CRS-related cytokines in pre-clinical studies (73), and has been shown to reduce grade ≥ 2 CRS when used as prophylaxis prior to axi-cel (79) • Ruxolitinib has demonstrated efficacy in several case reports (74–77)	

CRS, cytokine release syndrome; IV, intravenous; mg, milligrams; kg, kilogram; IL, interleukin; TNF, tumor necrosis factor; IFN, interferon.

effect on response or median progression-free survival outcomes. (80) There have been conflicting reports regarding corticosteroid therapy, with one retrospective study demonstrating no impact of corticosteroid therapy on response rate and CAR T expansion/persistence in B-ALL patients, whereas another retrospective analysis in LBCL patients suggested that a higher cumulative dose and prolonged use of corticosteroids had a negative impact on progression-free survival. (81, 82) Ultimately, randomized studies with long-term follow up will be required to discern whether certain toxicity management strategies have a positive or negative effect on CAR T cell therapy efficacy.

There has also been investigation into whether prophylactic or preemptive approaches to toxicity management can decrease the incidence and severity of both CRS and ICANS. Such attempts include early intervention with tocilizumab or corticosteroids for lower grade toxicity, as well as prophylaxis strategies with corticosteroids, tocilizumab, anakinra, or the JAK1 inhibitor itacitinib. (79, 83–90) Results from studies investigating prophylactic interventions are summarized in Table 4.

TABLE 4 Prophylactic approaches for mitigation of CRS/ICANS in patients treated with anti-CD19 CAR T.

Agent/Study	Outcomes	Comparator	Comments
Dexamethasone 10 mg on days 0, 1, and 2 ZUMA-1, cohort 6 (88)	CRS 80% G ≥ 3 CRS 0% ICANS 58% G ≥ 3 ICANS 13%	ZUMA-1, cohorts 1–2 (no prophylactic dex): CRS 93%, G ≥ 3 13% ICANS 64%, G ≥ 3 28%	Lower baseline tumor burden in prophylactic dex cohort compared to cohorts 1–2
Tocilizumab on day 2 after axi-cel infusion Safety expansion cohort of ZUMA-1 (87)	G ≥ 3 CRS 3% G ≥ 3 ICANS 35%	ZUMA-1, cohorts 1–2 (no prophylactic toci): G ≥ 3 CRS 13% G ≥ 3 ICANS 28%	Peak IL-6 levels were higher in prophylactic toci cohort
Anakinra on days 0–7 NCT04432506 (91)	CRS 95% G ≥ 2 CRS 40% G ≥ 3 CRS 5% ICANS 35% G ≥ 3 ICANS 20%	Tumor-burden matched retrospective cohort: G ≥ 2 CRS 50% G ≥ 3 ICANS 30%	No observed impact on expansion kinetics or CAR T efficacy
Anakinra on day 2 through at least day 10 post-CAR T NCT04148430 (92)	CRS 74% G ≥ 3 CRS 6.4% ICANS 19% G ≥ 3 ICANS 9.7%	No comparison cohort	Favorable reduction in all grades of ICANS compared to historical controls
Itacitinib (JAK1 inhibitor) starting day -3 through day +26 after CAR T NCT04071366 (79)	CRS 65% Grade 2 CRS 17% G ≥ 3 CRS 0% ICANS 13% G ≥ 2 ICANS 9%	Placebo-controlled cohort: CRS 87% Grade 2 CRS 57% G ≥ 3 CRS 0% ICANS 35% G ≥ 2 ICANS 22%	Tocilizumab use lower in itacitinib arm (17% v. 57%). Persistent G ≥ 3 neutropenia and TCP at day 28 higher in itacitinib arm
Defibrotide on days -5 to -3 pre-CAR T and days 0–7 post-CAR T; NCT03954106 (93)	ICANS 50% G ≥ 3 ICANS 25%	No comparison cohort	Study terminated early as unlikely to meet 1° endpoint
Lenzilumab (GM-CSF inhibitor) 6 hours prior to axi-cel ZUMA-19 (94)	G ≥ 3 CRS 0% G ≥ 3 ICANS 17%	No comparison cohort	Met 1° endpoint for safety, but terminated after only 6 patients

CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; G, grade; dex, dexamethasone; toci, tocilizumab; TCP, thrombocytopenia; 1°, primary; GM-CSF, granulocyte-macrophage colony-stimulating factor.

Immune effector cell-associated neurotoxicity syndrome (ICANS): clinical manifestations and mechanisms

The ASTCT consensus grading criteria defines ICANS as "a disorder characterized by a pathologic process involving the central

nervous system following any immune therapy that results in the activation or engagement of endogenous or infused T cells and/or other immune effector cells. Symptoms or signs can be progressive and may include aphasia, altered level of consciousness, impairment of cognitive skills, motor weakness, seizures, and cerebral edema." Other forms of neurotoxicity potentially attributable to CAR T cell therapies such as headache, tremor, myoclonus, and asterixis are not considered as ICANS-defining, are graded separately by NCI-CTCAE criteria, and are managed symptomatically. (18) ICANS has been noted to occur concurrently with CRS, shortly after CRS subsides, in the absence of CRS, and in some instances with delayed onset up to one month after infusion. It often first manifests with tremor, mild expressive aphasia, impaired attention, dysgraphia, apraxia, and mild lethargy. Expressive aphasia appears to be the most common and specific symptom of ICANS. At higher grades of severity, ICANS may result in refractory seizures, necessitate intubation for airway protection, and can rarely result in fatal cerebral edema (18, 61).

Observations from imaging of the central nervous system in patients with ICANS has generally observed that findings are symmetric and have a propensity to affect uniquely susceptible brain regions. Involvement of the thalami and deep gray matter indicate that ICANS is likely prompted by a systemic process such as the inflammation mediated by inflammatory cytokines. Specific imaging findings such as leptomeningeal enhancement and T2 hyperintensity of the cerebral sulci, T2 hyperintensity and swelling of the bilateral thalami, and T2 hyperintensities in the supratentorial white matter can bear resemblance to injury patterns seen in other infectious or inflammatory central nervous system (CNS) conditions. Elevation of cerebrospinal fluid (CSF) protein on lumbar puncture samples has been commonly observed in patients with ICANS, and elevated cytokine levels in the CSF typically mirror cytokine elevations observed in serum samples. (95) Electroencephalogram (EEG) findings in patients with ICANS have most commonly been notable for generalized periodic discharges generated from both cortical and subcortical regions which may be induced by proinflammatory cytokines or directly mediated by T cells (96).

Elevated serum and CSF cytokines have been implicated in the pathophysiology of ICANS. Despite the strong association with CRS and inflammatory cytokines, the pathophysiology remains poorly understood in that ICANS can occur without antecedent CRS, and patients with severe CRS do not always develop ICANS. Based on serum and CSF cytokine analyses pooled from multiple human studies, IFN-ɣ, IL-15, IL-6, IL-10, GM-CSF, and IL-1RA appear to be most strongly associated with ICANS. Additionally, IL-2, IL-2Rα, CXCL10, and Granzyme b seem to be possibly associated with ICANS. (95) In a retrospective analysis of 53 patients treated with anti-CD19 CAR T for B-ALL, higher serum elevations of the proinflammatory cytokines IL1α, IL2, IL3, IL5, IL6, IL10, IL15, INF-γ, interferon-gamma inducible protein-10 (IP10), G-CSF, GM-CSF, and MCP1 by day three after CAR T was associated with severe neurotoxicity. Those with severe neurotoxicity were observed to have disproportionately high CSF levels of IL6, IL8, MCP1, and IP10, potentially indicating localized CNS production rather than just mirroring serum cytokine elevations (97).

Studies from animal models have also supported cytokine elevations in association with neurotoxicity. In the murine model of CRS and ICANS which demonstrated that IL-1 and IL-6 were primarily derived from activated monocytes and macrophages, treatment with tocilizumab did not abate symptoms of neurotoxicity when given at the onset of fever, whereas anakinra was able to improve neurotoxicity severity and survival. Furthermore, prophylactic tocilizumab did not protect the mice from delayed lethal neurotoxicity, whereas prophylactic anakinra prevented both CRS and neurotoxicity development. These murine studies suggested that IL-1 may be more integral to the pathophysiology of ICANS and further supported investigation of IL-1 inhibition for ICANS treatment and prevention in subsequent human studies. (30) In a rhesus macaque model of neurotoxicity after adoptive transfer of anti-CD20 CAR T cells, neurotoxicity was associated with serum elevations in IL-8, IL-8, IL-1RA, CXCL9, and CXCL11, and with disproportionately elevated CSF levels of IL-6, IL-2, GM-CSF, and VEGF levels. Pan-T cell encephalitis was observed in the brain parenchyma of the non-human primates experiencing neurotoxicity as evidenced by the accumulation of both CAR T and non-CAR T cells (98).

As seen with CRS, evidence from multiple studies have demonstrated endothelial activation and disruption as a mechanism underlying the pathophysiology of ICANS. Endothelial activation resulting from systemic cytokine release after CAR T cell therapy may predispose to the CNS microvascular dysfunction noted in ICANS. Perturbations in the angiopoietin (Ang)-Tie 2 axis have been associated with ICANS, as Ang-2 is released from endothelial cells upon activation from proinflammatory cytokines. Ang-1 binding to the Tie-2 receptor typically facilitates vascular quiescence and integrity; however, Ang-2 displaces Ang-1 from its receptor and induces vascular permeability. (95) In two separate retrospective analyses of patients treated with CAR T for B-ALL, patients who developed severe neurotoxicity were more likely to have laboratory findings consistent with DIC and capillary leak, lowers levels of Ang-1, higher levels of Ang-2, and a higher Ang-2:Ang-1 ratio suggestive of endothelial activation. (97, 99) Another retrospective study found that ICANS occurrence was associated with an increased PT, D-dimer, Factor VII level, and vWF level, and with decreased serum levels of fibrinogen and platelet count. (28) CSF samples from patients experiencing ICANS compared to baseline were notable for elevated protein concentrations, leukocyte counts (including CAR T cells), and serum cytokines reflective of increased blood-brain barrier permeability. When human brain vascular pericytes were exposed *in vitro* to IFN-ɣ and TNF-α, pericyte secretion of IL-6 and VEGF was observed which further increased blood-brain barrier disruption. An autopsy specimen from a patient who developed fatal neurotoxicity after anti-CD19 CAR T was notable for platelet aggregation, vWF binding in small capillaries, and multifocal vascular disruption with multifocal hemorrhages indicative of endothelial activation (99).

A subset of patients with ICANS develop excitatory neurotoxicity manifested by seizures. Glutamate and quinolinic acid are endogenous excitatory agonists of the N-methyl-D-aspartate (NMDA) receptor. Analysis of CSF from 13 patients

with pre-treatment samples who then developed excitatory neurotoxicity revealed that both glutamate and quinolinic acid were significantly elevated during the neurotoxicity period compared to baseline. On the contrary, these endogenous NMDA agonists were not elevated in a patient without ICANS who underwent pre-treatment and day 14 lumbar punctures. These findings indicate that endothelial activation and increased blood-brain barrier permeability contributing to CSF cytokine elevation may be linked to elevations of these excitatory agonists in the CNS. (97) It has been proposed that neurotoxicity may reflect an on-target, off-tumor effect of anti-CD19 CAR T cell therapy given that CD19 expression was observed by single-cell RNA sequencing analysis of human brain mural cells, which support the vasculature. However, this appears to be an unlikely mechanism given that ICANS has subsequently been observed with CAR T therapies targeting alternative antigens such as BCMA and GPRC5D (11, 12, 100).

Factors associated with ICANS risk

Risk factors for ICANS intrinsic to the CAR T product identified from several patient cohorts include higher CAR T dose, higher peak CAR T expansion, and early/higher elevations of serum proinflammatory cytokines likely related to CAR T expansion kinetics. (97, 99) Additionally, the incorporation of a CD28 costimulatory domain as in axi-cel and brexu-cel appears to be associated with higher incidence and severity of ICANS compared to the other approved products that incorporate a 4–1BB costimulatory domain (Table 1) (4, 7, 9, 10).

In terms of patient and disease-specific factors, a high burden of disease prior to lymphodepletion has been associated with increased ICANS risk. While ICANS can occur in the absence of CRS, developing early and severe CRS after CAR T infusion has been associated with increased incidence and severity of ICANS. (97, 99) One analysis identified pre-existing neurologic comorbidities as a risk factor for subsequent ICANS development. (99) Another study observed that thrombocytopenia was significantly associated with an increased risk for grade ≥ 3 neurotoxicity. (101) The modified EASIX score at day three after CAR T infusion was found to be associated with onset of severe ICANS, though the pre-infusion m-EASIX score was not associated with the development of ICANS. Analyzing the individual components of the m-EASIX score, higher CRP and lower platelet count were both associated with ensuing onset of severe ICANS (46).

Grading and management of ICANS

While ICANS has become the preferred term for this specific manifestation of neurotoxicity due to immune effector cell therapies, the registrational trials for the approved CAR products were done at a time when the NCI-CTCAE criteria for neurotoxicity were being utilized and thus there is more heterogeneity in the way neurotoxicity has been defined, graded, and reported in the literature. (18, 55–60) The CARTOX criteria provided the first step toward streamlining the objective grading of neurotoxicity with the incorporation of a screening tool called the CARTOX-10, which included a ten point scale to assess the patient for alterations in speech, orientation, concentration, and handwriting. The complete CARTOX neurotoxicity grading system involved evaluation of level of consciousness, motor symptoms, seizures, and for signs of elevated intracranial pressure (ICP) with CSF opening pressure measurement and papilledema grading, which can be challenging to obtain and objectively quantify. (49) The ASTCT consensus criteria incorporates a modified version of the CARTOX-10 screening tool called the Immune Effector Cell-Associated Encephalopathy (ICE) score (Table 5), and simplifies assessment of the other domains to facilitate more objective ICANS grading (Table 6) (18).

TABLE 5 The immune effector cell-associated encephalopathy (ICE) score.

Orientation	Orientation to year, month, city, hospital: 4 points (1 point each)
Naming	Name 3 objects (e.g., clock, pen, button): 3 points
Following Commands	Ability to follow simple commands (e.g., Show me 2 fingers or close your eyes and stick out your tongue): 1 point
Writing	Ability to write a standard sentence (e.g., Our national bird is the bald eagle): 1 point
Attention	Count backwards from 100 by 10: 1 point

This table was adapted from the ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. (18)
10: No impairment.
7–9: Grade 1 ICANS.
3–6: Grade 2 ICANS.
0–2: Grade 3 ICANS.
0 due to patient unarousable and unable to perform ICE assessment: Grade 4 ICANS.

The incidence of neurotoxicity observed with each approved CAR T product in their respective registrational trials is summarized in Table 1; however, it is important to note that the ASTCT consensus criteria were not yet implemented for these trials. The ASTCT consensus grading criteria for ICANS is outlined in Table 6. The overall ICANS grade is dictated by the most severe grade observed across the five neurotoxicity domains (ICE score, depressed level of consciousness, seizure, motor findings, and elevated ICP/cerebral edema). Grade 1 ICANS is defined by an ICE score in the range of 7–9 and the patient must be able to awaken spontaneously. An ICE score of 3–6 constitutes grade 2 ICANS, with the patient being able to awaken to voice. Any clinical or electrical seizure that resolves rapidly with intervention or focal areas of edema on neuroimaging qualify as grade 3 ICANS, as does an ICE score ranging 0–2 if patient awakens only to tactile stimulus. A patient with an ICE score of 0 who is unarousable and unable to perform the ICE questionnaire is defined as having grade 4 ICANS. Other qualifiers for grade 4 ICANS include life-threatening prolonged seizures or repetitive clinical or electrical without interim return to baseline, deep focal motor weakness, diffuse cerebral edema on neuroimaging, decerebrate or decorticate posturing, cranial nerve VI palsy, papilledema, and Cushing's triad. Patients with grade 4 ICANS often require intubation and mechanical ventilation for airway protection and seizure

TABLE 6 ASTCT consensus grading for ICANS in adult patients.

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE score*	7–9	3–6	0–2	0 (patient is unarousable and unable to perform ICE
Depressed level of consciousness†	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse. Stupor or coma
Seizure	N/A	N/A	Any clinical seizure focal or generalized that resolves rapidly or nonconvulsive seizures on EEG that resolve with intervention	Life-threatening prolonged seizure (>5 min); or repetitive clinical or electrical seizures without return to baseline in between
Motor findings‡	N/A	N/A	N/A	Deep focal motor weakness such as hemiparesis or paraparesis
Elevated ICP/ cerebral edema	N/A	N/A	Focal/local edema on neuroimaging§	Diffuse cerebral edema on neuroimaging; decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad

ICANS grade is determined by the most severe event not attributable to any other cause.
*A patient with an ICE score of 0 may be classified as grade 3 ICANS if awake with global aphasia, but a patient with an ICE score of 0 may be classified as grade 4 ICANS if unarousable.
†Depressed level of consciousness should be attributable to no other cause (e.g., no sedating medication).
‡Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0, but they do not influence ICANS grading.
§Intracranial hemorrhage with or without associated edema is not considered a neurotoxicity feature and is excluded from ICANS grading. It may be graded according to CTCAE v5.0.
This table was adapted from the ASTCT Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells. (18)
N/A, not applicable.

management, but this should not also be classified as grade 4 CRS if the need for mechanical ventilation is not secondary to refractory hypoxia (18).

Pharmacotherapy for the management of ICANS is summarized in Table 7. The prescribing information for each approved CAR T includes guidance for the management of neurologic toxicities. (55–60) After CAR T infusion, patients should be monitored closely with documentation of the ICE score and assessment of motor strength at least twice daily. Patients should have serial laboratory monitoring (CBC, CMP, coagulation studies, inflammatory markers) and severe hyponatremia should be corrected. Initiating anti-epileptic therapy for seizure prophylaxis is common at most institutions. Neurology consultation should be sought after noted onset of ICANS, and grade ≥ 2 neurotoxicity should prompt further evaluation with neuroimaging and EEG. Lumbar puncture for CSF analysis and opening pressure can be considered for patients with grade 2 ICANS and strongly encouraged for those with grade ≥ 3 ICANS. Tocilizumab may be administered for situations where CRS is concurrent with ICANS, but in the absence of CRS the mainstay of ICANS treatment is supportive care and corticosteroid therapy (61).

Patients with grade 1 ICANS can often be monitored and managed with supportive care alone, but it is reasonable to treat with dexamethasone 10 mg and reassess. Increasingly severe ICANS is generally managed with increased corticosteroid dosing, such as dexamethasone 10 mg every 12 hours for grade 2 ICANS, and escalation to 10 mg every 6 hours for persistent grade 2 or development of grade 3 ICANS. Corticosteroids should be continued until improvement to grade 1 ICANS and then rapidly tapered as deemed clinically appropriate. Patients with grade ≥ 3 ICANS should be monitored in an ICU setting. Seizures and status epilepticus should be managed with neurology expertise per institutional guidelines. With persistent grade 3 ICANS or emergence of grade 4 toxicity, corticosteroid dose can be further increased to 1,000 mg methylprednisolone given two to three times daily. Alternative therapies should be considered for management of ICANS refractory to corticosteroids, which may include anakinra, ruxolitinib, siltuximab, cyclophosphamide, antithymocyte globulin, or intrathecal hydrocortisone with or without chemotherapy. (61) As with CRS, attempts to prevent or mitigate the severity of ICANS prior to its onset are being investigated. Table 4 summarizes recent investigations into such prophylactic approaches.

There is a lack of high-quality evidence to inform the management of ICANS refractory to corticosteroid therapy, and it can be challenging to isolate the effect of a particular intervention in the setting of patients receiving simultaneous or overlapping therapies. With preclinical studies suggesting a fundamental role for IL-1 in the pathogenesis of ICANS, anakinra has become a preferred agent for the management of refractory ICANS. (30) Clinical responses with ICANS improvement or resolution have been reported in retrospective analyses from multiple institutions, though timing of initiation, dosing, and concurrent therapies have been heterogeneous. (64, 102, 103) A recent retrospective analysis across nine institutions identified 40 patients treated with anakinra for grade ≥ 2 ICANS without improvement on corticosteroid therapy, which was demonstrated to be well tolerated. Treatment with a high-dose anakinra regimen (defined as >200 mg/day IV) was associated with improved clinical outcomes compared to a lower dose (100–200 mg/day), as evidenced by 0% treatment-related mortality in the high-dose group compared to 47% in the low-dose group, and a median cumulative incidence of CRS/ICANS resolution from the time of anakinra initiation of seven days in the high-dose group compared to median time to resolution not being reached in the low-dose due to the higher rate of treatment-related mortality (64).

The IL-6 receptor inhibitor tocilizumab has not been shown to be effective in the treatment of ICANS. Due to its inhibition of IL-6R rather than IL-6 itself, transient increases in serum and CSF IL-6

TABLE 7 Pharmacotherapy for the treatment of ICANS.

ICANS Grade	Pharmacotherapy Recommendations
Grade 1	• Supportive care, consider dexamethasone 10 mg and reassess
Grade 2	• Dexamethasone 10 mg IV every 12 hours, can escalate dosing to 10 mg every 6 hours for persistent grade 2 ICANS • Continue corticosteroids until improvement to grade 1 ICANS, then rapidly taper as clinically appropriate
Grade 3 or Grade 4	• Dexamethasone 10 mg IV every 6 hours • Can escalate up to methylprednisolone 1,000 mg IV given two to three times daily for refractory grade 3 or grade 4 ICANS • Seizures and/or status epilepticus should be managed with anti-epileptics with neurology assistance as per institutional guidelines • Alternative therapies should be considered for the treatment of ICANS refractory to corticosteroids
Alternative Therapies for Refractory ICANS	
Anakinra – IL-1 receptor antagonist, multiple retrospective studies demonstrating efficacy (64, 102, 103)	
Siltuximab – IL-6 inhibitor, pre-clinical rationale without significant clinical demonstration of efficacy. Note that tocilizumab (IL-6R inhibitor) should only be used for concomitant CRS as inhibition of the receptor causes transient increases in free IL-6 which may exacerbate ICANS (65, 97, 104)	
Dasatinib – tyrosine kinase inhibitor, demonstrated efficacy in case report (72)	
Intrathecal hydrocortisone and/or chemotherapy – direct targeting of CAR T cells in CSF, demonstrated efficacy in case reports and retrospective studies (105, 106)	
Antithymocyte globulin (ATG) – direct targeting of CAR T cells, demonstrated efficacy when used as part of multimodal therapy in case report (104)	
Cyclophosphamide – Chemotherapeutic targeting of CAR T cells, demonstrated efficacy in case report (107)	

ICANS, immune effector cell-associated neurotoxicity syndrome; IV, intravenous; IL, interleukin; CRS, cytokine release syndrome; CSF, cerebrospinal fluid.

have been documented after tocilizumab initiation and it has been hypothesized that this may exacerbate ICANS as tocilizumab is not thought to penetrate the blood-brain barrier well. For example, in 16 patients with severe neurotoxicity who received tocilizumab, 56% had their peak neurotoxicity grade occur after the first dose of tocilizumab administration. (97) Whereas siltuximab is an antagonist of IL-6, there is some rationale that treatment with this agent would be advantageous for ICANS outcomes compared to tocilizumab. (65) However, clinical experience with siltuximab specifically for the treatment of refractory ICANS has not been well documented. (65, 104) The tyrosine kinase inhibitor dasatinib has demonstrated an ability to impair CAR T proliferation and downstream cytokine secretion in both *in vitro* and *in vivo* models, supporting a rationale for clinical use. (70, 71) In one patient who received dasatinib for refractory grade 4 ICANS with concurrent grade 3 CRS, treatment with dasatinib led to a rapid improvement in neurologic function and allowed for extubation one day later (72).

In addition to interfering with cytokine signaling, direct targeting of CAR T cells has been explored for the management of refractory ICANS. A case report of two patients treated with intrathecal hydrocortisone plus chemotherapy for corticosteroid-refractory grade 3 and 4 ICANS respectively led to substantial clinical improvement. (105) A retrospective analysis noted that for seven patients who received early intrathecal hydrocortisone with or without intrathecal chemotherapy within five days of developing high-grade ICANS, all seven patients had subsequent resolution of ICANS. Of the four patients with refractory high-grade ICANS who did not receive intrathecal therapy or received it late (>5 days after onset), only two patients recovered from ICANS. Additionally, the median duration of steroid use and cumulative steroid dose were lower for the patients receiving early intrathecal therapy. (106) In one case of refractory grade 4 ICANS with cerebral edema, three doses of rabbit antithymocyte globulin (ATG) were given as part of the multimodal therapy with a temporal suggestion of benefit as the patient was extubated five days after starting ATG and ICANS had resolved completely within two weeks. (104) Another case described a patient who developed refractory rebound neurotoxicity characterized by the development of brain MRI findings consistent with posterior reversible encephalopathy syndrome (PRES) which coincided with a continued rise to peak circulating CAR T cell levels in the peripheral blood. The patient was treated with cyclophosphamide 1.5 g/m2 to target the CAR T cells and significant clinical improvement was noted over the subsequent days and with resolution of MRI abnormalities four weeks later (107).

Other neurotoxicities distinct from ICANS

Neurotoxicity distinct from ICANS has also been observed after CAR T cell therapies. Of particular concern are the described movement and neurocognitive treatment-emergent adverse events (MNTs) that have been described after administration of anti-BCMA CAR T. These defined MNTs consist of a potential range of symptoms falling into one of three categories: movement disorder (e.g., micrographia, resting tremor), cognitive impairment (e.g., memory loss, disturbance in attention), or personality changes (e.g., reduced facial expression, tremors). For patients treated with cilta-cel on the CARTITUDE-1 study, patients were defined as having MNTs if they reported symptoms in at least two of these categories. Symptoms must have occurred after recovery from CRS and/or ICANS and felt to be attributable to the CAR T cell therapy. Using these criteria, five patients (5%) developed MNTs after cilta-cel with median onset of 27 days post-infusion, and a median onset of 17 days (range, 3–94) after resolution from CRS/ICANS. These toxicities were generally not responsive to corticosteroids or other supportive measures, which included anakinra, siltuximab, systemic and intrathecal chemotherapy, dasatinib, and carbidopa/levodopa for parkinsonism. One patient's death was attributed to such neurotoxicity, and only one patient's MNTs were resolving at data cutoff. Patients who experienced MNTs were retrospectively determined to have at least two of the following features: high tumor burden, grade ≥ 2 CRS or any grade ICANS, and high CAR T

expansion/persistence in peripheral blood. After mitigation strategies were implemented, which included more aggressive bridging therapy to reduce disease burden prior to cilta-cel infusion, early intervention for CRS/ICANS, handwriting assessments for early detection, and an extended monitoring period for neurotoxicity, incidence of MNTs has been noted to be < 1% for patients treated with cilta-cel on subsequent trials. (108)

While the clinical manifestations of MNTs seen after anti-BCMA CAR T can have a presentation similar to Parkinson disease, autopsy results from two of the patients who developed such toxicity after cilta-cel suggest an alternative pathophysiology. Both patients were noted to have normal pigmentation in the substantia nigra and symptoms did not improve with a trial of carbidopa/levodopa. There was notable focal gliosis and T cell infiltrate (CD8 > CD4) in the basal ganglia. It was not determined whether these were CAR-positive T cells, however these patients did have persistent CAR T elevations in the blood and CSF. (108, 109)One patient was noted to have detectable BCMA expression on neurons and astrocytes in the caudate nucleus of the basal ganglia, as well as in neurons of the adjacent frontal cortex. (109) This autopsy finding coupled with RNA sequencing analysis of healthy donors observing low levels of BCMA RNA expression in the basal ganglia suggest a possible on-target, off-tumor mechanism for this form of neurotoxicity. (110) While more commonly observed after cilta-cel, MNTs manifesting as parkinsonism have also been observed after treatment with ide-cel. (59) Clinical management of MNTs remains challenging, as they have generally not been responsive to corticosteroids or anti-inflammatory therapies. One case report demonstrated that treatment with cyclophosphamide 2 grams/m^2 aimed at significantly reducing the persistent CAR T cell population resulted in resolution of refractory parkinsonism symptoms. (111)

In addition to ICANS and MNTs, other neurotoxicities observed after anti-BCMA CAR T cell therapy include peripheral sensory and motor neuropathies, ataxia, nystagmus, facial nerve paralysis and other cranial nerve palsies, diplopia, and concentration impairment. (108) CAR T cell therapy targeting the G protein-coupled receptor, class C, group 5, member D (GPRC5D) are under clinical investigation for the treatment of multiple myeloma with promising preliminary results. At the highest dose-level evaluated, two patients developed dose-limiting toxicities of cerebellar toxicity characterized by wide-based gait, saccadic eye movements, appendicular and truncal ataxia, and dysarthria. Treatment with steroids and intravenous immune globulin (IVIG) resulted in stabilization of symptoms but without resolution. Given that microarray data from six healthy donors noted that GPRC5D expression was most enriched in the olivary nucleus, it is possible that this represents on-target, off-tumor toxicity. (100) Other rare distinct neurotoxicities observed after CAR T cell therapy include transverse myelitis/myelopathy, human herpesvirus 6-associated myelitis, acute leukoencephalopathy, central diabetes insipidus, Guillain-Barre-like syndrome, persistent postural tremors resembling essential tremor, new onset migraine headaches, and various peripheral neuropathies (112–117).

Immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome (IEC-HS): clinical manifestations, risk factors, and mechanisms

Secondary hemophagocytic lymphohistiocytosis (HLH) can occur after CAR T due to overactivation of the immune system. Typically characterized by hyperferritinemia, coagulopathy, hepatic dysfunction and cytopenias among other HLH-like manifestations, this is a lesser known but potentially fatal complication of CAR T that has become increasingly recognized. Consequently, US Food and Drug Administration package inserts incorporate the black box warning risk of HLH for both commercially approved BCMA -targeted CAR T cell therapies. (59, 60) The chronology and symptomatology of HLH-like toxicities can be difficult to distinguish from severe CRS. The earliest of the reports described laboratory features mimicking HLH/MAS superimposed on severe CRS in a 7-year-old girl with recurrent B-ALL who was treated with CD19 CAR T cell therapy. (26) Subsequent reports have demonstrated that HLH-like syndrome can occur as a late manifestation when signs of conventional CRS appear to be resolving. (118, 119) Recognizing the need to better delineate HLH-like toxicities following CAR T and to provide a framework for cross-trial comparisons, an ASTCT expert panel recently named this complication as IEC-HS, which is defined as "the development of a pathological and biochemical hyperinflammatory syndrome independent from CRS and ICANS that (1) manifests with features of MAS/HLH (2), is attributable to immune effector cell therapy, and (3) is associated with progression or new onset of cytopenias, hyperferritinemia, coagulopathy with hypofibrinogenemia, and/or transaminitis." (120)

Clinical suspicion and early recognition of IEC-HS is critical. CRS in moderate to severe forms may manifest with overlapping features of IEC-HS (e.g., elevated ferritin, elevated transaminases, hemophagocytosis, coagulopathy, cytopenia, and multi-organ dysfunction) with indistinguishable cytokine and proteomic profiles. (18, 118, 121) Given significant overlap, it is critically important to distinguish between IEC-HS and prolonged severe or recurrent CRS as treatment implications differ between the two. Timing of IEC-HS in relation to antecedent CRS can vary, but it is a distinct manifestation independent from CRS that occurs once CRS is resolved/resolving. While CRS typically develops and resolves within 2 weeks of CAR-T administration, IEC-HS arises after CRS and may persist for weeks. The consensus panel strongly cautions against using IEC-HS nomenclature to describe patients with severe CRS involving multiorgan dysfunction. Malignancy progression, underlying rheumatologic/metabolic disorders, severe infections, or other etiologies that can trigger secondary HLH should be excluded (120).

The HLH-2004 criteria and the H score, the common scoring systems used for diagnosis of secondary HLH, have low validity and applicability in identifying IEC-HS as most patients treated with CAR T have alternative explanations for traditional criteria and thresholds used for diagnosing HLH (e.g., elevated ferritin, fever and cytopenias).

(122–124) In 2017, a CARTOX Working Group was formed that proposed a diagnosis of HLH should be made if the ferritin levels peak at >10,000 ng/mL within the CRS window of 5 days after CAR T-cell therapy, and if the patient develops any two of the following: grade 3 or greater organ toxicities involving the liver, kidneys, or lungs, or hemophagocytosis in the bone marrow or other organs (49). Considering vast heterogeneity in baseline ferritin values among patients undergoing CAR T, waiting to reach an arbitrarily set threshold for ferritin elevation could cause unnecessary delays in diagnosis of IEC-HS, therefore the ASTCT working group has not incorporated specific ferritin values in their proposed diagnostic criteria of IEC-HS. Although there is no specific cutoff, a substantial elevation (defined as >2 times the upper limit of normal or baseline at time of infusion) or rapidly rising serum ferritin is a prerequisite for the diagnosis of IEC-HS. Acknowledging that not all patients with antecedent or ongoing CRS will progress to IEC-HS, the ASTCT working group considers the timing as a key factor in diagnosing IEC-HS. While CRS develops within 14 days post-infusion, IEC-HS is often of delayed onset and is associated with progression or new onset laboratory abnormalities or clinical deterioration as CRS is resolved/resolving. Common manifestations of IEC-HS include hepatic transaminase elevation, hypofibrinogenemia, other coagulation abnormalities, hemophagocytes in bone marrow or other tissue, cytopenias (new onset, worsening, or refractory), elevated LDH, direct hyperbilirubinemia, and new onset splenomegaly. Other manifestations that may be present include renal insufficiency, pulmonary complications, neurotoxicity, fever, and hypertriglyceridemia (120).

Risk factors that have been implicated in the development of IEC-HS include baseline disease burden, CAR T proliferation dynamics, immune resistance mechanisms, target antigen, CAR T cell construct, costimulatory domain, T cell selection, cell dose and patient characteristics (baseline inflammation, immune suppression, cytopenias, genetic predisposition). (45, 118, 119, 125, 126). While the underlying pathophysiology of IEC-HS remains to be fully elucidated, similarities to CRS have been observed. The sustained T cell activation via engineered CAR recognition of tumor antigen results in a hyperinflammatory response, T cell activation and proliferation, cytokine release, and resultant macrophage activation and release of soluble factors creating a positive feedback loop. (120) Like primary and secondary HLH, where defects in cytolytic function of NK cells and CTLs can lead to pathologic T-cell expansion, it has been postulated that the profound and persistent NK cell lymphopenia in concert with heightened CAR T cell expansion and delayed T cell contraction can predispose select patients with CRS to develop a secondary hyperinflammatory response in form of IEC-HS. (118) In the context of 59 patients infused with CD22 CAR T cells where a substantial proportion developed IEC-HS, disproportionately low absolute number of NK cells relative to CD8 T cells in peripheral blood pre-infusion, and higher bone marrow T cell to NK cell ratio was noted in patients who later developed IEC-HS. (118) In line with this observation, a recent murine model of HLH demonstrated that both CTL and cytotoxic NK cells contribute to the development of HLH-like syndrome in mice after infection with lymphocytic choriomeningitis virus (127).

Certain cytokines (e.g., IFN-γ, IL-6, IL-10, IL-18, sIL-2R, CXCL9), and proteomic profiles overlap between HLH and severe CRS. (118, 121). Unlike in CRS, IL-1 appears to be a central player in IEC-HS. IL-1β levels were particularly high among those patients with IEC-HS, which supports the use of anakinra, an IL-1 receptor antagonist, for management of this syndrome. (118) IFN-ɣ, a mediator of systemic inflammation and macrophage activation, is thought to play a key role in development of IEC-HS, which is supported by the high serum level observed in a patient who developed IEC-HS post tisa-cel and was treated with emapalumab with rapid defervescence and improvement in clinical and laboratory parameters (128).

Grading and management of IEC-HS

The ASTCT expert panel developed a grading schema for IEC-HS formed based on the NCI-CTCAE category "Immune system disorder, other." Grades of IEC-HS range from 1–5 with grade 1 toxicity representative of asymptomatic or mild symptoms not requiring intervention, and grade 5 reflecting death due to IEC-HS. Toxicity warranting intervention is labeled as grade 2, with grade 3 toxicity being severe but not immediately life-threatening, and grade 4 IEC-HS is considered life-threatening (e.g., life-threatening bleeding or hypotension, respiratory distress requiring intubation, dialysis indicated for acute kidney injury) (120).

The ASTCT expert panel has proposed a stepwise approach to the treatment of IEC-HS. Therapy should be initiated prior to the development of life-threatening complications. The use of tocilizumab or siltuximab in patients with IEC-HS without CRS is discouraged. First line therapy with the IL-1 receptor antagonist anakinra, with or without corticosteroids, is recommended given its potential to disrupt secondary HLH/MAS associated with rheumatologic and other disorders. Anakinra has a short half-life allowing rapid titration to effect, a well-established side effect profile, and pharmacokinetic data to support both intravenous and subcutaneous use. (120) Anakinra has been observed to be efficacious in IEC-HS in patients treated with CD19 CAR T cell therapy for pediatric B-ALL, DLBCL, and mantle cell lymphoma. (129, 130) Anakinra can be given at a dose of 100–200 mg every 6–12 hours in adults, or up to 10 mg/kg IV daily. If there is no improvement within 48 hours despite escalation of anakinra to target dose with concurrent corticosteroids, initiation of the JAK1/2 inhibitor ruxolitinib as second line therapy is recommended. Ruxolitinib, via inhibition of JAK/STAT signaling pathways, can reduce secretion of the pro-inflammatory cytokines implicated in the development of IEC-HS such as IL-2, IL-6, and IFN-γ. (120) Case reports and retrospective studies have demonstrated its clinical efficacy for primary and secondary HLH (131, 132).

For severe IEC-HS refractory to anakinra and ruxolitinib, additional agents like the anti-IFN-γ monoclonal antibody emapalumab or low-dose etoposide chemotherapy can be considered. Treatment with emapalumab may be more appropriate in the context of significantly elevated serum IFN-γ, whereas etoposide to deplete T cells may be more applicable for a documented persistent elevation of CAR T cells. (120) Emapalumab

binds both free and receptor-bound IFN-γ, and elevated levels of each have been documented in both primary HLH and CAR-T associated toxicities. (133, 134) It is FDA-approved for primary HLH in children and adults in the setting of refractory, recurrent or progressive disease or intolerance to other standard treatments. The initial dosing is a twice-weekly intravenous infusion of 1 mg/kg. Evidence pertaining to its efficacy in IEC-HS is limited to case reports describing the clinical recovery of patients with severe IEC-HS and CRS refractory to corticosteroids and multiple anti-cytokine treatments who received emapalumab as salvage therapy. (68, 128) The topoisomerase inhibitor etoposide is cytotoxic to T cells and has been shown to have efficacy in primary and secondary HLH in protocols such as HLH-94. (135, 136) The suggested dosing in adults is 50–100 mg/m2 once to twice weekly. (137) Antithymocyte globulin (ATG) and alemtuzumab have been proposed as salvage therapy options in primary pediatric HLH, but their use for IEC-HS is not well established (120).

Patients with IEC-HS require frequent clinical assessment, daily laboratory studies (CBC, CMP, coagulation studies, inflammatory markers), and diligent supportive care. Such supportive care includes transfusion support for cytopenias, cryoprecipitate for hypofibrinogenemia, vitamin K and/or fresh frozen plasma for severe coagulopathy, and infectious disease consultation for both diagnostic purposes and infection prophylaxis recommendations given prolonged immunosuppression. As clinical and laboratory parameters stabilize, tapering immunosuppression is encouraged while monitoring for recrudescence of symptoms. Further retrospective and prospective studies are necessary to characterize and differentiate severe CRS complicated by HLH-like manifestations versus IEC-HS, and to inform optimal monitoring and therapeutic strategies (120).

Immune effector cell-associated hematotoxicity (ICAHT): clinical manifestations, risk factors, and mechanisms

Anemia, thrombocytopenia, and leukopenia with neutropenia are among the most common toxicities observed after treatment with CAR T cell therapy. The incidence of cytopenias observed with each approved CAR T cell therapy on the respective registrational study is summarized in Table 1, noting the limitations of comparisons among trials. In a pooled real-world analysis of 235 patients treated with anti-CD19 CAR T, incidence of grade ≥3 neutropenia, anemia, and thrombocytopenia were 91%, 69%, and 62% respectively. While early cytopenias can be attributed to the effects of lymphodepletion chemotherapy prior to CAR T, persistent or late recurrence of cytopenias is likely multifactorial in nature and can result in significant transfusion dependence, bleeding complications, and infectious sequelae (138–140).

Three distinct clinical phenotypes of neutrophil recovery have been observed after CAR T. The most common recovery pattern observed in a multicenter retrospective analysis was that of intermittent recovery in 52%, consisting of initial recovery with G-CSF stimulation for the neutropenia following lymphodepletion followed by a biphasic second reduction in absolute neutrophil count (ANC) in month two post-CAR T, and then with another recovery in the subsequent weeks. A subset of 25% demonstrated quick recovery from the initial neutropenia, whereas 23% of patients displayed an aplastic phenotype characterized by a protracted course of neutropenia despite G-CSF use (139).

A multitude of risk factors have been associated with the development of cytopenias after CAR T cell therapy. Such risk factors include malignancy-related features (e.g., underlying disease and disease burden), number and type of prior therapies, baseline bone marrow characteristics (e.g., pre-existing cytopenias, clonal hematopoiesis of indeterminate potential [CHIP]), inflammatory status prior to CAR T, and factors intrinsic to the CAR T product. Additionally, post-infusion toxicities such as CRS, IEC-HS, or infection influence the risk of developing prolonged cytopenias. (140) Calculated prior to lymphodepletion (day −5), the CAR-HEMATOTOX score incorporates both factors that relate to the baseline inflammatory state (e.g., CRP, ferritin) and the patient's hematopoietic reserve (e.g., hemoglobin, ANC, platelet count). This model was first developed to predict severe hematotoxicity in relapsed/refractory LBCL and was then subsequently extended to patients receiving CAR-T therapy for mantle cell lymphoma and multiple myeloma. High-risk patients with score ≥2 had a longer duration of neutropenia, higher incidence of severe thrombocytopenia and anemia, increased rates of severe infection, higher non-relapse mortality, and inferior treatment outcomes compared to their low-risk counterparts (score 0–1) (139–142).

There are several factors to consider regarding the pathophysiology of ICAHT. Baseline cytopenias prior to CAR T treatment likely reflect underlying impairment of the hematopoietic stem and progenitor cell compartment secondary to prior cytotoxic therapies. The presence of preexisting CHIP may contribute to an underlying inflammatory state and contribute to the subsequent development of prolonged cytopenias. (143, 144) Patients with cytopenia prior to CAR T are more likely to have increased bone marrow disease burden, which in turn is correlated with a longer time to hematologic recovery after CAR T. Patients with delayed or prolonged cytopenias after anti-BCMA CAR T were shown to have a higher percentage of persistent CAR T cells in the bone marrow aspirate two months after infusion, suggesting that CAR T-mediated inflammation may contribute to these cytopenias. (145) This is further supported by the observation that patients developing hyperinflammatory complications such as CRS and IEC-HS are more likely to develop prolonged cytopenias. (146, 147) In patients with an aplastic phenotype, single cell sequencing studies have shown an inflammatory micromilieu in the form of oligoclonal CAR-T-cell expansion, T-cell receptor restriction, clonally expanded CXCR1^{hi}, and IFN-γ expressing cytotoxic T cells (148, 149) Additionally, reactivation of certain viruses (human herpesvirus 6, cytomegalovirus, Epstein-Barr virus, adenovirus etc.) in the setting of immune suppression can directly suppress hematopoietic cell recovery (140).

Grading and management of ICAHT

The NCI-CTCAE criteria have been used for grading the hematologic adverse events in the respective registrational CAR T studies. With the goal of more accurately representing the neutropenia observed after T-cell-based immunotherapies, the European Hematology Association (EHA) and European Society for Blood and Marrow Transplantation (EBMT) formed an expert panel that established the terminology and grading system for ICAHT. Early ICAHT is defined as occurring within the first 30 days after CAR T, and late ICAHT refers to cytopenia observed after day 30. The grading system (grade 1–4) incorporates both depth and duration of neutropenia for early ICAHT, whereas late ICAHT grading is reflective of the depth of neutropenia (140).

The EHA/EBMT expert panel recommends the use of the CAR-HEMATOTOX score to identify patients at elevated risk for developing severe neutropenia and continued use of current grading systems for anemia and thrombocytopenia. A tiered approach to diagnostic evaluation has been proposed for persistent cytopenia. Initial workup includes ruling out common infectious etiologies, vitamin deficiencies, contribution from medications, and persistent inflammatory etiologies (CRS/IEC-HS). For those with sustained ICAHT, additional evaluation to rule out less common infectious etiologies and bone marrow aspiration and biopsy to investigate underlying bone marrow disease is warranted (140).

Management of ICAHT consists primarily of transfusion support, growth factor administration, and infectious prophylaxis. Administration of G-CSF has been demonstrated to shorten the duration of severe neutropenia and reduce infectious complications. (140) Retrospective analyses from real-word data sets have demonstrated faster neutrophil recovery and an acceptable safety profile with early G-CSF without increases in the rate of high-grade CRS or ICANS. (150–152) Notably, G-CSF did not impact CAR-T expansion or efficacy. (153) Most patients will adequately respond to growth factor support with count recovery. (146) While data for patients treated with CAR T remains limited, thrombopoietin (TPO) agonists have been given successfully to patients with prolonged or late thrombocytopenia, and have also been noted to improve anemia in some instances. (140) B-cell aplasia and hypogammaglobulinemia associated with CAR T can compound the risk of infections, therefore IgG replacement should be considered for those with high infection risk or experiencing recurrent infections with a recommended goal IgG trough > 400 mg/dL. (154) For patients with persistent ICAHT refractory to the aforementioned interventions, hematopoietic stem cell boost with cryopreserved autologous $CD34^{+}$ stem cells has been observed to result in improvement or resolution of neutropenia in a majority of cases (155–157).

Secondary malignancies observed after CAR T cell therapy

The US Food and Drug Administration (FDA) released a statement on November 28, 2023, regarding the potential risk of secondary T cell malignancies in patients treated with CAR T cell therapy, which include CAR-positive T cell lymphoma. There have been 20 cases of T cell malignancies reported after CAR T cell infusion in approximately 8,000 cases conveyed to the FDA Adverse Events Reporting System. With an estimated total number of commercial CAR T infusions greater than 30,000, the suggested incidence of secondary T cell malignancies is quite low, but further studies are needed to better define the true incidence and to further elucidate the pathogenesis. Furthermore, the Centers for International Blood and Marrow Transplant Research (CIBMTR) database has documented 565 cases of secondary or subsequent malignancies in 485 individual patients out of a total of 8,060 enrolled in post-authorization safety studies. Such malignancies are most commonly non-melanomatous skin cancers and therapy-related myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). (158) For example, 10 of the 97 patients who received cilta-cel on the CARTITUDE-1 study have since been noted to have secondary myeloid malignancies. (60, 159) As such, the FDA has stated that patients who have received CAR T cell therapy should be monitored indefinitely for the development of subsequent malignancies but that benefits of CAR T in treating the malignancy at hand continues to outweigh such risks. (158) It is particularly challenging to determine the causal association of CAR T with the development of secondary malignancy given the existing confounders related to prior systemic therapies received (including high-dose alkylating chemotherapy for autologous stem cell transplant), patient age, and immortal time bias. Long-term follow up of patients enrolled to studies with CAR T in earlier line treatment settings may help to elucidate this further (159).

Conclusion

In conclusion, CAR T cell therapy has revolutionized the treatment of hematologic malignancies and efforts are ongoing to extend the benefits of cellular therapies to patients with solid tumors and rheumatologic disease. Despite remarkable efficacy, the development of potentially severe immune effector cell-associated toxicities require that patients receive close monitoring at centers with expertise in the diagnosis and management of these adverse effects. Significant progress has been made in the understanding of the underlying pathophysiology of such toxicities by means of retrospective analyses and animal models, which have informed therapeutic approaches to toxicity management. The development of consensus guidelines for the definition and grading of these distinct toxicities will further facilitate future cross-trial comparisons and prospective studies, which will be critical for optimizing risk-stratification and management approaches. For the future, active areas of investigation include the optimization of preclinical models to better elucidate the mechanisms of CAR T toxicities, identification of predictive biomarkers, prospective trials of prophylactic or preemptive toxicity mitigation, and creative CAR designs to further ameliorate the development of such toxicities.

Author contributions

CF: Conceptualization, Writing – original draft, Writing – review & editing. MB: Conceptualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors express their deep appreciation to the Leon Levine Foundation and the Kerry and Simone Vickar Family Foundation for their support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J.* (2021) 11:69. doi: 10.1038/s41408–021-00459–7

2. 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. *New Engl J Med.* (2018) 378:439–48. doi: 10.1056/NEJMoa1709866

3. 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. *New Engl J Med.* (2019) 380:45–56. doi: 10.1056/NEJMoa1804980

4. 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. *New Engl J Med.* (2017) 377:2531–44. doi: 10.1056/NEJMoa1707447

5. 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) 396:839–52. doi: 10.1016/S0140–6736(20)31366–0

6. 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) 141:1675–84. doi: 10.1182/blood.2022018730

7. 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. *New Engl J Med.* (2022) 386:640–54. doi: 10.1056/NEJMoa2116133

8. 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) 23:91–103. doi: 10.1016/S1470-2045(21)00591-X

9. 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. *New Engl J Med.* (2020) 382:1331–42. doi: 10.1056/NEJMoa1914347

10. 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) 398:491–502. doi: 10.1016/S0140–6736(21)01222–8

11. Munshi NC, Anderson LD, Shah N, Madduri D, Berdeja J, Lonial S, et al. Idecabtagene vicleucel in relapsed and refractory multiple myeloma. *N Engl J Med.* (2021) 384:705–16. doi: 10.1056/NEJMoa2024850

12. 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) 398:314–24. doi: 10.1016/S0140–6736(21)00933–8

13. Brudno JN, Kochenderfer JN. Recent advances in CAR T-cell toxicity: Mechanisms, manifestations and management. *Blood Rev.* (2019) 34:45–55. doi: 10.1016/j.blre.2018.11.002

14. Zhu F, Wei G, Zhang M, Zhao H, Wu W, Yang L, et al. Factors associated with costs in chimeric antigen receptor T-cell therapy for patients with relapsed/refractory B-cell Malignancies. *Cell Transpl.* (2020) 29:963689720919434. doi: 10.1177/0963689720919434

15. Palomba ML, Jun MP, Lymp J, Nguyen A, McGarvey N, Gitlin M, et al. Postinfusion monitoring costs by site of care for patients with relapsed/refractory large B-cell lymphoma receiving third- or later-line treatment with lisocabtagene maraleucel in the TRANSCEND NHL 001 and OUTREACH trials. *Leuk Lymphoma.* (2021) 62:2169–76. doi: 10.1080/10428194.2021.1910686

16. Maalej KM, Merhi M, Inchakalody VP, Mestiri S, Alam M, Maccalli C, et al. CAR-cell therapy in the era of solid tumor treatment: current challenges and emerging therapeutic advances. *Mol Cancer.* (2023) 22:20. doi: 10.1186/s12943-023-01723-z

17. Schett G, Mackensen A, Mougiakakos D. CAR T-cell therapy in autoimmune diseases. *Lancet.* (2023) 402:2034–44. doi: 10.1016/S0140–6736(23)01126–1

18. Lee DW, Santomasso BD, Locke FL, Ghobadi A, Turtle CJ, Brudno JN, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. *Biol Blood Marrow Transpl.* (2019) 25:625–38. doi: 10.1016/j.bbmt.2018.12.758

19. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, et al. Chimeric antigen receptor T cells for sustained remissions in leukemia. *New Engl J Med.* (2014) 371:1507–17. doi: 10.1056/NEJMoa1407222

20. Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, et al. Efficacy and toxicity management of 19–28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med.* (2014) 6:224ra25. doi: 10.1126/scitranslmed.3008226

21. Brudno JN, Maric I, Hartman SD, Rose JJ, Wang M, Lam N, et al. T cells genetically modified to express an anti–B-Cell maturation antigen chimeric antigen receptor cause remissions of poor-prognosis relapsed multiple myeloma. *J Clin Oncol.* (2018) 36:2267–80. doi: 10.1200/JCO.2018.77.8084

22. Alvi RM, Frigault MJ, Fradley MG, Jain MD, Mahmood SS, Awadalla M, et al. Cardiovascular events among adults treated with chimeric antigen receptor T-cells (CAR-T). *J Am Coll Cardiol.* (2019) 74:3099–108. doi: 10.1016/j.jacc.2019.10.038

23. Wang Z, Han W. Biomarkers of cytokine release syndrome and neurotoxicity related to CAR-T cell therapy. *biomark Res.* (2018) 6:4. doi: 10.1186/s40364–018-0116–0

24. Shimabukuro-Vornhagen A, Gödel P, Subklewe M, Stemmler HJ, Schlößer HA, Schlaak M, et al. Cytokine release syndrome. *J Immunother Cancer.* (2018) 6:56. doi: 10.1186/s40425–018-0343–9

25. Porter DL, Levine BL, Kalos M, Bagg A, June CH. Chimeric antigen receptor–modified T cells in chronic lymphoid leukemia. *New Engl J Med.* (2011) 365:725–33. doi: 10.1056/NEJMoa1103849

26. Grupp SA, Kalos M, Barrett D, Aplenc R, Porter DL, Rheingold SR, et al. Chimeric antigen receptor–modified T cells for acute lymphoid leukemia. *New Engl J Med.* (2013) 368:1509–18. doi: 10.1056/NEJMoa1215134

27. Obstfeld AE, Frey NV, Mansfield K, Lacey SF, June CH, Porter DL, et al. Cytokine release syndrome associated with chimeric-antigen receptor T-cell therapy: clinicopathological insights. *Blood.* (2017) 130:2569–72. doi: 10.1182/blood-2017–08-802413

28. Galli E, Sorà F, Hohaus S, Fresa A, Pansini I, Autore F, et al. Endothelial activation predicts disseminated intravascular coagulopathy, cytokine release syndrome and prognosis in patients treated with anti-CD19 CAR-T cells. *Br J Haematol.* (2023) 201:86–94. doi: 10.1111/bjh.18596

29. Hay KA, Hanafi LA, Li D, Gust J, Liles WC, Wurfel MM, et al. Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor-modified T-cell therapy Key Points. *Blood*. (2017) 130:2295–306. doi: 10.1182/blood-2017–06-793141

30. Norelli M, Camisa B, Barbiera G, Falcone L, Purevdorj A, Genua M, et al. Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. *Nat Med*. (2018) 24:739–48. doi: 10.1038/s41591–018-0036–4

31. Giavridis T, van der Stegen SJC, Eyquem J, Hamieh M, Piersigilli A, Sadelain M. CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade letter. *Nat Med*. (2018) 24:731–8. doi: 10.1038/s41591–018-0041–7

32. Bailey SR, Vatsa S, Larson RC, Bouffard AA, Scarfò I, Kann MC, et al. Blockade or deletion of IFNγ reduces macrophage activation without compromising CAR T-cell function in hematologic Malignancies. *Blood Cancer Discovery*. (2022) 3:136–53. doi: 10.1158/2643–3230.BCD-21–0181

33. Manni S, Del Bufalo F, Merli P, Silvestris DA, Guercio M, Caruso S, et al. Neutralizing IFNγ improves safety without compromising efficacy of CAR-T cell therapy in B-cell Malignancies. *Nat Commun*. (2023) 14:3423. doi: 10.1038/s41467-023-38723-y

34. Boulch M, Cazaux M, Cuffel A, Ruggiu M, Allain V, Corre B, et al. A major role for CD4+ T cells in driving cytokine release syndrome during CAR T cell therapy. *Cell Rep Med*. (2023) 4:101161. doi: 10.1016/j.xcrm.2023.101161

35. Rosen RS, Yang JH, Peña JS, Schloss R, Yarmush ML. An in *vitro* model of the macrophage-endothelial interface to characterize CAR T-cell induced cytokine storm. *Sci Rep*. (2023) 13:18835. doi: 10.1038/s41598-023-46114-y

36. Raje N, Berdeja J, Lin Y, Siegel D, Jagannath S, Madduri D, et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. *New Engl J Med*. (2019) 380:1726–37. doi: 10.1056/NEJMoa1817226

37. 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) 28:2145–54. doi: 10.1038/s41591-022-01969-y

38. Bove C, Arcangeli S, Falcone L, Camisa B, El Khoury R, Greco B, et al. CD4 CAR-T cells targeting CD19 play a key role in exacerbating cytokine release syndrome, while maintaining long-term responses. *J Immunother Cancer*. (2023) 11:e005878. doi: 10.1136/jitc-2022–005878

39. Perez A, Johnson G, Patel K, Arciola B, Wood A, Bachmeier CA, et al. Primary progression during frontline CIT associates with decreased efficacy of subsequent CD19 CAR T-cell therapy in LBCL. *Blood Adv*. (2022) 6:3970–3. doi: 10.1182/bloodadvances.2022007006

40. Dean EA, Mhaskar RS, Lu H, Mousa MS, Krivenko GS, Lazaryan A, et al. High metabolic tumor volume is associated with decreased efficacy of axicabtagene ciloleucel in large B-cell lymphoma. *Blood Adv*. (2020) 4:3268–76. doi: 10.1182/bloodadvances.2020001900

41. Park JH, Rivière I, Gonen M, Wang X, Sénéchal B, Curran KJ, et al. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. *New Engl J Med*. (2018) 378:449–59. doi: 10.1056/NEJMoa1709919

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) 4:4898–911. doi: 10.1182/bloodadvances.2020002394

43. Lemoine J, Vic S, Houot R. Disease-specific outcomes after chimeric antigen receptor T-cell therapy. *Eur J Cancer*. (2022) 160:235–42. doi: 10.1016/j.ejca.2021.10.022

44. Scholler N, Perbost R, Locke FL, Jain MD, Turcan S, Danan C, et al. Tumor immune contexture is a determinant of anti-CD19 CAR T cell efficacy in large B cell lymphoma. *Nat Med*. (2022) 28:1872–82. doi: 10.1038/s41591-022-01916-x

45. 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) 26:4823–31. doi: 10.1158/1078–0432.CCR-20–1434

46. Pennisi M, Sanchez-Escamilla M, Flynn JR, Shouval R, Tomas AA, Silverberg ML, et al. Modified EASIX predicts severe cytokine release syndrome and neurotoxicity after chimeric antigen receptor T cells. *Blood Adv*. (2021) 5:3397–406. doi: 10.1182/bloodadvances.2020003885

47. National Cancer Institute. Common terminology criteria for adverse events (CTCAE). Version 5.0 . Available online at: https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03/CTCAE_4.03_2010–06-14_QuickReference_8.5x11.pdf.

48. Porter D, Frey N, Wood PA, Weng Y, Grupp SA. Grading of cytokine release syndrome associated with CAR T cell therapy tisagenlecleucel. *J Hematol Oncol*. (2018) 11:35. doi: 10.1186/s13045-018-0571-y

49. Neelapu SS, Tummala S, Kebriaei P, Wierda W, Gutierrez C, Locke FL, et al. Chimeric antigen receptor T-cell therapy - assessment and management of toxicities. *Nat Rev Clin Oncol*. (2018) 15:47–62. doi: 10.1038/nrclinonc.2017.148

50. Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, et al. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood*. (2014) 124:188–95. doi: 10.1182/blood-2014–05-552729

51. Myers GD, Verneris MR, Goy A, Maziarz RT. Perspectives on outpatient administration of CAR-T cell therapy in aggressive B-cell lymphoma and acute lymphoblastic leukemia. *J Immunother Cancer*. (2021) 9:e002056. doi: 10.1136/jitc-2020–002056

52. Nasta SD, Hughes ME, Namoglu EC, Garfall A, DiFilippo H, Ballard HJ, et al. Outcomes of tisagenlecleucel in lymphoma patients with predominant management in an ambulatory setting. *Clin Lymphoma Myeloma Leuk*. (2022) 22:e730–7. doi: 10.1016/j.clml.2022.04.012

53. Borogovac A, Keruakous A, Bycko M, Holter Chakrabarty J, Ibrahimi S, Khawandanah M, et al. Safety and feasibility of outpatient chimeric antigen receptor (CAR) T-cell therapy: experience from a tertiary care center. *Bone Marrow Transpl*. (2022) 57:1025–7. doi: 10.1038/s41409-022-01664-z

54. Le RQ, Li L, Yuan W, Shord SS, Nie L, Habtemariam BA, et al. FDA approval summary: tocilizumab for treatment of chimeric antigen receptor T cell-induced severe or life-threatening cytokine release syndrome. *Oncologist*. (2018) 23:943–7. doi: 10.1634/theoncologist.2018–0028

55. US Food and Drug Administration. *KYMRIAH® (tisagenlecleucel) suspension for intravenous infusion*. Initial U.S. Approval (2017).

56. US Food and Drug Administration. *YESCARTA® (axicabtagene ciloleucel) suspension for intravenous infusion*. Initial U.S. Approval (2017).

57. US Food and Drug Administration. *TECARTUS® (brexucabtagene autoleucel) suspension for intravenous infusion*. Initial U.S. Approval (2020).

58. US Food and Drug Administration. *BREYANZI® (lisocabtagene maraleucel) suspension for intravenous infusion*. Initial U.S. Approval (2021).

59. US Food and Drug Administration. *ABECMA® (idecabtagene vicleucel) suspension for intravenous infusion*. Initial U.S. Approval (2021).

60. US Food and Drug Administration. *CARVYKTI® (ciltacabtagene autoleucel) suspension for intravenous infusion*. Initial U.S. Approval (2022).

61. Santomasso BD, Nastoupil LJ, Adkins S, Lacchetti C, Schneider BJ, Anadkat M, et al. Management of immune-related adverse events in patients treated with chimeric antigen receptor T-cell therapy: ASCO Guideline. *J Clin Oncol*. (2021) 39:3978–92. doi: 10.1200/JCO.21.01992

62. Jain MD, Smith M, Shah NN. How I treat refractory CRS and ICANS after CAR T-cell therapy. *Blood*. (2023) 141:2430–42. doi: 10.1182/blood.2022017414

63. Jatiani SS, Aleman A, Madduri D, Chari A, Cho HJ, Richard S, et al. Myeloma CAR-T CRS management with IL-1R antagonist anakinra. *Clin Lymphoma Myeloma Leuk*. (2020) 20:632–636.e1. doi: 10.1016/j.clml.2020.04.020

64. Gazeau N, Liang EC, Wu Q"Vicky,", Voutsinas JM, Barba P, Iacoboni G, et al. Anakinra for refractory cytokine release syndrome or immune effector cell-associated neurotoxicity syndrome after chimeric antigen receptor T cell therapy. *Transplant Cell Ther*. (2023) 29:430–7. doi: 10.1016/j.jtct.2023.04.001

65. Chen F, Teachey DT, Pequignot E, Frey N, Porter D, Maude SL, et al. Measuring IL-6 and sIL-6R in serum from patients treated with tocilizumab and/or siltuximab following CAR T cell therapy. *J Immunol Methods*. (2016) 434:1–8. doi: 10.1016/j.jim.2016.03.005

66. Lipe BC, Renaud T. Siltuximab as a primary treatment for cytokine release syndrome in a patient receiving a bispecific antibody in a clinical trial setting. *J Oncol Pharm Pract*. (2023) 29:1006–10. doi: 10.1177/10781552221140320

67. Zhang L, Wang S, Xu J, Zhang R, Zhu H, Wu Y, et al. Etanercept as a new therapeutic option for cytokine release syndrome following chimeric antigen receptor T cell therapy. *Exp Hematol Oncol*. (2021) 10:16. doi: 10.1186/s40164–021-00209–2

68. McNerney KO, DiNofia AM, Teachey DT, Grupp SA, Maude SL. Potential role of IFNγ inhibition in refractory cytokine release syndrome associated with CAR T-cell therapy. *Blood Cancer Discovery*. (2022) 3:90–4. doi: 10.1158/2643–3230.BCD-21–0203

69. Schuelke MR, Bassiri H, Behrens EM, Canna S, Croy C, DiNofia A, et al. Emapalumab for the treatment of refractory cytokine release syndrome in pediatric patients. *Blood Adv*. (2023) 7:5603–7. doi: 10.1182/bloodadvances.2023010712

70. Mestermann K, Giavridis T, Weber J, Rydzek J, Frenz S, Nerreter T, et al. The tyrosine kinase inhibitor dasatinib acts as a pharmacologic on/off switch for CAR T cells. *Sci Transl Med*. (2019) 11:eaau5907. doi: 10.1126/scitranslmed.aau5907

71. Weber EW, Lynn RC, Sotillo E, Lattin J, Xu P, Mackall CL. Pharmacologic control of CAR-T cell function using dasatinib. *Blood Adv*. (2019) 3:711–7. doi: 10.1182/bloodadvances.2018028720

72. Baur K, Heim D, Beerlage A, Poerings AS, Kopp B, Medinger M, et al. Dasatinib for treatment of CAR T-cell therapy-related complications. *J Immunother Cancer*. (2022) 10:e005956. doi: 10.1136/jitc-2022–005956

73. Huarte E, O'Connor RS, Peel MT, Nunez-Cruz S, Leferovich J, Juvekar A, et al. Itacitinib (INCB039110), a JAK1 inhibitor, reduces cytokines associated with cytokine release syndrome induced by CAR T-cell therapy. *Clin Cancer Res*. (2020) 26:6299–309. doi: 10.1158/1078–0432.CCR-20–1739

74. Pan J, Deng B, Ling Z, Song W, Xu J, Duan J, et al. Ruxolitinib mitigates steroid-refractory CRS during CAR T therapy. *J Cell Mol Med*. (2021) 25:1089–99. doi: 10.1111/jcmm.16176

75. Zi FM, Ye LL, Zheng JF, Cheng J, Wang QM. Using JAK inhibitor to treat cytokine release syndrome developed after chimeric antigen receptor T cell therapy for patients with refractory acute lymphoblastic leukemia: A case report. *Med (Baltimore)*. (2021) 100:e25786. doi: 10.1097/MD.0000000000025786

76. Wei S, Gu R, Xu Y, Liu X, Xing Y, Gong X, et al. Adjuvant ruxolitinib therapy relieves steroid-refractory cytokine-release syndrome without impairing chimeric antigen receptor-modified T-cell function. *Immunotherapy*. (2020) 12:1047–52. doi: 10.2217/imt-2020–0116

77. Gu C, Wu Q, Zhang J, Kang L, Yu L, Qiu H, et al. Successful treatment of severe cytokine release syndrome after CAR-T therapy by ruxolitinib without compromising CAR-T efficacy. *Leuk Lymphoma*. (2023) 64:495–8. doi: 10.1080/10428194.2022.2148209

78. Sahillioglu AC, Schumacher TN. Safety switches for adoptive cell therapy. *Curr Opin Immunol*. (2022) 74:190–8. doi: 10.1016/j.coi.2021.07.002

79. Frigault MJ, Maziarz RT, Park JH, Lazaryan A, Shah NN, Svoboda J, et al. Itacitinib for the prevention of immune effector cell therapy-associated cytokine release syndrome: results from the phase 2 Incb 39110–211 placebo-controlled randomized cohort. *Blood*. (2023) 142:356. doi: 10.1182/blood-2023–180205

80. Banerjee R, Marsal J, Huang CY, Lo M, Kambhampati S, Kennedy VE, et al. Early time-to-tocilizumab after B cell maturation antigen-directed chimeric antigen receptor T cell therapy in myeloma. *Transplant Cell Ther*. (2021) 27:477.e1–7. doi: 10.1016/j.jtct.2021.03.004

81. Liu S, Deng B, Yin Z, Pan J, Lin Y, Ling Z, et al. Corticosteroids do not influence the efficacy and kinetics of CAR-T cells for B-cell acute lymphoblastic leukemia. *Blood Cancer J*. (2020) 10:15. doi: 10.1038/s41408-020-0280-y

82. Strati P, Ahmed S, Furqan F, Fayad LE, Lee HJ, Iyer SP, et al. Prognostic impact of corticosteroids on efficacy of chimeric antigen receptor T-cell therapy in large B-cell lymphoma. *Blood*. (2021) 137:3272–6. doi: 10.1182/blood.2020008865

83. Kadauke S, Myers RM, Li Y, Aplenc R, Baniewicz D, Barrett DM, et al. Risk-adapted preemptive tocilizumab to prevent severe cytokine release syndrome after CTL019 for pediatric B-cell acute lymphoblastic leukemia: a prospective clinical trial. *J Clin Oncol*. (2021) 39:920–30. doi: 10.1200/JCO.20.02477

84. Gardner RA, Ceppi F, Rivers J, Annesley C, Summers C, Taraseviciute A, et al. Preemptive mitigation of CD19 CAR T-cell cytokine release syndrome without attenuation of antileukemic efficacy. *Blood*. (2019) 134:2149–58. doi: 10.1182/blood.2019001463

85. Topp MS, van Meerten T, Houot R, Minnema MC, Bouabdallah K, Lugtenburg PJ, et al. Earlier corticosteroid use for adverse event management in patients receiving axicabtagene ciloleucel for large B-cell lymphoma. *Br J Haematol*. (2021) 195:388–98. doi: 10.1111/bjh.17673

86. Caimi PF, Pacheco Sanchez G, Sharma A, Otegbeye F, Ahmed N, Rojas P, et al. Prophylactic tocilizumab prior to anti-CD19 CAR-T cell therapy for non-Hodgkin lymphoma. *Front Immunol*. (2021) 12:745320. doi: 10.3389/fimmu.2021.745320

87. Locke FL, Neelapu SS, Bartlett NL, Lakakis LJ, Jacobson CA, Braunschweig I, et al. Preliminary results of prophylactic tocilizumab after axicabtagene ciloleucel (axi-cel; KTE-C19) treatment for patients with refractory, aggressive non-Hodgkin lymphoma (NHL). *Blood*. (2017) 130:1547. doi: 10.1182/blood.V130.Suppl_1.1547.1547

88. Oluwole OO, Bouabdallah K, Muñoz J, De Guibert S, Vose JM, Bartlett NL, et al. Prophylactic corticosteroid use in patients receiving axicabtagene ciloleucel for large B-cell lymphoma. *Br J Haematol*. (2021) 194:690–700. doi: 10.1111/bjh.17527

89. Strati P, Li X, Deng Q, Feng L, Sun R, Jallouk A, et al. Primary analysis of a pilot study of prophylactic anakinra to mitigate CAR T cell-associated toxicity in patients with relapsed or refractory large B-cell lymphoma. *Blood*. (2022) 140:7493–5. doi: 10.1182/blood-2022–157448

90. Park JH, Sauter CS, Palomba ML, Shah GL, Dahi PB, Lin RJ, et al. A phase II study of prophylactic anakinra to prevent CRS and neurotoxicity in patients receiving CD19 CAR T cell therapy for relapsed or refractory lymphoma. *Blood*. (2021) 138:96. doi: 10.1182/blood-2021–150431

91. Strati P, Jallouk A, Deng Q, Li X, Feng L, Sun R, et al. A phase 1 study of prophylactic anakinra to mitigate ICANS in patients with large B-cell lymphoma. *Blood Adv*. (2023) 7:6785–9. doi: 10.1182/bloodadvances.2023010653

92. Park JH, Nath K, Devlin SM, Sauter CS, Palomba ML, Shah G, et al. CD19 CAR T-cell therapy and prophylactic anakinra in relapsed or refractory lymphoma: phase 2 trial interim results. *Nat Med*. (2023) 29:1710–7. doi: 10.1038/s41591–023-02404–6

93. Jacobson CA, Rosenthal AC, Arnason J, Agarwal S, Zhang P, Wu W, et al. A phase 2 trial of defibrotide for the prevention of chimeric antigen receptor T-cell–associated neurotoxicity syndrome. *Blood Adv*. (2023) 7:6790–9. doi: 10.1182/bloodadvances.2023009961

94. Oluwole OO, Kenderian SS, Shiraz P, Karmali R, Reshef R, McCarthy PL, et al. ZUMA-19: A phase 1/2 study of axicabtagene ciloleucel plus lenzilumab in patients with relapsed or refractory large B-cell lymphoma. *Blood*. (2022) 140:10318–20. doi: 10.1182/blood-2022–167688

95. Gust J, Ponce R, Liles WC, Garden GA, Turtle CJ. Cytokines in CAR T cell–associated neurotoxicity. *Front Immunol*. (2020) 11:577027. doi: 10.3389/fimmu.2020.577027

96. Herlopian A, Dietrich J, Abramson JS, Cole AJ, Westover MB. EEG findings in CAR T-cell therapy-related encephalopathy. *Neurology*. (2018) 91:227–9. doi: 10.1212/WNL.0000000000005910

97. Santomasso BD, Park JH, Salloum D, Riviere I, Flynn J, Mead E, et al. Clinical and biological correlates of neurotoxicity associated with car t-cell therapy in patients with B-cell acute lymphoblastic leukemia. *Cancer Discovery*. (2018) 8:958–71. doi: 10.1158/2159–8290.CD-17–1319

98. Taraseviciute A, Tkachev V, Ponce R, Turtle CJ, Snyder JM, Liggitt HD, et al. Chimeric antigen receptor T cell–mediated neurotoxicity in nonhuman primates. *Cancer Discovery*. (2018) 8:750–63. doi: 10.1158/2159–8290.CD-17–1368

99. Gust J, Hay KA, Hanafi LA, Li D, Myerson D, Gonzalez-Cuyar LF, et al. Endothelial activation and blood–brain barrier disruption in neurotoxicity after adoptive immunotherapy with CD19 CAR-T cells. *Cancer Discovery*. (2017) 7:1404–19. doi: 10.1158/2159–8290.CD-17–0698

100. Mailankody S, Devlin SM, Landa J, Nath K, Diamonte C, Carstens EJ, et al. GPRC5D-targeted CAR T cells for myeloma. *New Engl J Med*. (2022) 387:1196–206. doi: 10.1056/NEJMoa2209900

101. Karschnia P, Jordan JT, Forst DA, Arrillaga-Romany IC, Batchelor TT, Baehring JM, et al. Clinical presentation, management, and biomarkers of neurotoxicity after adoptive immunotherapy with CAR T cells. *Blood*. (2019) 133:2212–21. doi: 10.1182/blood-2018–12-893396

102. Strati P, Ahmed S, Kebriaei P, Nastoupil LJ, Claussen CM, Watson G, et al. Clinical efficacy of anakinra to mitigate CAR T-cell therapy-associated toxicity in large B-cell lymphoma. *Blood Adv*. (2020) 4:3123–7. doi: 10.1182/bloodadvances.2020002328

103. Wehrli M, Gallagher K, Chen YB, Leick MB, McAfee SL, El-Jawahri AR, et al. Single-center experience using anakinra for steroid-refractory immune effector cell-Associated neurotoxicity syndrome (ICANS). *J Immunother Cancer*. (2022) 10:e003847. doi: 10.1136/jitc-2021–003847

104. Wang M, Jain P, Chi TL, Chen SE, Heimberger A, Weathers SP, et al. Management of a patient with mantle cell lymphoma who developed severe neurotoxicity after chimeric antigen receptor T-cell therapy in ZUMA-2. *J Immunother Cancer*. (2020) 8:e001114. doi: 10.1136/jitc-2020–001114

105. Shah NN, Johnson BD, Fenske TS, Raj RV, Hari P. Intrathecal chemotherapy for management of steroid-refractory CAR T-cell-associated neurotoxicity syndrome. *Blood Adv*. (2020) 4:2119–22. doi: 10.1182/bloodadvances.2020001626

106. Zurko JC, Johnson BD, Aschenbrenner E, Fenske TS, Hamadani M, Hari P, et al. Use of early intrathecal therapy to manage high-grade immune effector cell-associated neurotoxicity syndrome. *JAMA Oncol*. (2022) 8:773–5. doi: 10.1001/jamaoncol.2022.0070

107. Garfall AL, Lancaster E, Stadtmauer EA, Lacey SF, Dengel K, Ambrose DE, et al. Posterior reversible encephalopathy syndrome (PRES) after infusion of anti-BCMA CAR T cells (CART-BCMA) for multiple myeloma: successful treatment with cyclophosphamide. *Blood*. (2016) 128:5702. doi: 10.1182/blood.V128.22.5702.5702

108. Cohen AD, Parekh S, Santomasso BD, Pérez-Larraya JG, van de Donk NWCJ, Arnulf B, et al. Incidence and management of CAR-T neurotoxicity in patients with multiple myeloma treated with ciltacabtagene autoleucel in CARTITUDE studies. *Blood Cancer J*. (2022) 12:32. doi: 10.1038/s41408–022-00629–1

109. Van Oekelen O, Aleman A, Upadhyaya B, Schnakenberg S, Madduri D, Gavane S, et al. Neurocognitive and hypokinetic movement disorder with features of parkinsonism after BCMA-targeting CAR-T cell therapy. *Nat Med*. (2021) 27:2099–103. doi: 10.1038/s41591–021-01564–7

110. Marella M, Yao X, Carreira V, Bustamante MF, Clark HB, Jackson CC, et al. Comprehensive BCMA expression profiling in adult normal human brain suggests a low risk of on-target neurotoxicity in BCMA-targeting multiple myeloma therapy. *J Histochem Cytochem*. (2022) 70:273–87. doi: 10.1369/00221554221079579

111. Graham CE, Lee W, Wiggin HR, Supper VM, Leick MB, Birocchi F, et al. Chemotherapy-induced reversal of ciltacabtagene autoleucel-associated movement and neurocognitive toxicity. *Blood*. (2023) 142:1248–52. doi: 10.1182/blood.2023021429

112. Sheikh S, Mokhtari S, Silverman JA, Reid K, Faramand R, Davila ML, et al. Transverse myelitis after anti-CD19 directed CAR T cell therapy for relapsed large B cell lymphoma. *EJHaem*. (2022) 3:223–7. doi: 10.1002/jha2.286

113. Aghajan Y, Yu A, Jacobson CA, Kim AI, Kean L, Robertson M, et al. Myelopathy because of CAR-T-related neurotoxicity treated with siltuximab. *Neurol Clin Pract*. (2021) 11:e944–6. doi: 10.1212/CPJ.0000000000001078

114. Handley G, Khawaja F, Kondapi DS, Lee HJ, Kaufman GP, Neelapu SS, et al. Human herpesvirus 6 myelitis after chimeric antigen receptor T-cell therapy. *Int J Infect Dis*. (2021) 112:327–9. doi: 10.1016/j.ijid.2021.09.061

115. Nair R, Drillet G, Lhomme F, Le Bras A, Michel L, Rossi J, et al. Acute leucoencephalomyelopathy and quadriparesis after CAR T-cell therapy. *Haematologica*. (2021) 106:1504–6. doi: 10.3324/haematol.2020.259952

116. Koch C, Fleischer J, Popov T, Frontzek K, Schreiner B, Roth P, et al. Diabetes insipidus and Guillain-Barré-like syndrome following CAR-T cell therapy: a case report. *J Immunother Cancer*. (2023) 11:e006059. doi: 10.1136/jitc-2022–006059

117. Santomasso BD, Gust J, Perna F. How I treat unique and difficult-to-manage cases of CAR T-cell therapy-associated neurotoxicity. *Blood*. (2023) 141:2443–51. doi: 10.1182/blood.2022017604

118. Lichtenstein DA, Schischlik F, Shao L, Steinberg SM, Yates B, Wang HW, et al. Characterization of HLH-like manifestations as a CRS variant in patients receiving CD22 CAR T cells. *Blood*. (2021) 138:2469–84. doi: 10.1182/blood.2021011898

119. Hines MR, Keenan C, Maron Alfaro G, Cheng C, Zhou Y, Sharma A, et al. Hemophagocytic lymphohistiocytosis-like toxicity (carHLH) after CD19-specific CAR T-cell therapy. *Br J Haematol*. (2021) 194:701–7. doi: 10.1111/bjh.17662

120. Hines MR, Knight TE, McNerney KO, Leick MB, Jain T, Ahmed S, et al. Immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome. *Transplant Cell Ther*. (2023) 29:438.e1–438.e16. doi: 10.1016/j.jtct.2023.03.006

121. Diorio C, Shraim R, Myers R, Behrens EM, Canna S, Bassiri H, et al. Comprehensive serum proteome profiling of cytokine release syndrome and immune effector cell–associated neurotoxicity syndrome patients with B-cell ALL receiving CAR T19. *Clin Cancer Res*. (2022) 28:3804–13. doi: 10.1158/1078–0432.CCR-22–0822

122. Henter JI, Horne AC, Aricó M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: Diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. *Pediatr Blood Cancer*. (2007) 48:124–31. doi: 10.1002/pbc.21039

123. Fardet L, Galicier L, Lambotte O, Marzac C, Aumont C, Chahwan D, et al. Development and validation of the hscore, a score for the diagnosis of reactive hemophagocytic syndrome. *Arthritis Rheumatol*. (2014) 66:2613–20. doi: 10.1002/art.38690

124. Kim DW, Bukhari A, Lutfi F, Zafforoni F, Merechi F, Mustafa Ali MK, et al. Low utility of the H-Score and HLH-2004 criteria to identify patients with secondary hemophagocytic lymphohistiocytosis after CAR-T cell therapy for relapsed/refractory diffuse large B-Cell lymphoma. *Leuk Lymphoma*. (2022) 63:1339–47. doi: 10.1080/10428194.2021.2024817

125. Shah NN, Highfill SL, Shalabi H, Yates B, Jin J, Wolters PL, et al. CD4/CD8 T-cell selection affects chimeric antigen receptor (CAR) T-cell potency and toxicity: updated results from a phase I anti-CD22 CAR T-cell trial. *J Clin Oncol*. (2020) 38:1938–50. doi: 10.1200/JCO.19.03279

126. McNerney KO, Si Lim SJ, Ishikawa K, Dreyzin A, Vatsayan A, Chen JJ, et al. HLH-like toxicities predict poor survival after the use of tisagenlecleucel in children and young adults with B-ALL. *Blood Adv*. (2023) 7:2758–71. doi: 10.1182/bloodadvances.2022008893

127. Sepulveda FE, Maschalidi S, Vosshenrich CAJ, Garrigue A, Kurowska M, Enasche GM, et al. A novel immunoregulatory role for NK-cell cytotoxicity in protection from HLH-like immunopathology in mice. *Blood*. (2015) 125:1427–34. doi: 10.1182/blood-2014–09-602946

128. Rainone M, Ngo D, Baird JH, Budde LE, Htut M, Aldoss I, et al. Interferon-γ blockade in CAR T-cell therapy-associated macrophage activation syndrome/hemophagocytic lymphohistiocytosis. *Blood Adv*. (2023) 7:533–6. doi: 10.1182/bloodadvances.2022008256

129. Porter TJ, Lazarevic A, Ziggas JE, Fuchs E, Kim K, Byrnes H, et al. Hyperinflammatory syndrome resembling haemophagocytic lymphohistiocytosis following axicabtagene ciloleucel and brexucabtagene autoleucel. *Br J Haematol*. (2022) 199:720–7. doi: 10.1111/bjh.18454

130. Major A, Collins J, Craney C, Heitman AK, Bauer E, Zerante E, et al. Management of hemophagocytic lymphohistiocytosis (HLH) associated with chimeric antigen receptor T-cell (CAR-T) therapy using anti-cytokine therapy: an illustrative case and review of the literature. *Leuk Lymphoma*. (2021) 62:1765–9. doi: 10.1080/10428194.2021.1881507

131. Zhang Q, Zhao YZ, Ma HH, Wang D, Cui L, Li WJ, et al. A study of ruxolitinib response-based stratified treatment for pediatric hemophagocytic lymphohistiocytosis. *Blood*. (2022) 139:3493–504. doi: 10.1182/blood.2021014860

132. Wang J, Wang Y, Wu L, Wang X, Jin Z, Gao Z, et al. Ruxolitinib for refractory/relapsed hemophagocytic lymphohistiocytosis. *Haematologica*. (2020) 105:e210–2. doi: 10.3324/haematol.2019.222471

133. Larson RC, Kann MC, Bailey SR, Haradhvala NJ, Llopis PM, Bouffard AA, et al. CAR T cell killing requires the IFNγR pathway in solid but not liquid tumours. *Nature*. (2022) 604:563–70. doi: 10.1038/s41586–022-04585–5

134. Bracaglia C, De Graaf K, Marafon DP, Guilhot F, Ferlin W, Prencipe G, et al. Elevated circulating levels of interferon-γ and interferon-γ-induced chemokines characterize patients with macrophage activation syndrome complicating systemic juvenile idiopathic arthritis. *Ann Rheum Dis*. (2017) 76:166–72. doi: 10.1136/annrheumdis-2015–209020

135. Johnson TS, Terrell CE, Millen SH, Katz JD, Hildeman DA, Jordan MB. Etoposide selectively ablates activated T cells to control the immunoregulatory disorder hemophagocytic lymphohistiocytosis. *J Immunol*. (2014) 192:84–91. doi: 10.4049/jimmunol.1302282

136. Trottestam H, Horne AC, Aricò M, Egeler RM, Filipovich AH, Gadner H, et al. Chemoimmunotherapy for hemophagocytic lymphohistiocytosis: long-term results of the HLH-94 treatment protocol. *Blood*. (2011) 118:4577–84. doi: 10.1182/blood-2011–06-356261

137. Rosée P, Rosée R, Horne A, Hines M, Von Bahr Greenwood T, Machowicz R, et al. Recommendations for the management of hemophagocytic lymphohistiocytosis in adults. *Blood*. (2019) 133:2465–77. doi: 10.1182/blood.2018894618

138. Cordeiro A, Bezerra ED, Hirayama AV, Hill JA, Wu QV, Voutsinas J, et al. Late events after treatment with CD19-targeted chimeric antigen receptor modified T cells. *Biol Blood Marrow Transpl*. (2020) 26:26–33. doi: 10.1016/j.bbmt.2019.08.003

139. 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) 138:2499–513. doi: 10.1182/blood.2020010543

140. Rejeski K, Subklewe M, Aljurf M, Bachy E, Balduzzi A, Barba P, et al. Immune effector cell-associated hematotoxicity: EHA/EBMT consensus grading and best practice recommendations. *Blood*. (2023) 142:865–77. doi: 10.1182/blood.2023020578

141. 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) 98:1699–710. doi: 10.1002/ajh.27056

142. 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) 16:88. doi: 10.1186/s13045-023-01465-x

143. Miller PG, Sperling AS, Brea EJ, Leick MB, Fell GG, Jan M, et al. Clonal hematopoiesis in patients receiving chimeric antigen receptor T-cell therapy. *Blood Adv*. (2021) 5:2982–6. doi: 10.1182/bloodadvances.2021004554

144. Saini NY, Swoboda DM, Greenbaum U, Ma J, Patel RD, Devashish K, et al. Clonal hematopoiesis is associated with increased risk of severe neurotoxicity in axicabtagene ciloleucel therapy of large B-cell lymphoma. *Blood Cancer Discovery*. (2022) 3:385–93. doi: 10.1158/2643–3230.BCD-21–0177

145. Brudno JN, Natrakul D, Lam N, Dulau-Florea A, Yuan CM, Kochenderfer JN. Acute and delayed cytopenias following CAR T-cell therapy: an investigation of risk factors and mechanisms. *Leuk Lymphoma*. (2022) 63:1849–60. doi: 10.1080/10428194.2022.2056172

146. Jain T, Knezevic A, Pennisi M, Chen Y, Ruiz JD, Purdon TJ, et al. Hematopoietic recovery in patients receiving chimeric antigen receptor T-cell therapy for hematologic Malignancies. *Blood Adv*. (2020) 4:3776–87. doi: 10.1182/bloodadvances.2020002509

147. Juluri KR, Wu QV, Voutsinas J, Hou J, Hirayama AV, Mullane E, et al. Severe cytokine release syndrome is associated with hematologic toxicity following CD19 CAR T-cell therapy. *Blood Adv*. (2022) 6:2055–68. doi: 10.1182/bloodadvances.2020004142

148. Rejeski K, Wu Z, Blumenberg V, Kunz WG, Müller S, Kajigaya S, et al. Oligoclonal T-cell expansion in a patient with bone marrow failure after CD19 CAR-T therapy for Richter-transformed DLBCL. *Blood*. (2022) 140:2175–9. doi: 10.1182/blood.2022017015

149. 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 IFNγ-expressing CD8 T cells. *Cell Rep Med*. (2023) 4:101158. doi: 10.1016/j.xcrm.2023.101158

150. Barreto JN, Bansal R, Hathcock MA, Doleski CJ, Hayne JR, Truong TA, et al. The impact of granulocyte colony stimulating factor on patients receiving chimeric antigen receptor T-cell therapy. *Am J Hematol*. (2021) 96:E399–402. doi: 10.1002/ajh.26313

151. Galli E, Allain V, Di Blasi R, Bernard S, Vercellino L, Morin F, et al. G-CSF does not worsen toxicities and efficacy of CAR-T cells in refractory/relapsed B-cell lymphoma. *Bone Marrow Transpl*. (2020) 55:2347–9. doi: 10.1038/s41409-020-01006-x

152. Liévin R, Di Blasi R, Morin F, Galli E, Allain V, De Jorna R, et al. Effect of early granulocyte-colony-stimulating factor administration in the prevention of febrile neutropenia and impact on toxicity and efficacy of anti-CD19 CAR-T in patients with relapsed/refractory B-cell lymphoma. *Bone Marrow Transpl*. (2022) 57:431–9. doi: 10.1038/s41409–021-01526–0

153. Miller KC, Johnson PC, Abramson JS, Soumerai JD, Yee AJ, Branagan AR, et al. Effect of granulocyte colony-stimulating factor on toxicities after CAR T cell therapy for lymphoma and myeloma. *Blood Cancer J*. (2022) 12:146. doi: 10.1038/s41408–022-00741–2

154. Wat J, Barmettler S. Hypogammaglobulinemia after chimeric antigen receptor (CAR) T-cell therapy: characteristics, management, and future directions. *J Allergy Clin Immunol Pract*. (2022) 10:460–6. doi: 10.1016/j.jaip.2021.10.037

155. Rejeski K, Burchert A, Iacoboni G, Sesques P, Fransecky L, Bücklein V, et al. Safety and feasibility of stem cell boost as a salvage therapy for severe hematotoxicity after CD19 CAR T-cell therapy. *Blood Adv*. (2022) 6:4719–25. doi: 10.1182/bloodadvances.2022007776

156. Gagelmann N, Fischer M, Penack O, Holtick U, Thomson J, Dreger P, et al. Hematopoietic stem cell boost for persistent neutropenia after CAR-T cell therapy: a GLA/DRST study. *Blood Adv*. (2023) 7:555–9. doi: 10.1182/bloodadvances.2022008042

157. Davis JA, Sborov DW, Wesson W, Julian K, Abdallah AO, McGuirk JP, et al. Efficacy and safety of CD34+ stem cell boost for delayed hematopoietic recovery after BCMA directed CAR T-cell therapy. *Transplant Cell Ther*. (2023) 29:567–71. doi: 10.1016/j.jtct.2023.05.012

158. Levine BL, Pasquini MC, Connolly JE, Porter DL, Gustafson MP, Boelens JJ, et al. Unanswered questions following reports of secondary Malignancies after CAR-T cell therapy. *Nat Med*. (2024) 30(2):338–41. doi: 10.1038/s41591-023-02767-w

159. Banerjee R, Poh C, Hirayama AV, Gauthier J, Cassaday RD, Mazyar S, et al. Answering the "Doctor, can CAR-T therapy cause cancer?" question in clinic. *Blood Adv*. (2024) 8(4):895–8. doi: 10.1182/bloodadvances.2023012336

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 05 June 2024
DOI 10.3389/fonc.2024.1384600

OPEN ACCESS

EDITED BY
Marcos De Lima,
The Ohio State University, United States

REVIEWED BY
Laura Magnano,
Hospital Clinic of Barcelona, Spain
Jean Koff,
Emory University, United States

*CORRESPONDENCE
Ryan Jacobs
ryan.jacobs@atriumhealth.org

RECEIVED 09 February 2024
ACCEPTED 29 April 2024
PUBLISHED 05 June 2024

CITATION
Jacobs R and Jacobson C (2024) The treatment of follicular lymphoma with CD19-directed chimeric antigen receptor T-cell therapy.
Front. Oncol. 14:1384600.
doi: 10.3389/fonc.2024.1384600

The treatment of follicular lymphoma with CD19-directed chimeric antigen receptor T-cell therapy

Ryan Jacobs[1]* and Caron Jacobson[2]

[1]Levine Cancer Institute, Charlotte, NC, United States, [2]Dana–Farber Cancer Institute, Boston, MA, United States

Follicular lymphoma (FL) is the most common indolent non-Hodgkin lymphoma. Significant unmet need remains for patients with relapsed/refractory FL after ≥3 lines of prior therapy. While recent advancements have likely improved the survival of patients with FL, most patients will eventually relapse. The treatment of patients with FL after multiple relapses or those with refractory disease has historically led to lower overall response rates (ORR) and shorter progression-free survival (PFS) with each subsequent line of therapy. New treatments with high ORR and durable PFS are needed in this setting, particularly in patients that progress within 2 years of first line chemoimmunotherapy (POD24) and/or those refractory chemoimmunotherapy. Chimeric antigen receptor T-cell therapies targeting the B-cell antigen CD-19 have shown to be an efficacious treatment option for both heavily pretreated patients and/or patients with refractory FL, resulting in a high ORR and durable remissions.

KEYWORDS

lymphoma, follicular lymphoma, CAR T, axicabtagene ciloleucel, tisagenlecleucel (tisa-cel, Kymriah), lisocabtagene maraleucel, mosunetuzumab

Introduction

In the United States, follicular lymphoma (FL) is the most common indolent non-Hodgkin lymphoma (NHL), accounting for approximately 35% of NHLs, and has an estimated incidence of 3.18 cases per 100,000 people (1). Numerous risk factors have been purported to be linked to FL, but none have been validated, and the incidence has been stable over time. FL has increased prevalence in white populations where the incidence is more than twice that in African and Asian populations (2). The incidence of FL increases with age with the median age at diagnosis being 65 years (3).

Significant breakthroughs have been made in the treatment of FL in recent decades. Effective frontline treatment with chemoimmunotherapy involving anti-CD20 antibodies has led to durable remissions in most patients (4, 5). However, about 20%–25% of patients will have significantly shorter progression-free survival (PFS) and early progression after

chemoimmunotherapy within the first 2 years from initial treatment (POD24). These patients have been shown to have poor long-term outcomes and reduced overall survival (OS) (6). Additionally, the treatment of relapsed/refractory (R/R) FL after ≥2 lines of prior therapy is associated with progressive shortening of PFS with each line of treatment (7–9). The approval of new classes of drugs including immunomodulatory agents and epigenetic modulators has improved outcomes for patients with multiple R/R FL, but these patients continue to represent an unmet need (10).

Recently, three novel therapies that engage T cells have been approved by the Food and Drug Association (FDA) and have been incorporated into the treatment armamentarium of patients with FL with R/R disease. They include CD20/CD3-bispecific antibody mosunetuzumab and the CD19-directed chimeric antigen receptor (CAR) T-cell therapies axicabtagene ciloleucel (axi-cel) and tisagenlecleucel (tisa-cel) (11, 12). We will further discuss the commercial use of axi-cel and tisa-cel as well as review the available data on an additional CD19-directed CAR T-cell therapy lisocabtagene maraleucel (liso-cel) in the treatment of patients with R/R FL. Additionally, we will contrast the use of CAR T-cell therapy with mosunetuzumab as well as review some initial data of new investigational CAR T-cell technologies.

Early studies in CD19 CAR T cells used in the treatment of FL

Targeting CD19 with CAR T-cell therapy was initially explored in lymphoma in R/R diffuse large B-cell lymphoma (DLBCL), where it revolutionized the natural history of chemorefractory disease and, as a result, changed the treatment paradigm and outlook for these patients (13–15). As CD19 is widely expressed in B-cell malignancies, assessing the efficacy in high-risk multiple relapsed FL, where no standard therapy was established, was likewise investigated. Initial reports of activity in R/R FL came out of early-phase studies from the National Cancer Institute investigating the use of a CD19-targeted CAR T-cell product with a CD28 costimulatory domain that would become axi-cel. In an early report of activity seen in a patient with FL, Kochenderfer et al. described dramatic lymphoma regression and noted that B-cell precursors were selectively eliminated from the patient's bone marrow for approximately 39 weeks. The targeted and prolonged elimination of B-lineage cells indicated eradication of CD19+ B cells that were antigen-specific and that the adoptive transfer of anti–CD19-CAR T cells could be a promising approach for treating B-cell malignancies like FL (16). Longer-term follow-up confirmed activity in a larger number of patients with FL, where a 3-year duration of response (DOR) was 63% for the eight patients treated with low-grade lymphoma on this study (17, 18).

Tisa-cel was originally developed from CTL019, whose activity in FL was first reported by Schuster et al., where 15 patients with R/R FL were treated with CTL019 with high overall response rate (ORR) and a Complete Remission (CR) rate of 71% (19). Median peak expansion of the CTLO19 cells appeared to occur later (median of 8 days) than the CD28 co-stimulated axi-cel, with a more gradual expansion of CAR T cells over time but increased area under the curve (20). A 5-year update was later reported on these patient and was encouraging, with a 5-year PFS of 43% and median DOR having not been reached (21).

Hirayama et al. later reported the results from the Fred Hutchinson Cancer Research Center where eight patients with R/R FL were treated on a phase I/II trial with CD19 CAR T cells on a 1:1 ratio of CD4/CD8+ T cells and the co-stimulatory molecule 4-1BB that would later become liso-cel (22). The reported ORR was high, and the majority of the patients with FL treated (88%) obtained a CR. Of the patients with FL who achieved CR, all remained in remission at a median follow-up of 24 months. The tolerance appeared acceptable, and no severe cytokine release syndrome (CRS) or immune effector cell–associated neurotoxicity (ICANS) events were observed.

The promising results of these studies led to the pivotal, single-arm phase 2 studies of axi-cel, tisa-cel, and liso-cel in multiple relapsed and refractory FL, the results from which have either resulted in FDA approvals (axi-cel and tisa-cel) or are pending FDA review for approval (liso-cel).

Axicabtagene ciloleucel

Axi-cel, originally labeled as KTE-C19, is an autologous CAR composed of an extracellular domain targeting CD19, a transmembrane domain, and an intracellular signaling domain composed by a CD28 co-stimulatory molecule.

In March of 2021, the FDA granted accelerated approval to axi-cel for adult patients with R/R FL after two or more lines of systemic therapy based on the results from the ZUMA-5 study. ZUMA-5 was a single-arm, multicenter, phase II trial that included 124 patients with R/R FL requiring treatment as well as 24 patients with marginal zone lymphoma (12). The majority of the patients with FL in ZUMA-5 were stage IV (85%) and with bulky disease (52%). More than half of the patients were identified as POD24 (55%), with a median prior lines of treatment of 3 (range of 2–4) and 63% of patients having had three or more lines of therapy. After lymphodepleting chemotherapy (LDC) with fludarabine and cyclophosphamide, the patients received a single infusion of axi-cel. Among 84 patients with FL eligible for the primary analysis, Jacobson et al. reported a high ORR at 94%, with 79% of patients achieving a CR. Fifty-five percent of the patients with FL treated on the ZUMA-5 trial were identified as POD24, and the outcomes of POD24 patients after receiving axi-cel did not differ significantly from the overall patient population. With a median follow-up of 23.3 months in the original manuscript, the median PFS had not been reached. CRS occurred in 78% patients with FL. Most cases of CRS were grade 1 or 2 [89 (72%)], and grade 3 or worse CRS occurred in eight (6%) patients. For the management of CRS, tocilizumab was administered to 50% and corticosteroids to 18% of the total ZUMA-5 patient population. One grade 5 event of multisystem organ failure leading to death on day 7 was reported in a patient with FL with bulky disease at baseline per GELF criteria. ICANS occurred in 56% of patients with FL, with grade 1 or 2

events in 51 (41%) and grade 3 or 4 events occurring in 19 (15%). No grade 5 neurological events occurred. For management of ICANS, corticosteroids were used in 53 (36%) patients, and tocilizumab was used in nine (6%) of the total ZUMA-5 patient population. Median duration of ICANS was 14 days (IQR of 5–43) in patients with FL, and two patients had ongoing ICANS at the time of publication (one patient with ongoing memory loss and patient with persistent paresthesia).

With additional follow-up at 3 years (median of 40.5 months), Neelapu et al. reported on patients in ZUMA-5 who had exposure to bendamustine within 6 months of LDC had shorter PFS after axi-cel, thought possibly related to the lymphotoxic effects of bendamustine (23). Most recently, Neelapu et al. reported a 4-year follow-up (median of 52.5 months) where the median DOR in patients with FL had lengthened to 55.5 months (24). Patients with a best response of CR had excellent outcomes with median DOR of 60.4 months. Patients that did not achieve CR fared far worse, with a 4.9-month median DOR reported in patients that only achieved a partial response. This longer follow-up led to an improved median PFS of 57.3 months in the patients with FL with an estimated 48-month PFS rate of 53% and a median OS that had still not been reached. Remarkably, only one patient with FL had progressed in interim between the 28 month and 48 month analysis (Table 1).

Longer follow-up is needed to assess the curative potential of axi-cel in patients with FL. The ZUMA-22 trial is an ongoing phase 3 randomized study evaluating the benefit of axi-cel compared to standard-of-care therapy for patients with R/R FL. In the absence of available prospective data comparing axi-cel with standard of care (SOC), Ghione et al. compared the results from ZUMA-5 with the International Scholar-5 cohort of patients with R/R FL treated with a third or higher line of SOC at seven different multinational institutions as well data from the phase 2 study of idelalisib in r/r FL (27, 28). This comparative and weighted analysis showed superiority in outcomes related to axi-cel relative to SOC: the median PFS was 57.3 months *vs.* 13.0 months [hazard ratio (HR), 0.27; 95% confidence interval (CI), 0.18–0.40), respectively. Median OS was NR in either study, but, at 48 months, OS was 72.4% in ZUMA-5 compared to 61.4% in SCHOLAR-5. The results strongly suggest the superior efficacy of axi-cel for R/R FL compared to other available SOC therapies.

The activity and safety of axi-cel outside of a clinical trial have been investigated by Jacobson et al. through a CIBMTR registry study where 151 patients with R/R FL underwent treatment with axi-cel and had evaluable assessments post-infusion (29). Forty percent of the patients in this registry study would have been ineligible for ZUMA-5, mainly due to comorbidities. Patients had a median of 4 (range of 1–13) lines of prior therapy. ORR and CR rates were 93% and 84%, respectively, and estimated PFS and OS at 6 months were 88% and 96%, respectively. Grade ≥ 3 CRS and ICANS occurred in 2% and 13% of patents, respectively. The median cumulative CRS resolution was seen by 5 days, and ICANS resolution was resolved on average by day 4. Of the patients that were alive at day 30 (n = 150), 11% had prolonged cytopenias (9% thrombocytopenia and 4% neutropenia). PFS and OS at 6 months were comparable regardless of ZUMA-5 eligibility. The patients that would have met eligibility criteria for ZUMA-5 experienced fewer grade ≥ 3 ICANS (10% *vs.* 16%) and more rapid ICANS resolution (92% *vs.* 71% resolved within 2 weeks). Patients aged ≥ 65 years *vs.* < 65 years were shown to have comparable effectiveness and safety profiles, supporting the safety and efficacy of axi-cel older patients with FL. The CIBMTR registry study confirmed that axi-cel demonstrates effectiveness and safety profiles consistent with those observed in the ZUMA-5 when used in a broader patient population outside of clinical trial.

Tisagenleucel

Tisa-cel is an autologous CAR T-cell composed of an extracellular domain targeting CD19 like axi-cel but differs in that it is constructed with a 4-1BB co-stimulatory molecule as opposed to CD28. The encouraging efficacy and tolerability of CTL019 reported by Dr. Schuster et al. at the University of Pennsylvania spurred the further clinical development of tisa-cel in FL. The phase II ELARA trial investigated the efficacy of tisa-cel in a larger R/R FL patient population. Ninety-seven patients with FL with at least two lines of prior therapy or who were relapsing after autologous hematopoietic stem cell transplant (HSCT) received a single infusion of tisa-cel following LDC (fludarabine and cyclophosphamide). ORR was reported at 86%, with 69% of the patient population achieving a CR (30). CRS was seen in 49% and ICANS in 4% of patients, but these events were generally low grade; grade 3 or 4 CRS and ICANS were reported in 0% and 1% of patients, respectively. There were no treatment related deaths.

TABLE 1 Comparison of efficacy and safety of CAR T-cell therapy in patients with R/R FL.

Car T-cell product	Reference	Number of patients with R/R FL	ORR (%)	CR (%)	Median PFS [months (mo)]	Median OS	Median DOR Months (mo)	CRS (grade 3+)	ICANS (grade 3+)
Axi-cel	ZUMA-5 (4-year update) (24)	124	94	79	57.3 mo	NR	55 mo	78% (6%)	56% (18%)
Tisa-cel	ELARA (3-year update) (25)	97	86	68	37 mo	NR	NR	49% (0%)	23% (1%)
Liso-cel	TRANSCEND-FL (26)	101	97	94	12 mo, 81%	NR	12 mo, 82%	58% (1%)	15% (2%)

On 27 May 2022, the FDA granted accelerated approval to tisa-cel for adult patients with patients with R/R FL after two or more lines of systemic therapy based on the outcomes seen in the ELARA trial. The study was later updated with 3-year follow-up (median follow-up of 41 months), and, at 36 months, 53% of patients remained in CR and the median PFS of the patient population was 37 months (25). Median DOR was NR (Table 1). Sixty-three percent of the patients treated on ELARA were identified as POD24, and, within the POD24 subgroup, 36-month PFS was 50% (n = 61) compared with 59% for patients without POD24 (n = 33). Persistence of CAR transgene was observed for up to 1,290 days. The patients that were not POD24 had higher median *in vivo* CAR expansion and longer persistence than patients with POD24.

Salles et al. utilized the data from the ELARA study to perform a comparative effectiveness analysis that matched tisa-cel–treated patients to similar patients in a historical control. The data from the control was utilized to perform a matched adjusted retrospective comparison of patients with R/R FL treated with SOC interventions to similar patients treated with tisa-cel. This analysis showed tisa-cel to be more efficacious than SOC: ORR was 86% for tisa-cel versus 64% for SOC; 12 month PFS 70.5% versus 52%; and 12-month OS was 97% for tisa-cel compared to 72% SOC (31).

In the ELARA study, tisa-cel was administered in the outpatient setting in 18% (17/97) of patients (30). Fowler et al. later evaluated the hospitalization costs and the amount of healthcare resource utilization for the patients with R/R FL undergoing CAR T-cell therapy with tisa-cel comparing inpatient administration versus outpatient administration. Patients infused in the outpatient setting generally had favorable Eastern Cooperative Oncology Group (ECOG) performance status and Follicular Lymphoma International Prognostic Index scores and less bulky disease at baseline (32). For the patients treated in the outpatient setting, 41% did not require hospitalization within 30 days after infusion, and the patients who did ultimately require hospitalization had a shorter average length of stay compared with the patients that received inpatient tisa-cel administration (5 days versus 13 days). Efficacy between the two groups was similar, and the calculated cost of care was reduced for those patients that received treatment in the outpatient setting. These findings supported the premise that some R/R patients can be safely treated with tisa-cel in the outpatient setting, thus reducing hospitalization costs and healthcare resource utilization.

Dickinson et al. performed a match-adjusted indirect comparison (MAIC) of the results reported in the ELARA and ZUMA-5 trials. The results showed that tisa-cel (n = 52), compared with axi-cel (n = 86), had similar ORR (91.2% *vs.* 94.2%; p = 0.58), CR rate (74.0% *vs.* 79.1%; p = 0.60), PFS [HR (95% CI), 0.8 (0.4, 1.9); p = 0.67], and OS [HR (95% CI), 0.5 (0.2, 1.5); p = 0.21] (33). Tisa-cel was associated with more favorable safety outcomes than axi-cel, with lower rates of any grade and grade ≥3 CRS and ICANS seen in patients treated with tisa-cel. After matching, the rates for any grade and grade ≥3 CRS were 33.7% and 6.5% lower ($p < 0.01$), respectively, and any grade and grade ≥3 ICANS were 47.0% and 15.1% lower ($p < 0.001$), respectively, in patients infused with tisa-cel *vs.* axi-cel. The proportions of patients who received tocilizumab and corticosteroids for any grade CRS were 35.3% ($p < 0.001$) and 12.3% lower ($p < 0.01$), respectively, in patients infused with tisa-cel *vs.* axi-cel.

Lisocabtagene maraleucel

Liso-cel is also an autologous is a CD19 targeting CAR T-cell with a 4-1BB co-stimulatory molecule with the final product containing CD4:CD8 T cells in a 1:1 ratio. Liso-cel is currently approved by the FDA for second-line or later treatment of DLBCL and is under active investigation for the treatment of R/R FL.

The TRANSCEND FL study is a single-arm, multicenter phase II study where patients with R/R FL who had previously received 1 or 2+ lines of therapy were treated with a single infusion of liso-cel following LDC (fludarabine and cyclophosphamide). The primary analysis of 124 patients treated in the third line was presented with high ORR at 97%, and nearly all of the responding patients achieving a CR (94%) despite being a high-risk patient population where 43% of patients were POD24 (26). With approximately 17 months of median follow-up, 12-month DOR and PFS in patients treated with liso-cel were 81.9% and 80.7%, respectively. CRS occurred in 58% of patients (grade 3, 1%; no grade 4–5) and ICANS in 15% (grade 3, 2%; no grade 4–5). There was one death due to a treatment related adverse event from a grade 5 macrophage activation syndrome (Table 1).

Mosunetuzumab

Mosunetuzumab is a CD20/CD3-bispecific monoclonal antibody that engages endogenous T cells to attack malignant CD20-expressing B cells, showing high response rates and encouraging tolerability in a large phase 2 study. In contrast to the single infusion of CAR T-cell therapy, mosunetuzumab is administered intravenously weekly for the first 3 weeks as part of a double step-up dose and then every 3 weeks for a total of 8 cycles. Nine additional cycles can be administered if complete response is not initially achieved after eight cycles. Budde et al. reported outcomes on 90 patients with median follow-up of 18.3 months; ORR 78% and 60% of patients achieved a CR (11). CRS was the most common adverse event reported at 44% but was predominantly grade 1/2, with only 1% experiencing higher grade CRS. No treatment-related fatal adverse event occurred. Extended 3-year follow-up showed median DOR was 35.9 months, median duration of CR was NR, and the estimated 30-month duration of CR rate was 72.4% (34). Three years after the completion of treatment, 57% of 70 responding patients were alive and had not had disease progression, and the overall median PFS was 24.0 months (Table 2).

CAR T cells versus mosunetuzumab

The approvals of CD19 CAR T cells and CD20 bispecifics for multiple relapsed FL are transformative for this high-risk group and based on vastly improved depth and DOR and are expected to

TABLE 2 CAR T-cell therapy compared to mosunetuzumab.

	Mosunetuzumab	Axi-cel	Tisa-cel	Liso-cel
Trial	Schuster et al. 3-year update (34)	ZUMA-5 (4-year update) (24)	ELARA (3-year update) (25)	TRANSCEND-FL (26)
Number of patients with R/R FL	90	124	97	101
Median prior lines of therapy	3	3	3	3
Median PFS	24 mo (58% at 12 mo)	57 mo (80% at 12 mo)	37 mo (75% at 12 mo)	Median NR at 16 mo (81% at 12 mo)
ORR	78%	94%	86%	97%
CR	60%	79%	68%	94%
CRS (grade 3+)	44% (2%)	78% (6%)	49% (0%)	58% (1%)
ICANS (grade 3+)	5% (0%)	56% (18%)	23% (1%)	15% (2%)

improve survival for this group of patients. How to sequence these new therapies in the third line and beyond is not yet established and an area of debate. With a disease like FL, where treatments are not historically curative but patients live for a long time, balancing treatment efficacy and toxicity as well as cost becomes a priority. On the one hand, CD19 CAR T cells represent a one-time treatment with the longest PFS of any available therapies in this space. On the other, it is an expensive and logistically complicated therapy only offered at select centers and does carry risks of higher grade CRS and ICANS compared with the CD20 bispecifics. No randomized trials yet exist to determine which is best for these patients, and so treatment decisions are often made for individual patients, taking into account their disease risk features and their preferences in order to develop an individualized treatment plan.

Nastoupil et al. conducted a MAIC of liso-cel patients from the TRANSCEND FL trial versus published data of mosunetuzumab in patients with 3L+ FL. Their analysis showed liso-cel was associated with higher ORR [odds ratio (OR), 3.78 (95% CI, 1.48–9.67)] and CR [OR, 6.46 (2.85–14.65)], as well as improved PFS [HR, 0.28 (0.16–0.49)] with the results remaining consistent across all scenario analyses (35). Liso-cel was associated with higher incidence of CRS [OR, 1.86 (1.01–3.43)] and ICANS [OR, 2.16 (0.72–6.44)], but liso-cel demonstrated a lower incidence of grade ≥ 3 CRS [OR, 0.45 (0.04–5.13)] and grade 3–4 serious infections [OR, 0.35 (0.12–1.03)] compared with mosunetuzumab. Additionally, liso-cel was associated with overall lower use of steroids for CRS management [OR, 0.14 (0.03–0.65)], but the use of tocilizumab was higher in liso-cel treated patients [OR, 2.27 (0.86–5.99)]. Although imperfect, analyses such as these strongly suggest that logistical considerations and cost effectiveness aside, CD19 CAR T cells with a 4-1BB costimulatory domain are more effective and equally tolerated compared with CD20 bispecifics. Cost analyses have been conflicting, with some data suggesting CAR T-cell therapy is the more cost-effective choice, whereas other data suggesting the opposite (36, 37). At this time, there is no official guidance as to which therapeutic modality (CAR T-cell therapy versus mosunetuzumab) should be sequenced first in patients with R/R FL. With ongoing investigations of moving bispecific antibodies into earlier lines of treatment, both as single agents and in combinations with other treatments, the argument over which treatment to sequence first may soon become irrelevant.

Future directions

CD19 antigen loss has been reported as a mechanism of resistance for patients that relapse after receiving CD19-directed CAR T-cell therapy. Development of CAR T cells that are directed at other antigens or bispecific CAR T cells with more than one target provides a possible answer for patients with relapses driven from CD19 antigen loss. CD20 CAR T cells and dual targeting of CD20 and CD19 with both bispecifics and CAR T cells are also in development. Shadman et al. reported on a pilot study investigating MB-106, which is a third-generation CD20-directed CAR T-cell constructed with both 4-1BB and CD28 co-stimulatory domains. Three patients with FL were part of the initial 11-patient NHL cohort reported, and two of the three patients with FL achieved a CR, whereas the third patient progressed (38). This was followed by a subsequent report where MB-106 was trialed specifically in 20 patients with R/R FL with a median of 4 prior lines of therapy. ORR was 95%, and the rate of CR was 80%, with overall better responses seen in patients treated at the higher doses of MB-106 (39). Notably, there was one patient on the study who had progressed after previously receiving a CD19-directed CAR, and this patient achieved a CR with MB-106. No grade 3 or 4 CRS or ICANS was observed. Both Shah and Tong et al. have reported separate first in human data on trials of bispecific anti-CD20, anti-CD19 CAR T cells. While these studies only included a small number of patients with R/R FL, they do provide a proof of concept in showing efficacy of these bispecific CAR-T agents in this patient population (40, 41).

Potential limitations of autologous CAR T-cell agents include logistics, product availability manufacturing, and quality consistency. Allogeneic CAR T-cell therapy derived from healthy donors offer an alternative to the available autologous CAR T-cell products and may be able to circumvent these challenges. The ability to infuse treatment to the patient more quickly with these products being available off the shelf and not requiring apheresis and subsequent cell manufacturing may be advantageous in some circumstances, such as patients with

very aggressive disease. Locke et al. have reported on safety outcomes of the ALPHA trial, a phase 1 trial exploring the safety and efficacy of an anti-CD19 allogeneic CAR T-cell administered over split doses that utilizes gene editing to control for host lymphocyte rejection. The trial included both R/R DLBCL (n = 61) and FL (n = 26) where 20 patients (23%) experienced CRS, which were low grade except for one (1%) grade 3 event. No graft-versus-host disease or grade ≥3 ICANS occurred (42). These safety data are encouraging, and we will await further reports of efficacy of allogeneic CAR T-cell therapies in patients with R/R FL.

Expert opinion

CAR T-cell therapies now represent an additional effective tool in the treatment armamentarium of patients with R/R FL. When considering CAR T-cell therapy alongside other approved options for 3L+ FL, the one-time infusion of CAR T cells is unique to the other available options with more protracted treatment schedules, which may have implications for toxicity risks that accumulate over time, like infection. The high rates of responses to CAR T-cell therapy and its ability to produce durable responses in heavily pretreated patients make it appealing and appear to be superior to the SOC in indirect comparisons. The activity of CAR T-cell therapy is particularly encouraging for patients' refractory to chemoimmunotherapy and POD24 patients who had historically had a poor prognosis with SOC therapies. The side effects overall appear to be acceptable, with low rates of grade 3+ CRS or ICANS, particularly with the 4-1BB CAR T cells' tisa-cel and liso-cel. The real-world analyses of the efficacy and safety of patients treated with axi-cel outside of clinical trials are reassuring that these treatments can be safely administered to patients commercially with similar efficacy observed, even in patients that would not have qualified for the clinical trials that led to their approval.

With these data in mind, it is our general approach to prioritize CAR T-cell therapy for all patients with 3L+ FL who have a history of POD24 or refractory disease, as well as for patients who are both suitable candidates and for whom a one-time therapy or a therapy with the longest PFS is preferred. This is especially true for younger patients whose life expectancy is anticipated to be shortened by their multiple relapsed FL. We previously referenced reports from patients in ZUMA-5 who had exposure to bendamustine within 6 months of LDC had shorter PFS after axi-cel, and similar concerning findings regarding bendamustine's negative impact on the outcome of CAR T-cell therapy in DLBCL have been reported (23, 43). Based on these data, we avoid the use of bendamustine within 6 months of leukapheresis for CAR T-cell therapy. Thankfully, this rarely occurs in 3L+ FL given the common use of bendamustine in the frontline and the long median DOR to frontline bendamustine and CD20 monoclonal antibody therapy.

Barriers remain for the widespread utilization of CAR T cell, however, with the high cost of treatment and lack of access to CAR T-cell centers proving to be insurmountable barriers to many patients. Thus, for older patients, for patients with certain medical comorbidities for whom CAR T-cell therapy would be contraindicated, and for lower-risk patients, including those who have enjoyed long remissions from their first- and second line-therapies, and those who prefer a therapy that can be given locally and without hospitalization, we prioritize treatment with mosunetuzumab.

Conclusion

Patients with R/R FL have a growing number of heterogeneous treatment options. Historically, treatments in the 3L+ setting often involved additional rounds of immunochemotherapy or anti-CD20 monotherapy, lenalidomide, PI3K inhibitors (which subsequently have been withdrawn from the marketplace), as well as HSCT. No consensus on the sequencing of these agents exists at this time. While high response rates to some of these above therapies have been observed, the DOR was usually short and diminished with each subsequent line of therapy (44). Both CD20 bispecifics and CD19 CAR T cells have made significant inroads in the treatment landscape for FL compared to these previous standards.

Data from the most recent follow-up of mosunetuzumab treatment in patients with R/R FL have shown that many responses to this CD20-bispecific also appear durable. It is likely that both bispecific antibodies and CAR T-cell therapies will have significant roles in the future management of R/R FL, but their sequencing remains to be defined. Mosunetuzumab has the benefit of being an off-the-shelf immunotherapy that avoids some of the challenges with logistics and product availability associated with current CAR T-cell therapies, including distance from a CAR T-cell treatment center and the need to relocate there, time to insurance approval, pheresis, manufacturing time, lymphodepleting chemotherapy, and potential hospitalization. The need for LDC also contributes to protracted cytopenias and T-cell lymphopenia in a portion of patients, and the long-term incidence of myeloid dysplasias and risk for T-cell malignancies has not been comprehensively documented and understood (45). No doubt, these risks, once defined, will help shape this debate. However, CAR T-cell therapy is a one-time therapy and offers the longest PFS of any therapy in the multiple relapsed setting, bispecifics included, and, for some patients, this may represent a definitive therapy. Encouragingly, the more typical CAR T-cell side effects of CAR T cells, CRS and ICANS, are lower than those seen in LBCL, particularly with the 4-1BB CAR T cells where these risks seem similar to those seen with mosunetuzumab. How oncologists and patients balance these relative risks and benefits will shape how these therapies are used; by the time this is sorted out, however, the debate is likely to be moot given the likely use of bispecifics in 1L FL, with CAR T cells reserved for select patients in the 2L and 3L settings. Regardless of sequencing preferences, we are better off for having a multitude of options for patients who need them.

Author contributions

RJ: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. CJ: Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

RJ: Consulting for KITE/Gilead and Genentech CJ: Consulting for Kite/Gilead, BMS/Celgene, Novartis, ImmPACT Bio, ADC Therapeutics, Abbvie, AstraZeneca, Caribou Bio, Galapagos, Appia Bio, Synthekine, Janssen, Sana.

Publisher's note

References

1. Morton LM, Wang SS, Devesa SS, Hartge P, Weisenburger DD, Linet MS. Lymphoma incidence patterns by WHO subtype in the United States, 1992-2001. *Blood*. (2006) 107:265–76. doi: 10.1182/blood-2005-06-2508

2. Biagi JJ, Seymour JF. Insights into the molecular pathogenesis of follicular lymphoma arising from analysis of geographic variation. *Blood*. (2002) 99:4265–75. doi: 10.1182/blood.V99.12.4265

3. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. The non-hodgkin's lymphoma classification project. *Blood*. (1997) 89:3909–18. doi: 10.1182/blood.V89.11.3909

4. Flinn IW, van der Jagt R, Kahl BS, et al. Randomized trial of bendamustine-rituximab or R-CHOP/R-CVP in first-line treatment of indolent NHL or MCL: the BRIGHT study. *Blood*. (2014) 123:2944–52. doi: 10.1182/blood-2013-11-531327

5. Rummel MJ, Niederle N, Maschmeyer G, et al. Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase 3 non-inferiority trial. *Lancet*. (2013) 381:1203–10. doi: 10.1016/S0140-6736(12)61763-2

6. Gao F, Zhang T, Liu H, et al. Risk factors for POD24 in patients with previously untreated follicular lymphoma: a systematic review and meta-analysis. *Ann Hematol*. (2022) 101:2383–92. doi: 10.1007/s00277-022-04914-8

7. Batlevi CL, Sha F, Alperovich A, et al. Follicular lymphoma in the modern era: survival, treatment outcomes, and identification of high-risk subgroups. *Blood Cancer J*. (2020) 10:74. doi: 10.1038/s41408-020-00340-z

8. Ghione P, Palomba ML, Ghesquieres H, et al. Treatment patterns and outcomes in relapsed/refractory follicular lymphoma: results from the international SCHOLAR-5 study. *Haematologica*. (2023) 108:822–32. doi: 10.3324/haematol.2022.281421

9. Link BK, Day BM, Zhou X, et al. Second-line and subsequent therapy and outcomes for follicular lymphoma in the United States: data from the observational National LymphoCare Study. *Br J haematology*. (2019) 184:660–3. doi: 10.1111/bjh.15149

10. Qualls D, Salles G. Prospects in the management of patients with follicular lymphoma beyond first-line therapy. *Haematologica*. (2022) 107:19–34. doi: 10.3324/haematol.2021.278717

11. Budde LE, Sehn LH, Matasar M, et al. Safety and efficacy of mosunetuzumab, a bispecific antibody, in patients with relapsed or refractory follicular lymphoma: a single-arm, multicentre, phase 2 study. *Lancet Oncol*. (2022) 23:1055–65. doi: 10.1016/S1470-2045(22)00335-7

12. Jacobson CA, Chavez JC, Sehgal AR, et al. Axicabtagene ciloleucel in relapsed or refractory indolent non-Hodgkin lymphoma (ZUMA-5): a single-arm, multicentre, phase 2 trial. *Lancet Oncol*. (2022) 23:91–103. doi: 10.1016/S1470-2045(21)00591-X

13. Abramson JS, Palomba ML, Gordon LI, et al. Lisocabtagene maraleucel for patients with relapsed or refractory large B-cell lymphomas (TRANSCEND NHL 001): a multicentre seamless design study. *Lancet*. (2020) 396:839–52. doi: 10.1016/S0140-6736(20)31366-0

14. 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) 20:31–42.

15. Schuster SJ, Bishop MR, Tam CS, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *New Engl J Med*. (2019) 380:45–56. doi: 10.1056/NEJMoa1804980

16. Kochenderfer JN, Wilson WH, Janik JE, et al. Eradication of B-lineage cells and regression of lymphoma in a patient treated with autologous T cells genetically engineered to recognize CD19. *Blood*. (2010) 116:4099–102. doi: 10.1182/blood-2010-04-281931

17. Cappell KM, Sherry RM, Yang JC, et al. Long-term follow-up of anti-CD19 chimeric antigen receptor T-cell therapy. *J Clin Oncol Off J Am Soc Clin Oncol*. (2020) 38:3805–15. doi: 10.1200/JCO.20.01467

18. Kochenderfer JN, Dudley ME, Kassim SH, et al. Chemotherapy-refractory diffuse large B-cell lymphoma and indolent B-cell Malignancies can be effectively treated with autologous T cells expressing an anti-CD19 chimeric antigen receptor. *J Clin Oncol Off J Am Soc Clin Oncol*. (2015) 33:540–9. doi: 10.1200/JCO.2014.56.2025

19. Schuster SJ, Svoboda J, Chong EA, et al. Chimeric antigen receptor T cells in refractory B-cell lymphomas. *New Engl J Med*. (2017) 377:2545–54. doi: 10.1056/NEJMoa1708566

20. Ayuk FA, Berger C, Badbaran A, et al. Axicabtagene ciloleucel in *vivo* expansion and treatment outcome in aggressive B-cell lymphoma in a real-world setting. *Blood Adv*. (2021) 5:2523–7. doi: 10.1182/bloodadvances.2020003959

21. Chong EA, Ruella M, Schuster SJ. Five-year outcomes for refractory B-cell lymphomas with CAR T-cell therapy. *New Engl J Med*. (2021) 384:673–4. doi: 10.1056/NEJMc2030164

22. Hirayama AV, Gauthier J, Hay KA, et al. High rate of durable complete remission in follicular lymphoma after CD19 CAR-T cell immunotherapy. *Blood*. (2019) 134:636–40. doi: 10.1182/blood.2019000905

23. Neelapu SS, Chavez J, Sehgal AR, et al. 3-year follow-up analysis of ZUMA-5: A phase 2 study of axicabtagene ciloleucel (Axi-cel) in patients with relapsed/refractory (R/R) indolent non-hodgkin lymphoma (iNHL). *Blood*. (2022) 140:10380–3. doi: 10.1182/blood-2022-156120

24. Neelapu SS, Chavez JC, Sehgal AR, et al. Axicabtagene ciloleucel (Axi-cel) in patients with relapsed/refractory indolent non-hodgkin lymphoma: 4-year follow-up from the phase 2 ZUMA-5 trial. *Blood*. (2023) 142:4868–. doi: 10.1182/blood-2023-174914

25. Schuster SJ, Fowler N, Dickinson M, et al. Clinical outcomes of patients with relapsed/refractory follicular lymphoma treated with tisagenlecleucel: phase 2 elara 3-year follow-up. *Blood*. (2023) 142:601–. doi: 10.1182/blood-2023-180936

26. Morschhauser F, Dahiya S, Palomba ML, et al. TRANSCEND FL: PHASE 2 STUDY RESULTS OF LISOCABTAGENE MARALEUCEL (LISO-CEL) IN PATIENTS (PTS) WITH RELAPSED/REFRACTORY (R/R) FOLLICULAR LYMPHOMA (FL). *Hematological Oncol*. (2023) 41:877–80. doi: 10.1002/hon.3196_LBA4

27. Ghione P, Palomba ML, Patel AR, et al. Comparative effectiveness of ZUMA-5 (axi-cel) vs SCHOLAR-5 external control in relapsed/refractory follicular lymphoma. *Blood*. (2022) 140:851–60. doi: 10.1182/blood.2021014375

28. Gribben JG, Ghione P, Palomba ML, et al. An updated comparison of clinical outcomes from 4-year follow-up of zuma-5 (Axicabtagene ciloleucel) and the international scholar-5 external control cohort in relapsed/refractory follicular lymphoma. *Blood*. (2023) 142:4869–. doi: 10.1182/blood-2023-186842

29. Jacobson CA, Hemmer MT, Hu Z-H, et al. Real-world early outcomes of axicabtagene ciloleucel for relapsed or refractory (R/R) follicular lymphoma (FL). *J Clin Oncol*. (2023) 41:7509–. doi: 10.1200/JCO.2023.41.16_suppl.7509

30. Fowler NH, Dickinson M, Dreyling M, et al. Tisagenlecleucel in adult relapsed or refractory follicular lymphoma: the phase 2 ELARA trial. *Nat Med*. (2022) 28:325–32.

31. Salles G, Schuster SJ, Dreyling M, et al. Efficacy comparison of tisagenlecleucel vs usual care in patients with relapsed or refractory follicular lymphoma. *Blood Adv*. (2022) 6:5835–43. doi: 10.1182/bloodadvances.2022008150

32. Fowler NH, Dickinson M, Ghosh M, et al. Assessment of healthcare resource utilization and hospitalization costs in patients with relapsed or refractory follicular lymphoma undergoing CAR-T cell therapy with tisagenlecleucel: results from the ELARA study. *Transplant Cell Ther*. (2023) 29:60.e1–.e4. doi: 10.1016/j.jtct.2022.09.022

33. Dickinson M, Martinez-Lopez J, Jousseaume E, et al. Comparative efficacy and safety of tisagenlecleucel and axicabtagene ciloleucel among adults with r/r follicular lymphoma. *Leuk Lymphoma*. (2024), 1–10. doi: 10.1080/10428194.2023.2289854

34. Schuster SJ, Sehn LH, Bartlett NL, et al. Mosunetuzumab Monotherapy Continues to Demonstrate Durable Responses in Patients with Relapsed and/or Refractory Follicular Lymphoma after ≥2 Prior Therapies: 3-Year Follow-up from a Pivotal Phase II Study. *Blood*. (2023) 142:603–. doi: 10.1182/blood-2023-173692

35. Nastoupil LJ, Bonner A, Wang P, et al. Matching-adjusted indirect comparison (MAIC) of efficacy and safety of lisocabtagene maraleucel (liso-cel) and mosunetuzumab for the treatment (Tx) of third line or later (3L+) relapsed or refractory (R/R) follicular lymphoma (FL). *Blood*. (2023) 142:2338–. doi: 10.1182/blood-2023-178786

36. Lin M, Weiss J, Phillips TJ, et al. Cost effectiveness of mosunetuzumab and CAR-T cell therapy in relapsed/refractory follicular lymphoma. *Blood*. (2023) 142:256–. doi: 10.1182/blood-2023-182244

37. Oluwole OO, Ray MD, Zur R, et al. Cost-effectiveness of axicabtagene ciloleucel versus mosunetuzumab in relapsed/refractory follicular lymphoma in the US. *Blood*. (2023) 142:5082–. doi: 10.1182/blood-2023-186548

38. Shadman M, Yeung C, Redman MW, et al. Third generation CD20 targeted CAR T-cell therapy (MB-106) for treatment of patients with relapsed/refractory B-cell non-hodgkin lymphoma. *Blood*. (2020) 136:38–9. doi: 10.1182/blood-2020-136440

39. Shadman M, Yeung C, Redman M, et al. HIGH EFFICACY AND FAVORABLE SAFETY OF 3RD GENERATION CD20 CAR-T (MB-106) FOR OUTPATIENT TREATMENT OF FOLLICULAR LYMPHOMA (FL)—RESULTS OF A SINGLE-INSTITUTION TRIAL. *Hematological Oncol*. (2023) 41:87–8. doi: 10.1002/hon.3163_49

40. Shah NN, Johnson BD, Schneider D, 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) 26:1569–75. doi: 10.1038/s41591-020-1081-3

41. Tong C, Zhang Y, Liu Y, et al. Optimized tandem CD19/CD20 CAR-engineered T cells in refractory/relapsed B-cell lymphoma. *Blood*. (2020) 136:1632–44. doi: 10.1182/blood.2020005278

42. Locke FL, Munoz JL, Tees MT, et al. ALLO-647 for lymphodepletion in the allogeneic CAR T setting: safety experience with ALLO-501/501A in patients (Pts) with relapsed/refractory (r/r) large B-cell and follicular lymphomas. *Blood*. (2023) 142:2095. doi: 10.1182/blood-2023-189196

43. Iacoboni G, Navarro V, Martín-López A, 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 Off J Am Soc Clin Oncol*. (2024) 42:205–17. doi: 10.1200/JCO.23.01097

44. Casulo C, Larson MC, Lunde JJ, et al. Treatment patterns and outcomes of patients with relapsed or refractory follicular lymphoma receiving three or more lines of systemic therapy (LEO CReWE): a multicentre cohort study. *Lancet Haematology*. (2022) 9:e289–300. doi: 10.1016/S2352-3026(22)00033-3

45. Verdun N, Marks P. Secondary cancers after chimeric antigen receptor T-cell therapy. *New Engl J Med*. (2024). doi: 10.1056/NEJMp2400209

frontiers | Frontiers in Oncology

TYPE Mini Review
PUBLISHED 28 June 2024
DOI 10.3389/fonc.2024.1425521

OPEN ACCESS

EDITED BY
Edward Copelan,
Atrium Healthcare, United States

REVIEWED BY
Jack Khouri,
Cleveland Clinic, United States

*CORRESPONDENCE
Heather Landau
landauh@mskcc.org

RECEIVED 29 April 2024
ACCEPTED 15 May 2024
PUBLISHED 28 June 2024

CITATION
Sarubbi C, Abowali H, Varga C and Landau H (2024) Treatment of AL amyloidosis in the era of novel immune and cellular therapies. *Front. Oncol.* 14:1425521. doi: 10.3389/fonc.2024.1425521

Treatment of AL amyloidosis in the era of novel immune and cellular therapies

Caitlin Sarubbi[1], Hesham Abowali[2], Cindy Varga[3] and Heather Landau[4*]

[1]Department of Medicine, Montefiore Medical Center/Albert Einstein College of Medicine, Bronx, NY, United States, [2]Department of Hematology/Oncology, Brookdale University Medical Center, Brooklyn, NY, United States, [3]Department of Hematology, Levine Cancer Institute Atrium Health, Charlotte, NC, United States, [4]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, United States

Light chain (AL) amyloidosis is a plasma cell disorder distinguished from multiple myeloma (MM) by the degree of organ involvement due to tissue deposition of misfolded proteins. Treatments for AL amyloidosis have largely been borrowed from those developed for patients with MM. High-dose chemotherapy followed by autologous stem cell transplant (ASCT) has historically been associated with the best outcomes. The recent incorporation of daratumumab into up front therapy represents a significant advance and has changed the treatment paradigm, calling into question the role of ASCT. The development of very active novel immune and cellular therapies, specifically B cell maturation antigen (BCMA)-directed therapies, has similarly been transformative for patients with MM and is now being studied in patients with AL amyloidosis. These include chimeric antigen receptor (CAR) T cells, bispecific antibodies, and antibody drug conjugates. Although limited, preliminary data in patients with relapsed and refractory AL amyloidosis are showing promising results, and it is expected that the treatment landscape for AL amyloidosis will continue to evolve. Particular attention to safety, potential for organ recovery, and quality of life will be important when evaluating new treatments and/or treatment paradigms.

KEYWORDS

AL amyloidosis, bispecific antibodies, CAR T cell therapy, stem cell transplant, daratumumab

Introduction

Light chain (AL) amyloidosis is a plasma cell disease in which light chains that are produced misfold and deposit in various tissues and organs throughout the body. Although the degree of plasma cell burden is often minimal compared to multiple myeloma, the considerable morbidity and mortality associated with AL amyloidosis result from organ dysfunction (1, 2). While light chain proteins can deposit in any organ, the most affected

organs are the heart (80% of patients), followed by the kidneys (60%–70% of patients) (3). The amyloid proteins that are circulating and not yet deposited in the organs are also toxic. In the case of AL amyloidosis, serum free light chain levels are closely correlated with the degree of disease (2, 3).

Most treatment options for AL amyloidosis are aimed at decreasing the serum levels of light chains with the use of anti-plasma cell agents. Survival is predicted by the organ affected as well as the depth and speed of hematologic response, which are necessary for organ function to be restored (4). Autologous stem cell transplant (ASCT) has been associated with rapid and durable suppression of the plasma cell clone and therefore, has been a commonly used therapeutic option in AL amyloidosis. Many aspects of this treatment paradigm, such as patient selection, melphalan dosing, and peri-transplant supportive care, have been adapted over time to reduce toxicity and improve outcomes. However, with the availability of novel plasma cell-directed therapies such as anti-CD38 antibodies, there is ongoing research looking at the optimal use and timing of ASCT as well as alternative treatment options. This review will examine the role of ASCT and anti-BCMA agents, including T cell redirecting therapies, in the treatment of patients with AL amyloidosis.

Autologous stem cell transplant

High-dose melphalan conditioned ASCT has been a mainstay of treatment for patients with AL amyloidosis for nearly 30 years, modeled after its use in multiple myeloma. In 1996 a pilot study was performed in which five patients with amyloid underwent ASCT, of which 60% achieved a hematologic complete response (CR), and all five patients had improvement in their involved organ function (5). These results were expanded upon in 2004 when a group of 312 patients underwent ASCT, resulting in a 40% CR rate and 66% organ response (6). Recent data indicate that approximately two-thirds of patients who undergo ASCT are alive 10 years following transplant, and among those who achieve a CR, almost 50% are event-free 15 years later (7).

While high-dose melphalan was the first treatment to result in meaningful hematologic responses, transplant related mortality (TRM) was higher in patients with AL amyloidosis than had been observed in MM patients (8). Patients with cardiac disease, renal impairment, multiorgan involvement, and/or advanced age were at particular risk for transplant related morbidity and mortality. To reduce toxicity, the melphalan dose was attenuated with melphalan 200 mg/m^2 or 100–140 mg/m^2 given to patients without risk factors versus those with one or more risk factors, respectively. Several studies have reported higher CR rates and better outcomes with melphalan 200 mg/m^2 when compared to reduced doses of melphalan (9, 10). Lower response rates in patients receiving attenuated doses of melphalan have led to the notion that high-dose therapy should be reserved for patients who can receive the full dose (11). However, several publications show the benefit of modified melphalan dosing in patients with AL amyloidosis (12–14). Among a series of 143 patients who received risk-adapted melphalan dosing, only 34% of patients received melphalan 200 mg/m^2. The OS of all patients surpassed 10 years (15). The absence of early death in patients who received a full dose of melphalan suggests that host factors necessitating dose reductions were primarily responsible for TRM rather than the dose of melphalan itself. The 100-day TRM rate was only 5% in that series, compared to up to 30% when dose reduction was not considered, indicating the safety of this approach (15, 16).

The advent of propylene glycol-free (PG-free) melphalan (Evomela^{TM}), resulting in improved stability compared to standard melphalan, has allowed for pharmacokinetically (Pk)-directed dosing in patients with plasma cell disorders (17). Safety and efficacy outcomes in patients with AL amyloidosis conditioned with PG-free melphalan are similar to those using the standard formulation (18). Pk-directed dosing has the potential to optimize and individualize melphalan conditioning, which may be particularly important in patients with AL amyloidosis but will require further study in randomized trials (19).

In addition to attenuated melphalan dosing, stricter selection criteria are associated with reduced toxicity of ASCT for patients with AL amyloidosis and an improved safety profile. A working group designated by the International Society of Amyloidosis (ISA) and the European Hematology Association (EHA) recently put forth eligibility guidelines (see Table 1). Definite exclusions include decompensated heart failure, pleural effusions, medically refractory

TABLE 1 Broad eligibility criteria for ASCT for AL amyloidosis (8).

Diagnostic criteria	Confirmed tissue amyloid diagnosis and typing
	Evidence of plasma cell disorder
	At least 1 organ involved
Performance criteria	ECOG performance status < 2 (unless limited by peripheral neuropathy)
Age	18–70
Cardiac and pulmonary criteria	Left ventricular ejection fraction > 40%
	NYHA class < III
	DLCO > 50%
	Supine systolic blood pressure > 90 mmHg
	NTpro BNP < 5,000 pg/mL
	Troponin I < 0.1 ng/mL and troponin *T* < 60 ng/mL and hs-troponin *T* < 75 ng/mL
	Oxygen saturation > 95% on room air
Hepatic criteria	Direct bilirubin < 2 mg/dL
Renal criteria	eGFR > 30 mL/min/m^2

Melphalan 140 mg/m^2 for estimated glomerular filtration rate (eGFR) < 50 mL/min/m^2, age > 70, and/or Mayo cardiac stage > II.

ventricular arrhythmias and/or orthostatic hypotension, and extensive gastrointestinal involvement or Factor X deficiencies that would pose an excess risk of bleeding. While compromised renal function is not a definite exclusion, melphalan should be adjusted based on a reduced estimated glomerular filtration rate (eGFR), and the risk of worsening renal function in the peri-ASCT period must be contemplated. Given these considerations, only 20%–30% of newly diagnosed patients with AL amyloidosis are eligible for transplantation, but that percentage is likely dynamic based on recent advances in the field (8).

Given the multiorgan involvement and complex pathophysiology associated with AL amyloidosis, a multidisciplinary care team is ideal to properly support patients, and thus, the experience of the transplant center matters. Institutes that perform less than 4 transplants for AL amyloid patients per year have more than double the TRM at 100 days post-transplant, compared to experienced centers that perform more (20).

The preponderance of evidence suggests that the incorporation of a fixed and limited number of cycles of bortezomib-based induction prior to ASCT has led to more frequent hematologic responses and superior outcomes following ASCT compared to ASCT alone (21–24). The risk of toxicity from induction therapy should be individually weighed against the benefit so as not to compromise a patient's eligibility for ASCT.

In 2021, the historic ANDROMEDA study demonstrated that the addition of daratumumab to bortezomib, cyclophosphamide, and dexamethasone (VCd) as induction therapy resulted in deeper hematologic responses, increased organ responses, and better outcomes compared to VCd alone (25). Remarkably, hematologic CRs were achieved in over 50% of patients who received Dara-VCd, and these responses occurred quickly (the median time to CR was 60 days). Based on these data, Dara-VCd has become the first (and only) FDA-approved induction regimen for the treatment of AL amyloidosis and is now widely accepted as a standard of care. With such effective induction therapy, the role of ASCT in the frontline setting is uncertain. Prompt hematologic disease control with Dara-VCd may improve a patient's condition, potentially increasing the number of patients who are transplant-eligible. Alternatively, if a CR is achieved with two to four cycles of induction, some physicians and patients may opt to defer ASCT (8). Although the results of the ANDROMEDA study are promising, it should be recognized that there are no data on the long-term outcomes of daratumumab-based induction therapies, and whether ASCT following Dara-VCd induction will improve the depth and duration of response has not been tested. The recently initiated SWOG trial (S2213) (NCT06022939), a phase III randomized study of Dara-VCd induction followed by ASCT versus Dara-VCd consolidation in patients with newly diagnosed AL amyloidosis, was designed to answer this question, but results are not expected for several years.

The results of the ANDROMEDA study have positively impacted the treatment of patients with AL amyloidosis. With the rapid development of alternate immune-based and cellular therapies for plasma cell disease, the treatment landscape will continue to evolve. Particular attention to safety, potential for organ recovery, and quality of life will be important when evaluating new treatments and/or treatment paradigms.

Novel immune and cellular therapies

CAR T cell therapy

While daratumumab-based initial therapy represents a significant advance in the treatment of AL amyloidosis, nearly half of patients do not achieve CR (47%), and organ responses are not guaranteed (25). Hematologic response rates in patients who fail to respond or relapse following daratumumab-based therapies are even lower (26). High-dose melphalan and ASCT are options for salvage therapy, but only for the percentage of patients who are transplant-eligible. Thus, second-line therapy for patients with AL amyloidosis is a critical unmet medical need.

Chimeric antigen receptor (CAR) T cell therapy is a novel therapeutic strategy utilizing T cells that are genetically engineered to target and destroy cells or tissues expressing a particular antigen. The unprecedented efficacy of B cell maturation antigen (BCMA)-directed CAR T cells in heavily pre-treated MM has led to the FDA-approval of two products, idecabtagene vicleucel and citacabtagene autoleucel, for this indication. While overall and complete hematologic response rates were notably high, ranging from 73% to 97% and 33% to 67%, respectively, these products have unique toxicities, including cytokine release syndrome (CRS) and immune effector cell associated neurotoxicity syndrome (ICANS), that require specialized management (27–29).

Similar to MM, BCMA is highly expressed on amyloidogenic plasma cells and also retained at relapse (30). As the risk of CRS and ICANS was expected to pose a significant risk to patients with AL amyloidosis, these patients have been notably excluded from initial studies. However, emerging data suggest that CAR T cells can be safely administered and have promising activity in this patient population.

The first case report from Spain described a patient with MM who developed AL amyloidosis with renal involvement in the context of relapsing disease (31). The patient received an academic BCMA CAR T cell product under compassionate use (NCT04309981) and developed grade 1 CRS that resolved within 48 h without treatment without signs or symptoms of ICANS. By D+28, the patient achieved a hematologic very good partial response (VGPR) (normal sFLCs and small residual M-spike) and had no detectable disease (minimal residual disease (MRD) negative) in the bone marrow. The M-spike resolved by D +90, and by 6 months following CAR T cell infusion, she achieved a renal response. It is now almost 4 years (46 months) following her CAR T cell infusion, and this patient remains in CR with minimal proteinuria (< 200 mg).

Two other patients with relapsed refractory MM and coexisting cardiac and renal AL amyloidosis were reported by the Mayo Jacksonville group (32). Given the initial concern for adverse effects, patients were given CRS prophylaxis according to the ZUMA-1 trial in the form of dexamethasone (33). The first patient was penta-refractory after eight lines of therapy and received commercially available idecabtagene vicleucel. During the hospitalization, the patient developed anemia and neutropenia but no evidence of CRS, ICANS, or organ dysfunction. By D+30, the

patient achieved a hematologic VGPR with no MRD in the bone marrow. She had a cardiac response (> 30% reduction in NT-ProBNP) and stable renal disease following CAR T cells (32). The second patient received ciltacatagene autoleucel after four prior lines of treatment. Despite pre-treatment with dexamethasone, the patient developed grade 3 CRS and required ICU admission. With the administration of tocilizumab and IV steroids, CRS resolved within 24 h. The patient developed pancytopenia but no ICANS or cardiac dysfunction. At D+30, he achieved MRD negative disease in the bone marrow and a serologic CR. At 9 months, following CAR T cells, the patient achieved a cardiac response (32).

A phase Ia/b study (NCT04720313) of a novel anti-BCMA CAR T cell therapy developed at Hadassah Medical Center was the first prospective trial to include patients with AL amyloidosis and reported on the safety and feasibility of this approach (34). These data were updated at the American Society of Hematology meeting (December 2023) and included eleven patients (median age: 64, range: 55–82), with either cardiac (*N*= 9; Mayo stage 2 (*N*= 4), 3a (*N*= 3), or 3b (*N*= 1)) or renal (*N*= 7) involvement. The median time from diagnosis was 4.5 years (range: 0.8–11 years), median lines of prior therapy were six (range: 3–10); and all were refractory to their last line of therapy. Patients received doses of 150×10^6(*N*= 1), 450×10^6(*N*= 2), and 800×10^6(*N*= 8).

CRS was seen in nine of eleven patients (grade 1 (*N*= 3), grade 2 (*N*= 4), grade 3 (*N*= 2)) and occurred within 1–3 days (median: 1.5) and persisted 1–4 days (median 1 day). Tociluzimab was used in seven patients and dexamethasone in two patients. No grade 4 CRS or ICANS were seen. Ten patients were evaluable for hematologic response, and 100% of patients responded including nine of ten with VGPR (*N*= 2) or CR (*N*= 7), and 67% (six of nine) had no evidence of MRD in the bone marrow by D+30. In total, 60% of patients achieved organ responses, including four cardiac and two renal responses. Follow up ranged from 0.5 to 25.2 months, and five patients remained alive with the longest response duration and survival of 25.2 months. Six patients died during the follow up period (median OS: 6.9 months, range: 5.2–12.2), five of six due to cardiac disease, three in the setting of hematologic progression, and one due to COVID while still in a CR (35).

These data provide evidence that this novel therapy is safe for patients with AL amyloidosis, including those with advanced cardiac disease, without early mortality. Hematologic responses are seen in most patients and can be deep and durable. Deaths were overwhelmingly cardiac-related, arguing for earlier use in the course of the disease.

Bispecific antibodies

Bispecific antibodies targeting CD3 expressed on the surface of T cells and BCMA expressed on MM cells represent another effective T cell redirecting strategy that has demonstrated promising results in MM, leading to the FDA approval of two commercially available products, teclistimab and elranatamab (36, 37). In heavily pre-treated patients, both BCMA-directed bispecific antibodies showed rapid and deep responses with ORR ~ 61%–63%, including 35%–39% CR rates, with a median time to first response of 1.2 months. While CRS occurred in 56%–72% of patients, these events occurred early and were almost exclusively grades 1–2.

Patients with AL amyloidosis were excluded from the pivotal trials that led to the regulatory approval of these drugs in MM. However, once commercially available, there have been several reports demonstrating the safety and efficacy of teclistimab in patients with AL amyloidosis (38–40). A 76-year-old woman with Mayo cardiac stage IIIb (by modified European criteria), renal stage II, and soft tissue involvement with AL amyloidosis was treated with teclistimab after receiving six prior lines of therapy. She had no CRS, neurotoxicity, or other adverse effects and, after two cycles, achieved a hematologic CR and a cardiac biomarker response (38).

In another series, seven patients with AL amyloidosis from two US medical centers with cardiac (*N*= 5, Mayo cardiac stage II (2), IIIa (1), or IIIb (2)) or renal (*N*= 5) involvement, having received a median of six prior lines of therapy (range: 2–7), including four with prior BCMA-targeted therapy, were reported by Chakraborty et al. (39). All seven patients achieved at least a VGPR with a median time to response of only 0.6 months, including six of seven with a stringent dFLC response (defined by dFLC < 1 mg/dL). Among patients evaluable for organ response, three of four achieved a cardiac response. CRS occurred in four patients, all grade 1, with only one patient requiring tocilizumab. No patients had ICANS. Two patients experienced grade 3 infections, but there were no infection-related deaths. One patient with Mayo stage IIIb cardiac disease died ~ 40 days after teclistimab due to progressive cardiac dysfunction despite achieving a hematologic VGPR (39).

Forgeard et al. identified seventeen patients with AL amyloidosis from ten university hospitals in Europe who were treated with teclistimab. In total, 94% of patients had cardiac involvement (including Mayo cardiac stages IIIa (*N*= 6) and IIIb (*N*= 4), and 59% had renal involvement. After a median of three cycles (range: 0.25–10) of teclistamab, 88% of patients achieved a VGPR or better, including 41% with CR. The median time to hematologic response was approximately 1 month, and five patients achieved organ responses, four of which were cardiac responses. CRS occurred in nine patients (53%), all grade 1. One patient with a pre-existing inflammatory syndrome developed grade 3 ICANS and discontinued treatment; one patient died from cardiac progression; and one patient died from infection (40).

The advantage of bispecific antibodies over CAR T cell therapy is that they are an "off-the-shelf" product, available for immediate administration. CAR T cell therapy is hampered by significant logistical considerations, including the requirement for apheresis, prolonged waiting periods for manufacturing, the need for bridging therapy, and the high cost of treatment. However, like CAR T cells, CRS, ICANS, and infections are potential toxicities of bispecific antibodies that will require careful attention and prompt management in patients with AL amyloidosis.

Antibody drug conjugates

Antibody drug conjugates (ADCs) are composed of an antibody bound to a cytotoxic drug through a chemical linker. Belantamab mafadotin is an ADC with an antibody against BCMA that delivers

a microtubule inhibitor, monomethyl auristatin F, to pathologic plasma cells (41).

Zhang et al. identified six patients with relapsed or refractory AL amyloidosis, with a median age of 61 years (range: 51–74), a median of six lines of prior therapy (range: 5–10), who were treated with belantamab mafadotin. Five of these patients had cardiac involvement. Overall, five patients (83%) achieved a hematologic response, including (three of six) 50% achieving a CR. Time to response was rapid (ranging from 1 week to 5 months), and four of five evaluable patients achieved a cardiac response. Keratopathy occurred in five of six (83%) patients but was all grade 1 (*N*= 2) and 2 (*N*= 3) (42).

A larger retrospective series that included 31 patients with relapsed and refractory AL amyloidosis treated in the UK with belantamab mafadotin was reported by Khwaja et al. (43). Patients had a median age of 65 years (range: 41–78), received a median of three prior lines of therapy (range: 1–6), and had renal (*N*= 23, 74%) and cardiac (*N*= 18, 58%) involvement, including Mayo stages IIIa (*N*= 5) and IIIb (*N*= 4). The ORR was 71% (22/31) and included 35% (11/31) and 23% (7/31) with CR and VGPR, respectively. The median time to hematologic response was 2 months. At 12 months following initiation of treatment, 65% of patients remained on belantamab. The most common reasons for treatment discontinuation were inadequate response or progression (*N*= 7) and physician choice (*N*= 3). Two patients died, one due to the progression of the disease and another due to an unrelated cause. The most frequent toxicity was corneal keratopathy, which was observed in (21/31) 68%. Keratopathy was mostly low grade (grades 1/2/3/4; *N*= 7/10/3/4, respectively) and improved in all patients after a delay in treatment (43).

An ongoing phase II clinical trial studying belantamab in AL amyloidosis patients is being conducted by the European Myeloma Network (EMN27; NCT04617925), and interim results were presented at the American Society of Hematology meeting in December 2023. At the time, 28 patients with a median age of 66 years (range: 46–80) and a median of four (range: 1–10) prior lines of therapy were enrolled. Cardiac and renal involvement were present in 22 (79%) and 17 (61%) of the patients, respectively. Overall hematologic response was achieved in 54% (15/28) of patients, including nine (32%) with VGPR, with a median time to first response of 15 days (range: 7–148). Ocular adverse events were common, observed in 96% of patients, including grade 3/4 events in up to 40% of patients. Visual symptoms were frequent in this study despite extending the dosing from the standard every 3 weeks administration schedule to every 6 weeks from treatment initiation and dose reductions/delays (44).

Table 2 summarizes the available literature on BCMA-targeted therapies in AL amyloidosis.

Discussion

With the advent of potent immune and cellular therapies, the treatment landscape for patients with AL amyloidosis will continue to evolve. The most mature data suggest that high-dose melphalan and ASCT can provide long-term disease control in carefully selected patients with AL amyloidosis. With improvements in supportive care, more refined eligibility criteria, and transplants at experienced centers, outcomes are ever-improving. However, only a

TABLE 2 BCMA-directed therapies in AL amyloidosis: literature review.

	N	Follow up (median; months)	Organs involved	Hematologic response	Organ response	Adverse effects of interest (CRS, ICANS or neurotoxicity, serious infection, ocular toxicity)
CAR T cell therapy						
Oliver-Caldes et al.	1	12	Renal	CR	Renal	CRS (grade 1)
Das et al.	2	9	Pt 1: cardiac and renal Pt 2: cardiac	Pt 1: VGPR[c] Pt 2: CR[c]	Pt 1: cardiac Pt 2: cardiac	Pt 2: CRS (grade 3)
Lebel et al.[a]	11	6 (range: 0.5–25.2)	Cardiac: 9/11 Renal: 7/11	CR: 7/10[a] VGPR: 2/10[a]	Cardiac: 4/9 Renal: 2/7	CRS: 9/11 (grades 1/2/3:3/4/2) 1 grade 5 COVID infection
Bispecific antibodies						
Chakraborty et al.	7	3.2 (range: 1.4–9)	Cardiac: 5/7 Renal: 5/7	CR: 3/7 VGPR: 4/7[d]	Cardiac: 3/4 Renal: 1/2	CRS: 4/7 (all 4 grade 1)
Forgeard et al.	17	N/A	Cardiac: 16/17 Renal: 10/17	CR: 7/17 VGPR: 8/17	Cardiac: 4/16 Renal: 1/10	CRS: 9/17 (all 9 grade 1) ICANS: 1/17 (grade 3) 1 grade 5 bacterial infection
Leung et al.	1	6	Cardiac Renal	CR	Cardiac	None

(Continued)

TABLE 2 Continued

	N	Follow up (median; months)	Organs involved	Hematologic response	Organ response	Adverse effects of interest (CRS, ICANS or neurotoxicity, serious infection, ocular toxicity)
Antibody drug conjugates						
Zhang et al.	6	4.5	Cardiac: 5/6	CR: 3/6 VGPR: 2/6	Cardiac: 4/5	Keratopathy: 5/6 (grades 1/2: 2/3)
Khwaja et al.	31	12 (range: 1–29)	Cardiac: 18/31 Renal: 23/31	CR: 11/31 VGPR: 7/31	Cardiac: 1/1[g]	Keratopathy: 21/31 (grades 1/2/3/4: 7/10/3/1)
Kastritis et al.[b]	28	16	Cardiac: 22/28 Renal: 17/28 Peripheral nerve: 6/28	VGPR: 9/28 > PR: 15/28	Cardiac[e]: 3 Renal[e]: 2	Keratopathy[f]: 22/28 Grade 1/2/3/4: 10/4/4/0

[a]Lebel et al. phase 1a/b study; 10/11 patients are evaluable for hematologic response and six of seven patients who achieved CR had no detectable disease (MRD negative) in the bone marrow.
[b]Kastritis et al. phase II study.
[c]Both patients with hematologic VGPR and CR had no detectable disease (MRD negative) in the bone marrow.
[d]All patients achieved at least VGPR; CR was limited by the absence of urine protein electrophoresis/immunofixation in four patients.
[e]The authors report that at 3 months, three patients with cardiac and two patients with renal involvement.
[f]Ocular disorders including reduced visual acuity, visual impairment, and keratopathy occurred in 27/28 patients.
[g]Only one patient was evaluable.

minority of patients are eligible for ASCT in the upfront setting. It remains unclear whether achieving rapid disease control with the combination of Dara-VCd will result in comparable outcomes to ASCT in eligible patients or if consolidation with ASCT following Dara-VCd will result in additional benefits. This remains the most pressing unanswered question clinicians face in practice today. The recently initiated SWOG trial (S2213) (NCT06022939) is randomizing newly diagnosed transplant-eligible patients with AL amyloidosis to Dara-VCd with or without ASCT and will provide critically important insight on this topic. For patients who are not transplant-eligible and receive upfront Dara-VCd, the high hematologic response rate and subsequent organ improvement may increase the pool of patients who become transplant-eligible. Understanding which patients and at what level of response the risk: benefit profile favors proceeding with ASCT will continue to pose a challenge as active salvage therapies emerge.

A key advantage of CAR T cell therapy is the current "one and done" paradigm, where responding patients can experience a real treatment-free interval. Most bispecific antibodies have been studied as ongoing therapy in MM; however, in a disease like AL amyloidosis with minimal tumor burden in the bone marrow, the need for continuous therapy comes into question and should be weighed against the long-term toxicity, most notably the high risk of infections. Given the deep responses that have been reported in AL patients, perhaps a MRD-driven approach would be the most sensical: discontinuation of the drug if an MRD negative status is achieved in the bone marrow or even a de-escalation to monthly dosing once light chains have normalized. Nevertheless, aggressive supportive care to prevent infections needs to be adopted when administering T cell redirecting therapy, and this includes prophylactic antiviral therapy, medications to prevent PJP pneumonia, and IVIG replacement. While the activity of belantamab mafadotin in patients with AL amyloidosis is promising, strategies to mitigate ocular toxicity will be important to prevent decrements in patients' quality of life and are currently being studied in MM.

Other targets that have been studied in MM, such as GPRC5D, FCHR5, and CS1/SLAM F7, may also be important in patients with AL amyloidosis, and novel therapies directed at these antigens may further expand the treatment armamentarium.

In summary, the upcoming SWOG trial (S2213) will be important to understand the role of ASCT in the setting of daratumumab-based induction and identify subgroups most likely to benefit. In the absence of a standard of care beyond first-line therapy, it is expected that CAR T cells and other immunotherapies will be studied in patients who fail to achieve an optimal hematologic or organ response to initial therapy. Patient selection, safety considerations, resistance mechanisms, organ involvement, potential for organ recovery, and quality of life implications will be critical to determining how to use and sequence novel therapies.

Author contributions

HL: Writing – original draft, Writing – review & editing. CS: Writing – original draft, Writing – review & editing. HA: Writing – original draft, Writing – review & editing. CV: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This research was funded in part through the NIH/NCI Cancer Center Support Grant P30 CA008748.

Acknowledgments

The authors express their deep appreciation to the Leon Levine Foundation and the Kerry and Simone Vickar Family Foundation for their support.

Conflict of interest

HL: Research funding: Janssen, Alexion, Protego, Prothena, Advisory role: Abbvie, Immix Biopharma, Legend Biotech, Honararia: Alexion, Prothena.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

1. Muchtar E, Gertz MA, Kourelis TV, Sidana S, Go RS, Lacy MQ, et al. Bone marrow plasma cells 20% or greater discriminate presentation, response, and survival in AL amyloidosis. *Leukemia*. (2020) 34:1135–43. doi: 10.1038/s41375–019-0655-x

2. Palladini G, Milani P. Advances in the treatment of light chain amyloidosis. *Curr Opin Oncol*. (2022) 34:748–56. doi: 10.1097/CCO.0000000000000881

3. Merlini G, Dispenzieri A, Sanchorawala V, Schönland SO, Palladini G, Hawkins PN, et al. Systemic immunoglobulin light chain amyloidosis. *Nat Rev Dis Primers*. (2018) 4. doi: 10.1038/s41572–018-0034–3

4. Palladini G, Milani P, Merlini G. Management of AL amyloidosis in 2020. *Blood*. (2020) 136:2620–7. doi: 10.1182/blood.2020006913

5. Comenzo RL, Vosburgh E, Simms RW, Bergethon P, Sarnacki D, Finn K, et al. Dose-intensive melphalan with blood stem cell support for the treatment of AL amyloidosis: one-year follow-up in five patients. *Blood*. (1996) 88:2801–6. doi: 10.1182/blood.V88.7.2801.bloodjournal8872801

6. Skinner M, Sanchorawala V, Seldin DC, Dember LM, Falk RH, Berk JL, et al. High-dose melphalan and autologous stem-cell transplantation in patients with AL amyloidosis: an 8-year study. *Ann Internal Med*. (2004) 140:85. doi: 10.7326/0003–4819-140–2-200401200–00008

7. Gustine JN, Staron A, Szalat RE, Mendelson LM, Joshi T, Ruberg FL, et al. Predictors of hematologic response and survival with stem cell transplantation in AL amyloidosis: A 25-year longitudinal study. *Am J Hematol*. (2022) 97:1189–99. doi: 10.1002/ajh.26641

8. Sanchorawala V, Boccadoro M, Gertz M, Hegenbart U, Kastritis E, Landau H, et al. Guidelines for high dose chemotherapy and stem cell transplantation for systemic AL amyloidosis: EHA-ISA working group guidelines. *Amyloid*. (2022) 29:1–7. doi: 10.1080/13506129.2021.2002841

9. Sanchorawala V, Sun F, Quillen K, Sloan JA, Berk JL, Seldin DC. Long-term outcome of patients with AL amyloidosis treated with high-dose melphalan and stem cell transplantation: 20-year experience. *Blood*. (2015) 126:2345–7. doi: 10.1182/blood-2015–08-662726

10. Tandon N, Muchtar E, Sidana S, Dispenzieri A, Lacy MQ, Dingli D, et al. Revisiting conditioning dose in newly diagnosed light chain amyloidosis undergoing frontline autologous stem cell transplant: impact on response and survival. *Bone Marrow Transplant*. (2017) 52:1126–32. doi: 10.1038/bmt.2017.68

11. Tandon N, Sidana S, Dispenzieri A, Gertz MA, Buadi FK, Lacy MQ, et al. Effect of standard dose versus risk adapted melphalan conditioning on outcomes in systemic AL amyloidosis patients undergoing frontline autologous stem cell transplant based on revised mayo stage. *Blood*. (2016) 128:4627–7. doi: 10.1182/blood.v128.22.4627.4627

12. Nguyen VP, Landau H, Quillen K, Brauneis D, Shelton AC, Mendelson L, et al. Modified high-dose melphalan and autologous stem cell transplantation for immunoglobulin light chain amyloidosis. *Biol Blood Marrow Transplant*. (2018) 24:1823–7. doi: 10.1016/j.bbmt.2018.06.018

13. Landau H, Hassoun H, Rosenzweig MA, Maurer M, Liu J, Flombaum C, et al. Bortezomib and dexamethasone consolidation following risk-adapted melphalan and stem cell transplantation for patients with newly diagnosed light-chain amyloidosis. *Leukemia*. (2013) 27:823–8. doi: 10.1038/leu.2012.274

14. Cohen AD, Zhou P, Chou J, Teruya-Feldstein J, Reich L, Hassoun H, et al. Risk-adapted autologous stem cell transplantation with adjuvant dexamethasone ± thalidomide for systemic light-chain amyloidosis: results of a phase II trial. *Br J Haematol*. (2007) 139:224–33. doi: 10.1111/j.1365-2141.2007.06783.x

15. Landau H, Smith M, Landry C, Chou JF, Devlin SM, Hassoun H, et al. Long-term event-free and overall survival after risk-adapted melphalan and SCT for systemic light chain amyloidosis. *Leukemia*. (2017) 31:136–42. doi: 10.1038/leu.2016.229

16. Jaccard A, Moreau P, Leblond V, Leleu X, Benboubker L, Hermine O, et al. High-Dose Melphalan versus Melphalan plus Dexamethasone for AL Amyloidosis. *N Engl J Med*. (2007) 357:1083–93. doi: 10.1056/nejmoa070484

17. Hari P, Aljitawi OS, Arce-Lara C, Nath R, Callander N, Bhat G, et al. A phase IIb, multicenter, open-label, safety, and efficacy study of high-dose, propylene glycol-free melphalan hydrochloride for injection (EVOMELA) for myeloablative conditioning in multiple myeloma patients undergoing autologous transplantation. *Biol Blood Marrow Transplantation: J Am Soc Blood Marrow Transplant*. (2015) 21:2100–5. doi: 10.1016/j.bbmt.2015.08.026

18. Sidiqi MH, Aljama MA, Muchtar E, Buadi FK, Warsame R, Lacy MQ, et al. Safety and efficacy of propylene glycol-free melphalan as conditioning in patients with AL amyloidosis undergoing stem cell transplantation. *Bone Marrow Transplant*. (2019) 54:1077–81. doi: 10.1038/s41409-018-0388-x

19. Shah GL, Lin A, Kamrowski A, Carino CA, Schofield R, Devlin SM, et al. Successful personalization of propylene glycol free melphalan (PGF-MEL) for multiple myeloma (MM) and AL amyloidosis (AL) patients undergoing autologous hematopoietic stem cell transplant (AHCT) using pharmacokinetic (PK)-directed dosing. *Biol Blood Marrow Transplant*. (2020) 26:S154–5. doi: 10.1016/j.bbmt.2019.12.707

20. D'Souza A, Dispenzieri A, Wirk B, Zhang MJ, Huang J, Gertz MA, et al. Improved outcomes after autologous hematopoietic cell transplantation for light chain amyloidosis: A center for international blood and marrow transplant research study. *J Clin Oncol*. (2015) 33:3741–9. doi: 10.1200/jco.2015.62.4015

21. Sanchorawala V, Brauneis D, Shelton AC, Lo S, Sun F, Sloan JM, et al. Induction therapy with bortezomib followed by bortezomib-high dose melphalan and stem cell transplantation for light chain amyloidosis: results of a prospective clinical trial. *Biol Blood Marrow Transplantation: J Am Soc Blood Marrow Transplant*. (2015) 21:1445–51. doi: 10.1016/j.bbmt.2015.04.001

22. Afrough A, Saliba RM, Hamdi A, Honhar M, Varma A, Cornelison AM, et al. Impact of induction therapy on the outcome of immunoglobulin light chain amyloidosis after autologous hematopoietic stem cell transplantation. *Biol Blood Marrow Transplantation: J Am Soc Blood Marrow Transplant*. (2018) 24:2197–203. doi: 10.1016/j.bbmt.2018.07.010

23. Scott EC, Heitner SB, Dibb W, Meyers G, Smith SD, Abar F, et al. Induction bortezomib in AL amyloidosis followed by high dose melphalan and autologous stem cell transplantation: A single institution retrospective study. *Clin Lymphoma Myeloma Leukemia*. (2014) 14:424–430.e1. doi: 10.1016/j.clml.2014.02.003

24. Huang X, Wang Q, Chen W, Zeng C, Chen Z, Gong D, et al. Induction therapy with bortezomib and dexamethasone followed by autologous stem cell transplantation versus autologous stem cell transplantation alone in the treatment of renal AL amyloidosis: a randomized controlled trial. *BMC Med*. (2014) 12. doi: 10.1186/1741–7015-12–2

25. Kastritis E, Palladini G, Minnema MC, Wechalekar AD, Jaccard A, Lee HC, et al. Daratumumab-based treatment for immunoglobulin light-chain amyloidosis. *New Engl J Med*. (2021) 385:46–58. doi: 10.1056/nejmoa2028631

26. Theodorakakou F, Fotiou D, Spiliopoulou V, Roussou M, Malandrakis P, Ntanasis-Stathopoulos I, et al. Outcomes of patients with light chain (AL) amyloidosis after failure of daratumumab-based therapy. *Br J Haematol*. (2023) 203:411–5. doi: 10.1111/bjh.19042

27. Munshi NC, Anderson LD, Shah N, Madduri D, Berdeja J, Lonial S, et al. Idecabtagene vicleucel in relapsed and refractory multiple myeloma. *N Engl J Med*. (2021) 384:705–16. doi: 10.1056/nejmoa2024850

28. 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) 41:1265-1274. doi: 10.1200/jco.22.00842

29. 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) 398:314–24. doi: 10.1016/s0140–6736(21)00933–8

30. Bal S, Landau H. AL amyloidosis: untangling new therapies. *Hematology*. (2021) 2021:682–8. doi: 10.1182/hematology.2021000305

31. Oliver-Caldés A, Jiménez R, Español-Rego M, Cibeira MT, Ortiz-Maldonado V, Quintana LF, et al. First report of CART treatment in AL amyloidosis and relapsed/refractory multiple myeloma. *J ImmunoTher Cancer*. (2021) 9:e003783–e003783. doi: 10.1136/jitc-2021–003783

32. Das S, Ailawadhi S, Sher T, Roy V, Fernandez A, Parrondo RD. Anti-B cell maturation antigen chimeric antigen receptor T cell therapy for the treatment of AL amyloidosis and concurrent relapsed/refractory multiple myeloma: preliminary efficacy and safety. *Curr Oncol (Toronto Ont)*. (2023) 30:9627–33. doi: 10.3390/curroncol30110697

33. Oluwole OO, Bouabdallah K, Muñoz J, De Guibert S, Vose JM, Bartlett NL, et al. Prophylactic corticosteroid use in patients receiving axicabtagene ciloleucel for large B-cell lymphoma. *Br J Haematol*. (2021) 194:690–700. doi: 10.1111/bjh.17527

34. Kfir-Erenfeld S, Asherie N, Grisariu S, Avni B, Zimran E, Assayag M, et al. Feasibility of a novel academic BCMA-CART (HBI0101) for the treatment of relapsed and refractory AL amyloidosis. *Clin Cancer Res*. (2022) 28:5156–66. doi: 10.1158/1078–0432.ccr-22–0637

35. Lebel E, Kfir-Erenfeld S, Asherie N, Grisariu S, Avni B, Elias S, et al. Feasibility of a novel academic anti-BCMA chimeric antigen receptor T-cell (CART) (HBI0101) for the treatment of relapsed and refractory AL amyloidosis. *Blood*. (2023) 142:538–8. doi: 10.1182/blood-2023–186450

36. Moreau P, Garfall AL, van de Donk NWCJ, Nahi H, San-Miguel JF, Oriol A, et al. Teclistamab in relapsed or refractory multiple myeloma. *N Engl J Med*. (2022) 387:495-505. doi: 10.1056/nejmoa2203478

37. Lesokhin AM, Tomasson MH, Arnulf B, Bahlis NJ, Miles Prince H, Niesvizky R, et al. Elranatamab in relapsed or refractory multiple myeloma: phase 2 MagnetisMM-3 trial results. *Nat Med*. (2023) 29:2259-2267. doi: 10.1038/s41591–023-02528–9

38. Leung N, Chapman JR, Bhatia S. First report of teclistamab in a patient with relapsed AL amyloidosis and multiple myeloma. *EJHaem*. (2023) 4:1157–9. doi: 10.1002/jha2.772

39. Chakraborty R, Bhutani D, Maurer MS, Mohan M, Lentzsch S, D'Souza A. Safety and efficacy of teclistamab in systemic immunoglobulin light chain amyloidosis. *Blood Cancer J*. (2023) 13:1–4. doi: 10.1038/s41408–023-00950–3

40. Forgeard N, Elessa D, Carpinteiro A, Belhadj K, Minnema MC, Roussel M, et al. Teclistamab in relapsed or refractory AL amyloidosis, a multinational retrospective case series. *Blood*. (2024) 143:734-737. doi: 10.1182/blood.2023022937

41. Shah UA, Mailankody S. Emerging immunotherapies in multiple myeloma. *BMJ*. (2020) 370:m3176. doi: 10.1136/bmj.m3176

42. Zhang Y, Godara A, Pan S, Toskic D, Mann H, Sborov DW, et al. Belantamab mafodotin in patients with relapsed/refractory AL amyloidosis with myeloma. *Ann Hematol*. (2022) 101:2119–21. doi: 10.1007/s00277-022-04890-z

43. Khwaja J, Bomsztyk J, Atta M, Bygrave C, Forbes A, Durairaj S, et al. Real-world efficacy of single-agent belantamab mafodotin in relapsed systemic AL amyloidosis. *Br J Haematol*. (2024) 204(5):1811-1815. doi: 10.1111/bjh.19286

44. Kastritis E, Palladini G, Dimopoulos M-A, Jaccard A, Merlini G, Theodorakakou F, et al. Efficacy and safety of belantamab mafodotin monotherapy in patients with relapsed or refractory light chain amyloidosis: A phase 2 study by the european myeloma network. *Blood*. (2022) 140:7294–6. doi: 10.1182/blood-2022-169688

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 01 July 2024
DOI 10.3389/fonc.2024.1394057

OPEN ACCESS

EDITED BY
Narendranath Epperla,
The Ohio State University, United States

REVIEWED BY
Hema Dave,
Merck, United States
Jacopo Mariotti,
Humanitas Research Hospital, Italy

*CORRESPONDENCE
Bei Hu
Bei.Hu@atriumhealth.org

RECEIVED 29 February 2024
ACCEPTED 21 May 2024
PUBLISHED 01 July 2024

CITATION
Hu B, Korsos V and Palomba ML (2024) Chimeric antigen receptor T-cell therapy for aggressive B-cell lymphomas.
Front. Oncol. 14:1394057.
doi: 10.3389/fonc.2024.1394057

Chimeric antigen receptor T-cell therapy for aggressive B-cell lymphomas

Bei Hu[1]*, Victoria Korsos[2] and M. Lia Palomba[2,3]

[1]Department of Hematologic Oncology and Blood Disorders, Atrium Health Levine Cancer Institute/ Wake Forest School of Medicine, Charlotte, NC, United States, [2]Cellular Therapy Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, United States, [3]Lymphoma Service, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, United States

Chimeric antigen receptor (CAR) T-cell therapy is a revolutionary approach in the treatment of lymphoma. This review article provides an overview of the four FDA-approved CAR T-cell products for aggressive B-cell lymphoma, including diffuse large B-cell lymphoma and mantle cell lymphoma, highlighting their efficacy and toxicity as well as discussing future directions.

KEYWORDS

chimeric antigen receptor T cell therapy (CAR T cell therapy), diffuse large B cell lymphoma (DLBCL), high grade B cell lymphoma, mantle cell lymphoma (MCL), relapsed and refractory lymphoma

Introduction

Aggressive B-cell lymphomas are a heterogenous group of cancers arising from B lymphocytes that are typically fatal without treatment. The most common is diffuse large B-cell lymphoma (DLBCL), which is cured with rituximab and anthracycline-based chemoimmunotherapy in over 60% of patients. However, those with primary refractory disease, early relapse, or relapse after autologous stem cell transplant (ASCT) have a dismal prognosis with overall survival measured in months, based on the SCHOLAR-1 study (1). Although relatively indolent in some cases, mantle cell lymphoma (MCL) is typically aggressive, and while most patients respond well to frontline chemoimmunotherapy, all patients eventually relapse. Patients who progress on Bruton's tyrosine kinase (BTK) inhibitor survive a median of 3–11 months (2–5). A more recent study for patients with relapsed or refractory MCL who progressed on BTK inhibition in the pre-CAR T-cell era is the retrospective SCHOLAR-2 study, which showed that the median overall survival (OS) from initiation of the first post-BTK inhibition therapy was 9.7 months (6).

Chimeric antigen receptor T-cell (CAR T) therapy has been one of the most revolutionary treatments for hematologic malignancies that have not responded to conventional therapy. CD19-directed CAR T-cell therapy is a type of immunotherapy that uses genetically modified T cells to target and kill cancer cells that express CD19, a protein found on most B-cell lymphomas. Herein we will review the currently Food and

Drug Administration (FDA)-approved CAR T-cell therapies for DLBCL and MCL as of Jan 2024 and discuss the management of its toxicities.

CAR T-cell therapy

CAR T-cell therapy is a multi-step process. Initially, the patient undergoes collection of autologous T cells through a process called leukapheresis. The cells are then shipped to the manufacturing site. CAR T cells are made by transduction of an inactivated viral vector into the patient's autologous T cells to express proteins called chimeric antigen receptors (CARs) which then recognize and bind to the CD19 proteins expressed on the patient's lymphoma cells. The final CAR T-cell product consists of the CD19 antigen domain, transmembrane spacer, a co-stimulatory domain, and finally the CD3ζ intracellular signaling domain (7). It is the presence of the co-stimulatory domains, in addition to the primary signal through the T-cell receptor, that results in full T-cell activation and CAR T-cell expansion and persistence, allowing for the improved efficacy of second-generation CAR T-cell therapy over the first-generation CARs (8). The type of co-stimulatory domain (CD28 or 4–1BB) is what accounts for the differences in toxicity in CAR T-cell products (9), with the CD28 co-stimulatory domain being associated with rapid and high peak expansion, thus resulting in more severe toxicities earlier on when compared with the 4–1BB costimulatory domain (10, 11). The manufacturing process takes 3–5 weeks depending on the product. During this time, patients may or may not receive bridging therapy in the form of systemic therapy or radiation to control the lymphoma. The cells are then shipped back to the treatment center, and prior to infusion of the CAR T cells, the patients receive lymphodepleting chemotherapy, which creates a favorable immune environment for CAR T-cell expansion and efficacy (12, 13). After the infusion of CAR T cells, the patients were then monitored for toxicity.

FDA-approved CAR T-cell products

Diffuse large B-cell lymphoma

Axicabtagene ciloleucel

Axicabtagene ciloleucel (axi-cel) is generated by using a retroviral vector and includes a CD28 transmembrane domain and a CD28 co-stimulatory domain. Axi-cel was the first CAR T-cell therapy approved for relapsed/refractory (R/R) DLBCL, high-grade B-cell lymphoma, primary mediastinal B-cell lymphoma, and transformed follicular lymphoma after the failure of two lines of therapy based on the results of the phase 1/2 ZUMA 1 trial (Table 1) (14). Of the 111 patients enrolled in the study, product was successfully manufactured in 110 patients and infused in 101 patients. Bridging therapy was not allowed in the study. The median time from leukapheresis to the delivery of cells was 17 days (Table 2). The overall response rate (ORR) was 82%, with 54% achieving a complete response (CR). At median follow-up of 15.4 months, 40% of the patients continued to be in CR. The OS at 18 months was 52%. When compared with historical control with ORR of 26% (CR of 7%) and a median OS of 6.3 months as described in the SCHOLAR-1 study (1), the results of the ZUMA-1 trial were practice-changing and thus led to the approval of the first gene-based therapy by the FDA for large B-cell lymphoma. In a longer follow-up study with a median follow-up of 63.1 months, the median OS was 25.8 months, with estimated 5-year OS of 42.6% and disease-specific survival of 51% (15). The median duration of CR was 62.2 months and of those who achieved CR, the median OS was not reached with 5-year OS of 64%, supporting the curative potential of axi-cel.

Given the success of CAR T-cell therapy in the third-line setting, recent efforts have focused on CAR T-cell therapy earlier due to concerns about T-cell exhaustion with multiple lines of therapy and given the poor prognosis in primary refractory disease or early relapse. ZUMA-7 was a phase 3 trial that randomized patients with large B-cell lymphoma who had primary refractory disease or had relapsed within 12 months of first-line therapy 1:1 to receive (1) axi-cel or (2) standard-of-care (SOC) chemoimmunotherapy followed by high-dose chemotherapy and followed by autologous stem cell transplantation (ASCT) (16). Of the 359 patients who underwent randomization, 180 were randomized to axi-cel, with 170 patients actually receiving the infusion (94%). The baseline characteristics revealed a high-risk patient population as 74% had primary refractory disease and 17% were double-hit. Of the patients who underwent leukapheresis, the manufacturing success rate of axi-cel was 100%. The median time from leukapheresis to release of axi-cel to the investigator was 13 days. It is worth noting that only 36% of patients in the SOC arm went on to receive high-dose chemotherapy with ASCT, signifying that most patients continued to have a chemo-refractory disease. With a median follow-up of 24.9 months, the median event-free survival (EFS) was 8.3 months in the axi-cel arm vs. 2.0 months in the standard-of-care (SOC) arm. The 2-year EFS was 41% and 16% in the axi-cel and SOC arm, respectively. The ORR was 83% with 65% CR rate in the axi-cel arm compared with 50% ORR and 32% CR in the SOC arm. Given the clear improvement with CAR T-cell therapy in this high-risk patient population, axi-cel was approved in 2022 by the FDA for patients with large cell lymphoma that was primary refractory or relapsed within 1 year.

Tisagenlecleucel

Tisagenlecleucel (tisa-cel) is made by using a lentiviral vector and includes a CD8a transmembrane domain and a 4–1BB costimulatory domain. Tisa-cel was the second CAR T-cell product approved by the FDA for relapsed/refractory DLBCL, transformed follicular lymphoma, or high-grade B-cell lymphoma after failure of two lines of therapy following the results of the phase 2 JULIET trial (17). Of the 165 patients enrolled in the study, only 111 received infusion of tisa-cel (17). For 12 patients, tisa-cel was unable to be manufactured. The median time from enrollment to infusion was 54 days. Among the 93 patients with evaluable responses, ORR was 52%, with 40% achieving a CR. The 1-year PFS was 35%, with OS of 49% for patients who underwent infusion. Unlike the ZUMA-1 trial, the JULIET trial did allow patients to undergo bridging therapy which occurred in 92% of patients given the long period between leukapheresis and infusion of product. A

TABLE 1 CAR T-cell therapy in aggressive B-cell lymphomas: clinical trials.

Drug	Trial	Indication	Dose	Bridging therapy (% received)
Axicabtagene ciloleucel	ZUMA-1 (14, 15)	R/R DLBCL after 2 failed lines of therapy	2×10^6/kg	No
	ZUMA-7 (16)	Primary refractory or relapsed DLBCL within 12 months	2×10^6/kg	Limited to steroids (36%)
Tisagenlecleucel	JULIET (17, 18)	R/R DLBCL after 2 failed lines of therapy	0.6 to 6×10^8	Yes (92%)
	BELINDA (19)	Primary refractory or relapsed DLBCL within 12 months	0.6 to 6×10^8	Yes (83%)
Lisocabtagene maraleucel	TRANSCEND NHL 001 (20, 21)	R/R DLBCL after 2 failed lines of therapy	50 to 110×10^6	Yes (59%)
	TRANSFORM (22)	Primary refractory or relapsed DLBCL within 12 months	90 to 110×10^6	Yes (63%)
	PILOT (23)	Primary refractory or relapsed DLBCL transplant ineligible	90 to 110×10^6	Yes (52%)
	TRANSCEND NHL 001 MCL cohort (24)	R/R MCL	50 to 100×10^6	Yes (66%)
Brexucabtagene autoleucel	ZUMA-2 (25, 26)	R/R MCL	2×10^6/kg	Yes (37%)

CAR T cell, chimeric antigen receptor T cell; DLBCL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma.

longer follow-up study of JULIET showed a median OS of 11.1 months (18). However, median PFS and OS were not reached for those achieving CR at 3 and 6 months, also demonstrating the curative potential of tisa-cel.

Like axi-cel, there was interest in bringing tisa-cel to the second-line setting. The BELINDA trial was a phase 3 trial comparing tisa-cel *versus* SOC salvage chemo-immunotherapy followed by ASCT in patients with aggressive B-cell lymphoma that was refractory or relapsed within 12 months of first-line chemo-immunotherapy (19). Of the 322 patients who underwent leukapheresis and randomization, 162 were assigned to receive tisa-cel (83% received bridging therapy). The baseline characteristics revealed a high-risk patient population as two-thirds had a primary refractory disease. The manufacturing success rate of tisa-cel was 97% and was infused in 96% of patients assigned to the experimental arm. The median time from leukapheresis to tisa-cel infusion was 52 days (range of 31 to 135), which is significantly longer than axi-cel. Like the ZUMA-7 trial, a minority of patients (32%) in the SOC arm in the BELINDA trial underwent autologous stem cell transplant as most had a chemo-refractory disease. Interestingly, the response assessment was performed prior to infusion, and a progressive disease was noted to be higher in the patients randomized to receive tisa-cel compared with those assigned to the SOC (26% vs. 14%). The best ORR was 46% (28.4% CR) in the tisa-cel group and 42% (15% CR) in the SOC group at 12 weeks. EFS was not significantly different between the treatment arms, and the median EFS was 3 months in both groups. A major reason thought to contribute to the negative results is the long manufacturing time of tisa-cel, which translated to some patients not having adequate time to respond to tisa-cel at week 12 assessment. While the authors of the trial noted that some patients had a response at later time points in the absence of lymphoma-directed therapy—thus suggesting the efficacy of tisa-cel—unfortunately, failure to respond at week 12 was counted as a negative event per the trial's definition of EFS. The long manufacturing also resulted in some patients becoming refractory to bridging therapy or worsening performance status by the time the product was delivered, which may have also contributed to worse outcomes in the tisa-cel arm. Additionally, after randomization, the tisa-cel arm had patients with a higher-risk disease as 26% had progressive disease pre-infusion compared to the 14% in the SOC arm, which may have also contributed to the negative results as some studies have noted that a higher disease burden was associated with a lower chance of long-term remissions with CAR T-cell therapy (27). To date, tisa-cel is only approved after two failed lines of therapy.

Lisocabtagene maraleucel

Lisocabtagene maraleucel (liso-cel) is made by using a lentiviral vector and includes a CD28 transmembrane domain and a 4–1BB costimulatory domain. However, unlike axi-cel and tisa-cel, T cells are separated to CD4+ and CD8+ CAR T cells and infused to patients as a sequential infusion at equal target doses (28). Liso-cel was approved by the FDA following the results of the TRANSCEND NHL 001 study which evaluated the efficacy in patients with R/R DLBCL, high-grade B-cell lymphoma, transformed from indolent lymphoma, primary mediastinal B-cell lymphoma, and follicular lymphoma grade 3B following failure of two or more lines of treatment (20). Two-thirds of the study population were chemo-refractory. Of the 344 patients who underwent leukapheresis, only 294 received CAR T-cell product (of which 25 received a non-conforming product). In two patients, product was unable to be manufactured, and 33 patients died prior to receipt of CAR T-cell therapy, indicating the high-risk patient population. Bridging therapy (given to 59% of patients) was allowed. The median time

TABLE 2 Efficacy of CAR T-cell therapy in aggressive B-cell lymphoma.

Trial	Ratio	Time (days)	ORR; CR	Median outcomes (months)	Long-term outcomes
ZUMA-1 (14, 15)	#infused/#leukapheresed: 62/74	Leukapheresis to delivery: 17	82%; 58%	PFS: 5.9 OS: 25.8	5-year PFS: 31.8% 5-year OS: 42.6%
ZUMA-7 (16)	#infused/#leukapheresed: 170/178	Leukapheresis to delivery: 13	ORR: axi-cel vs. SOC: 83% vs. 50% CR: axi-cel vs. SOC: 65% vs. 32%	Median EFS axi-cel vs. SOC: 8.3 vs. 2.0 HR = 0.4 95% CI 0.31 to 0.51 Median OS axi-cel vs. SOC: NR vs. 35.1 HR = 0.73 95% CI 0.53 to 1.01	2 year EFS axi-cel vs. SOC: 41% vs. 16% 2 year OS axi-cel vs. SOC: 61% vs. 52%
JULIET (17, 18)	#infused/#enrolled: 111/165	Enrollment to infusion: 54	52%; 40%	PFS: 2.9 OS: 11.1	
BELINDA (19)	#infused/#assigned: 155/162	Leukapheresis to infusion: 52	ORR: tisa-cel vs. SOC at week 12: 46% vs. 42% CR: tisa-cel vs. SOC at week 12: 28% vs. 27.5%	Median EFS tisa-cel vs. SOC: 3 months for both HR = 1.1 95% CI 0.8 to 1.4	
TRANSCEND NHL 001 (20, 21)	#infused/#leukapheresed: 294/344	Leukapheresis to delivery: 24	73%; 53%	DOR: 23.1 PFS: 6.8 OS: 27.3	2 year DOR: 49.5% 2 year PFS: 40.6% 2 year OS: 50.5%
TRANSFORM (22)	#infused/#leukapheresed (randomized to liso-cel): 90/92	Leukapheresis to product availability: 26	ORR liso-cel vs. SOC: 86% vs. 48% CR liso-cel vs. SOC: 66% vs. 39%	EFS: liso-cel vs. SOC: 10.1 vs. 2.3 HR = 0.35 95% CI 0.23 to 0.53 PFS: liso-cel vs. SOC: 14.8 vs. 5.7 HR = 0.41 95% CI 0.25 to 0.66 OS: liso-cel vs. SOC: NR vs. 16.4 HR = 0.51 95% CI 0.26 to 1.00	1 year EFS liso-cel vs. SOC: 44.5% vs. 23.7% 1 year PFS liso-cel vs. SOC: 52.3% vs. 33.9% 1 year OS liso-cel vs. SOC: 79.1% vs. 64.2%
PILOT (23)	#infused/#leukapheresed: 62/74	Leukapheresis to delivery: 24	80%; 54%	DOR: 12.1 PFS: 9 OS: NR	
TRANSCEND NHL 001 MCL cohort (24)	#infused/#leukapheresed: 88/104	Leukapheresis to delivery: 24.5	83%; 72%	DOR: 15.7 PFS: 15.3 OS: 18.2	
ZUMA-2 (25, 26)	#infused/#leukapheresed: 68/74	Leukapheresis to delivery: 16	93%; 67%	DOR: 28.2 PFS: 25.8 OS: 46.6	

CAR T cell, chimeric antigen receptor T cell; DLBCL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; ORR, overall response rate; CR, complete response; SOC, standard of care; NR, not reached; HR, hazard ratio; CI, confidence interval; PFS, progression-free survival; OS, overall survival; DOR, duration of response.

from leukapheresis to availability for shipment was 24 days (range, 17–51), while the time to leukapheresis to infusion was 37 days (range, 27–224). Unlike the ZUMA-1 and JULIET trials, the patients with secondary CNS involvement were eligible (3% of patient population). ORR was 73%, and 53% achieved CR. The 1-year OS was 58% for the total population and not reached for those with CR. The efficacy of those who received a non-conforming product was similar to those who received liso-cel. Re-treatment with liso-cel occurred in 16 patients who relapsed after an initial response, but ORR was low at 19% and response to re-treatment was not durable. In the 2-year follow-up study, the median duration of response (DOR), PFS, and OS were 23.1, 6.8, and 27.3 months (21). However, the median OS of those who achieved a CR was 48.5 months, demonstrating the long-term remission of CAR T-cell therapy for large B-cell lymphoma.

Much like axi-cel and tisa-cel, liso-cel was also studied in the second-line setting for those patients with high-risk aggressive B-cell lymphoma with refractory disease. The TRANSFORM study was the liso-cel equivalent of the ZUMA-7 and BELINDA trials: a phase 3 study comparing liso-cel with SOC salvage chemoimmunotherapy followed by ASCT (22) in patients with large B-cell lymphoma with primary refractory disease or relapse within 1 year of first-line chemoimmunotherapy. This study also allowed crossover to receive liso-cel if patients in the SOC arm failed to achieve a response to salvage chemoimmunotherapy, had a progressive disease, or failed to achieve CR at 18 weeks post-randomization. A total of 184 patients were randomized (92 per arm), with nearly three quarters of patients having a refractory disease in each arm. All patients who were randomized underwent leukapheresis. Of the 92 patients who were in the liso-cel arm, 89

patients (97%) received liso-cel and one patient (1%) received a non-conforming product. There was manufacturing failure in one patient (1%). The median time from leukapheresis to product availability was 26 days (range, 19–84) and from leukapheresis to infusion was 36 days (range, 25–91). Bridging therapy was allowed and occurred in 63% of patients in the liso-cel group. Of the 92 patients in the SOC arm, only 46% achieved a response and received ASCT. A total of 50 of the 92 patients in the SOC were approved for crossover, 46 patients received liso-cel, and one received a non-conforming product. ORR was 86% (CR of 66%) in the liso-cel arm and 48% (CR of 39%) in the SOC arm. The median EFS was 10.1 months for liso-cel vs. 2.3 months for SOC with respective 12-month EFS of 44.5% and 23.7%. The 1-year PFS and OS was 52.3% and 79.1% for liso-cel and 33.9% and 64.2% for SOC, respectively. Given the efficacy of liso-cel over SOC, liso-cel is now approved by the FDA in the second-line setting for patients with primary refractory large B-cell lymphoma or relapse within 12 months of finishing frontline treatment.

Liso-cel is also approved for first relapses in patients with large B-cell lymphoma who are ineligible for ASCT due to age or other comorbidities based on the results of the phase 2 PILOT study (23). Of the 74 patients who underwent leukapheresis, 62 received CAR T cells (one of whom received a non-conforming product). Manufacturing success was 100%. The median time from leukapheresis to product release was 24 days, and the median time to infusion was 25.5 days. Bridging therapy was allowed and occurred in 52% of patients. Unlike the ZUMA-7, BELINDA, and TRANSFORM studies, the median age was much older at 74 years as the patients were transplant ineligible. About one-third of the patients were double or triple hit, and 54% were refractory to their last treatment. ORR was 80%, and 54% achieved CR. The median PFS was 9 months, and the median EFS was 7.2 months; the median OS was not reached. In those with CR, the median PFS was 22.6 months and the median OS was not reached. Given these efficacy results in a population who were not transplant eligible and thus without a curative option, the FDA approved liso-cel for transplant-ineligible patients with large B-cell lymphoma who failed in first-line therapy.

Mantle cell lymphoma

Brexucabtagene autoleucel

Brexucabtagene autoleucel (brexu-cel, previously KTE-X19) is a CD-19-directed second-generation CAR T-cell therapy with the co-stimulatory domain CD28 but removes circulating CD19+ malignant B cells to reduce possible CAR T-cell activation and exhaustion (25). ZUMA-2 is a phase 2 trial which evaluated the efficacy of brexu-cel in patients with relapsed or refractory MCL who had received up to five previous therapies, including a monoclonal antibody, anthracycline- or bendamustine-based chemotherapy, and a BTK inhibitor. Bridging therapy with steroids or BTK inhibition was allowed and was received by 37% of patients. The primary end point was ORR. A total of 74 patients were enrolled. Brexu-cel was manufactured for 71 patients (96%) and administered to 68 patients. The median time from leukapheresis to product delivery was 16 days. In a pre-specified primary efficacy analysis of the first 60 treated patients who had at least 7 months of follow-up, 93% had an ORR as assessed by an independent radiologic review, with 67% having a complete response (CR). In the intention-to-treat analysis, 85% had an ORR; 59% had a CR. At a median follow-up of 12.3 months, 57% of the 60 patients in the primary efficacy analysis were in CR. At 12 months, the estimated progression-free survival and overall survival were 61% and 83%, respectively.

Importantly, these remarkable and durable remissions in ZUMA-2 were the same across all poor prognosis subgroups, including age >65, blastoid or pleomorphic variants, high Ki-67, TP53-mutated, and high MIPI score. These findings are salient for patients with TP53 mutations and blastoid or pleomorphic subtypes who traditionally have not had sustainable long-term therapeutic options (29, 30). At 3 years of follow-up of the ZUMA-2 study, the median duration of response was 28.2 months, with median PFS of 25.8 months and OS of 44.6 months (26). Brexu-cel was approved for the treatment of relapsed and refractory MCL following two lines of therapy by the FDA in July 2020 based on ZUMA-2. In retrospective studies looking at the real-world experience of brexu-cel in the standard-of-care practice in both the US and Europe, results and toxicities were similar to ZUMA-2 despite longer manufacturing times and a higher risk profile of patients who would not have been eligible for ZUMA-2 (31–33).

Lisocabtagene maraleucel

TRANSCEND NHL 001 was a seamless design study which evaluated the safety and efficacy of liso-cel in patients with relapsed or refractory large B-cell lymphomas and included a MCL cohort of patients after two prior lines of therapy including a BTK inhibitor, an alkylator, and an anti-CD20 monoclonal antibody (24). Bridging therapy was also allowed in this study. The primary endpoints were safety and ORR. Among the 104 patients with MCL who were leukapheresed, 88 patients received liso-cel, 83 patients were part of the efficacy analysis set, and 74 patients were part of the primary analysis set. A substantial number of these patients had high risk features, including 75% with a Ki67 greater than 30%, 23% with a TP53 mutation, 31% with blastoid morphology, and 8% with secondary CNS lymphoma at the time of infusion. The overall ORR was 86.5%, with 74.3% achieving a CR in the primary analysis set and was similar across all high-risk groups. The median duration of response (DOR) was 15.7 months, with a median PFS of 15.3 months and a median OS of 18.2 months at a median follow-up of 22.8–24 months (20). Based on these data, the FDA approval of liso-cel for MCL is expected in 2024.

CNS involvement

CNS involvement represents a specific therapeutic challenge in the treatment of patients with relapsed and refractory aggressive B-cell lymphoma. The investigators were initially hesitant to include patients with CNS involvement in the landmark CAR T-cell therapy trials over concerns of a higher risk of neurological events. TRANSCEND NHL included a small number of large B-cell

lymphoma patients with CNS involvement (20). More recently, several retrospective single-institution small case series of primary and secondary CNS DLBCL have shown safety and efficacy in the use of CAR T-cell therapy (34–38). A meta-analysis of 128 patients showed that those with primary CNS lymphoma had a CR of 56% and 37% remained in remission at 6 months (39). For those with secondary CNS lymphoma, CR was 47% and 37% were in remission at 6 months (39). CRS was 77% (13% grade 3 or higher) and 72% (11% grade 3 or higher) in primary CNS lymphoma and secondary CNS lymphoma, respectively. Immune-effector cell-associated neurotoxicity syndrome (ICANS) was experienced by 53% (18% grade 3 or higher) and 48% (26% grade 3 or higher), respectively. A second multicenter study of 61 patients with secondary CNS lymphoma who underwent CAR T-cell therapy had ORR of 68% and CR of 57% (40). The median PFS and OS were 3.3 and 7.6 months, respectively (40). CRS was 70% (16% grade 3 or higher), and ICANS was 57% (44% grade 3 or higher) (40). Recent case reports have specifically pointed to the safety of CAR T-therapy with brexu-cel in the treatment of MCL with CNS involvement, even in one patient whose primary presentation of CNS involvement was seizures and in another patient with blastoid MCL and neurolymphomatosis (41–43). Recently, a subgroup analysis of patients with secondary CNS lymphoma in TRANSCEND showed high response rates, with 86% of patients (6/7) achieving a CR (44). The ability of CAR T-cell therapy to be a potential therapeutic option for aggressive B-cell lymphoma patients with CNS involvement meets a clinical need which has, up until now, remained unmet.

CAR T-cell toxicities

Early toxicities

CAR T-cell therapies cause predictable toxicities following their administration. Two unique early toxicities are known as cytokine release syndrome (CRS) and immune-effector cell-associated neurotoxicity syndrome for which patients must be monitored within the first 30 days following receipt of therapy. CRS is the immune system's response to the *in vivo* activation and expansion of the CAR T cells. CRS is the more common early toxicity and is graded on a scale of 1–4 per American Society for Transplantation and Cellular Therapy (ASTCT) (45). CRS manifests with fever, hypotension, and hypoxia. In its most severe forms, it requires intensive care monitoring and support due to end-organ damage. Ruling out infection in this immunocompromised population is also essential. Incidence and grading of CRS differ between CAR T-cell products. Axi-cel and brexu-cel have CD28 co-stimulation which results in rapid peak expansion of CAR T cells compared to those with 4–1BB co-stimulation (9). This often results in quicker onset and a higher incidence of CRS. High tumor burden is also associated with higher incidence and severity of CRS and neurotoxicity (46). Liso-cel and tisa-cel have 4–1BB co-stimulation, which results in more gradual expansion and longer persistence of T cells and have delayed CRS that are not as severe. Indeed this is what we see in clinical practice. In the ZUMA-1 trial (Table 3), CRS was observed in 93% of patients (13% were grade 3 or higher) at a median onset of 2 days with axi-cel (14). In the ZUMA-5 trial, CRS occurred in 91% (15% were grade 3 or higher) with a median onset of 2 days (25). In contrast, the incidence of CRS was 42% (2% grade 3 or higher) with a median onset of 5 days with liso-cel in the TRASCEND study (20). Similar incidences of CRS and its onset with liso-cel were reported in the TRANSFORM and PILOT studies (22, 23). In the JULIET study, CRS occurred in 58% of the patients (22% were grade 3 or higher) with a median onset of 3 days for tisa-cel (17). CRS is managed with supportive care such as anti-pyretics, fluids, and supplemental oxygen as well as early administration of steroids and tocilizumab, an IL-6 inhibitor. IL-6 is one of the many driving cytokines of this toxicity (47, 48). While close monitoring is required of patients experiencing CRS, it is reversible with early and appropriate treatment and supportive care and is experienced for a limited duration of time. In severe cases, vasopressors, mechanical ventilation, and high doses of steroids are used. Siltuximab, another IL-6 inhibitor that binds directly to IL-6 (unlike tocilizumab which binds to the IL-6 receptor) (49), is used off-label for tocilizumab-refractory CRS (50). Etanercept, infliximab, and anakinra have also been used off-label for tocilizumab-refractory CRS as tumor necrosis factor alpha (TNFα), and IL-1 also contributes to CRS (51–54).

ICANS is the brain's response to the exposure of cytokines from surrounding immune cells secondary to CAR T-cell activation and expansion. ICANS is generally less common than CRS and can manifests with a wide range of neurological symptoms, including tremor, headache, aphasia, inattention, confusion, somnolence, coma, and/or seizures in its most severe forms. It generally occurs after CRS symptoms. ICANS is graded on a scale of 1–4 using a standardized immune effector encephalopathy (ICE) scoring system which evaluates alterations in speech, orientation, handwriting, attention, and receptive aphasia and is traditionally effectively managed using steroids +/- levetiracetam prophylaxis (45). In the majority of cases, ICANS is reversible, though less severe symptoms can linger in approximately 10% of patients. Like CRS, the incidence and the severity of ICANS are higher and occur earlier with CAR T-cell products with C28 co-stimulation. ICANs occurred in 64% (28% were grade 3 or higher) of patients receiving axi-cel in ZUMA 1 trial (14) with a median onset of 5 days and with similar results in the ZUMA-7 study (16). In the ZUMA-5 study, the incidence of ICANS with brexu-cel was 64% (32% grade 3 or higher) with a median onset of 7 days (25). In contrast, liso-cel was associated with ICANs incidence of 30% (10% grade 3 or higher) with a median onset of 9 days in the TRANSCEND study (20) and with similar results in the MCL cohort (24) and in the PILOT study (23). The incidence of ICANS was far lower in the TRANSFORM study with liso-cel with incidence of 12% (2% grade 3 or higher) at a median onset of 11 days (22). ICANS occurred in 21% of patients (12% grade 3 or higher) with a median onset of 6 days in patients who received tisa-cel in the JULIET trial (17).

Optimizing prevention strategies for CRS and ICANS is an ongoing area of research. Recently, Park et al. published the interim results of their phase 2 study looking at the efficacy of prophylactic anakinra, a commercially available IL-1 receptor antagonist, in

TABLE 3 Toxicity of CAR T-cell therapy in aggressive B-cell lymphoma.

Trial	CRS (≥ grade 3)	ICANS (≥ grade 3)	Grade 5; other comments
ZUMA-1 (14, 15)	93% (13%) Median onset: 2 days	64% (28%) Median onset: 5 days	1% HLH/ cardiac arrest
ZUMA-7 (16)	92% (6%) Median onset: 3 days	60% (21%) Median onset: 7 days	0%
JULIET (17, 18)	58% (22%) Median onset: 3 days	21% (12%) Median onset: 6 days	0%
BELINDA (19)	61% (5%) Median onset: 4 days	10% (2%) Median onset: 5 days	6%
TRANSCEND NHL 001 (20, 21)	42% (2%) Median onset: 5 days	30% (10%) Median onset: 9 days	6%
TRANSFORM (22)	49% (1%) Median onset: 5 days	12% (4%) Median onset: 11 days	14% (13) in liso-cel arm, 4 were from COVID 19, 7 due to disease progression
PILOT (23)	49% (2%)	31% (5%)	0%
TRANSCEND NHL 001 MCL cohort (24)	61% (1%) Median onset: 4 days	31% (9%) Median onset: 8 days	4% (infection, tumor lysis, unrelated cardiopulmonary arrest)
ZUMA-2 (25, 26)	91% (15%) Median onset: 2 days	63% (31%) Median onset: 7 days	3% (due to infections)

CAR T cell, chimeric antigen receptor T cell; DLBCL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome.

participants with LBCLs, including MCL receiving CD-19-directed CAR T-cell therapies (axi-cel, brexu-cel, or tisa-cel) (55). In this study, 74% of the participants experienced CRS, with 6.4% experiencing grade 3 or greater, and 19% of the participants experienced ICANS, with 9.7% experiencing grade 3 or greater. Of the participants receiving axi-cel and brexu-cel, ICANS occurred in 22% of the participants, with 11% experiencing grade 3 or greater compared to over 60% overall and 28%–31% greater than grade 3 reported in ZUMA-1 and ZUMA-2 trials (14, 25).

The rationale for the use of anakinra, a commercially available IL-1 inhibitor, is based on pre-clinical models in mice, trends observed in the CSF of patients experiencing ICANS, and the ability of IL-1 receptor inhibitors to cross the blood–brain barrier (56–60). In both pre-clinical murine models, the mice were treated with CAR T cells and clinically manifested CRS. Monocytes were the source of both IL-6 and IL-1 driving the CRS. While IL-6 blockade with tocilizumab was effective at controlling the manifestations of CRS, it was not protective of neurotoxicity and inflammation. IL-1 blockade, however, was effective at mitigating the manifestations of both CRS and ICANS (56). Similarly, the CSF of patients with acute lymphoblastic leukemia experiencing ICANS was high in specific cytokines, including both IL-6 and IL-1 (57). These early findings represent a potential option for effective ICANS prophylaxis, especially in high-risk patient groups such as high-risk MCL patients and those with bulky disease burden or CNS involvement.

Late toxicities

The most common toxicities of CAR T-cell therapy are cytopenias. Indeed, at 1 month post-infusion, only 61%, 51%, and 33% of patients receiving CAR T-cell therapies were found to have recovered their hemoglobin, platelet, and neutrophil counts in an early retrospective study looking at hematological toxicity (61). Factors associated with a lower likelihood of hematopoietic recovery included baseline cytopenias, CAR construct, higher peak C-reactive protein and ferritin levels, and increasing-grade ICANS with a similar trend in CRS. Protracted cytopenias can cause significant co-morbidity to patients receiving CAR T-cell therapies.

The most morbid cytopenia is prolonged and severe neutropenia, which puts patients receiving CAR T-cell therapy at an increased risk for serious infection. Indeed advances in the management of both CRS and ICANS have led to fatal infections currently representing the most common cause of non-relapse mortality (NRM) in patients receiving this therapy (62, 63). All patients receive lymphodepleting chemotherapy prior to receipt of CAR T cells to provide an optimal environment for their expansion. This naturally leads to a transient period of cytopenia with expected recovery within 7–14 days post-chemotherapy. Protracted cytopenias, however, occur several weeks beyond this expected time frame and are felt to be due to immune dysregulation and inflammation occurring in the bone marrow following the administration of CAR T cells, though our understanding of this toxicity is evolving (63). Neutrophil recovery following infusion of CAR T-cells has been shown to exhibit quick, intermittent, or aplastic patterns (64, 65). The quick pattern shows sustained neutrophil recovery without any subsequent dips. The intermittent pattern shows neutrophil recovery followed by a second dip in neutrophil counts following day 21. Finally, the aplastic pattern shows continuous and severe neutropenia for greater than 14 days. Interestingly, an association between clinical outcomes and neutrophil recovery patterns has been found. The best clinical outcomes are associated to the intermittent neutrophil recovery pattern. The poorest clinical outcomes are associated to the aplastic neutrophil recovery pattern thought to be secondary to the presence of immune dysregulation which suppresses the expansion of CAR T cells (66).

In September 2023, the European Hematology Association/ European Society for Blood and Marrow Transplantation (EBMT) released consensus grading and practice recommendations for immune effector cell-associated hematotoxicity (ICAHT) (67). ICAHT grading is based on the duration and severity of neutropenia. As part of the practice recommendations, the CAR-HEMATOTOX score is used to identify patients at a high risk of prolonged neutropenia and aplastic phenotype of neutrophil

recovery (64). The score is calculated by looking at baseline bone marrow reserve (absolute neutrophil count, hemoglobin, and platelet count) and baseline inflammatory state (C-reactive protein and ferritin) prior to the receipt of lymphodepletion and places patients in either low risk or high risk categories. Based on this risk stratification, recommendations for anti-microbial prophylaxis, transfusion, and growth factor support have been suggested (68). The use of the CAR-HEMATOTOX score represents an important avenue to improve the supportive management of the infectious complications associated to CAR T-cell therapy. The association between clinical outcomes, baseline bone marrow reserve and inflammatory state, and hematological toxicity in patients receiving CAR T-cell therapy is an evolving area of research.

As more longitudinal experience is gained with CAR T-cell therapies, rare complications have emerged. While initially thought only to occur in conjunction with CRS, a life-threatening hemophagocytic lymphohistiocytosis (HLH)-like syndrome is increasingly being recognized post-CAR T-cell therapy. This entity often presenting as CRS is resolving or resolved and is believed to be associated to a protracted and exaggerated immune response which can cause end-organ damage. Current management strategies are derived from the expert opinion of those who have experiences this rare presentation and include the prompt initiation of anakinra and steroids with the addition of ruxolitinib or emapalumab if the case is progressively life-threatening (69). In addition, CAR T-cell therapies have recently been associated to a risk of secondary T-cell malignancies manifesting within 2 years of their receipt. Of the 22 cases known to the FDA as of December 2023, three had genetic sequencing performed, which detected the CAR transgene in the malignant clone, suggesting that the product was directly implicated in producing the cancer (70). Close monitoring of these rare but serious toxicities is warranted as well as the strategies to prevent them.

Bridging therapy

The administration of CAR T-cell therapies poses unique challenges. CAR T-cell manufacturing, depending on the CAR T-cell product, can take several weeks to months to complete. Clinically, this means that there is a period of time where patients progressing on their last line of therapy must wait and remain stable until they can receive their CAR T cells. This period is supported by "bridging therapy" for disease control and can include steroids, chemotherapy, radiation, or targeted therapies. Given the aggressiveness of aggressive B-cell lymphoma and the limited therapeutic options, this poses a specific challenge to these patients. Manufacturing time and burden of disease at relapse are particularly salient to differences between the administration of cellular therapies in the clinical trial *versus* real-world setting (71). In the ZUMA-2 trial, bridging therapies were limited to steroids and BTK inhibitors (on patients already having progressed on BTK inhibition), and only 37% of patients required bridging, suggesting a population with less disease burden (25). In the benchmark retrospective studies looking at outcomes post-ibrutinib in the pre-CAR T cell era, 29.8–37.9% of patients progressing on BTK inhibitors never received subsequent therapies as they rapidly deteriorated and died (5, 6). Early signs of progression on BTK inhibition or suboptimal clinical response should prompt referral for CAR T-cell therapy in MCL. Even in the ZUMA-1, TRANSCEND NHL 001, and JULIET studies, a significant number of patients did not receive CAR T-cell therapy due to complications related to disease progression or death (14, 17, 20). While bridging therapy prior to CAR T cell varies, one retrospective review of 439 patients with 80 receiving bendamustine prior to leukapheresis was associated with lower ORR (53% vs. 72%) as well as shorter PFS (3.1 vs. 6.2 months) and OS (10.3 vs. 23.5 months) with CAR T-cell therapy (72). The authors of the study noted that bendamustine use within 9 months of leukapheresis was also associated with worse outcomes in terms of ORR, PFS, and OS with CAR T-cell therapy, suggesting that its use should be avoided in CAR T-cell eligible patients. Radiation has also been used as an effective bridging strategy in several retrospective studies (73, 74). Radiation is thought to work synergistically with CAR T-cell therapy by increasing the release of tumor-specific antigens, thus improving tumor recognition by immune cells as well as increasing the sensitivity of tumor to the cytotoxic effects by CAR T cells (75, 76).

Predictors of success and failure of CAR T-cell therapy can be patient, disease, or CAR T-cell product-related. Both patient fitness prior to therapy and the degree of tumor burden at cell infusion impact the efficacy of CAR T cells, making effective bridging and conditioning strategies a key factor in success treatment (77). In addition, the cellular starting material and T-cell fitness impact the cell manufacturing process and the efficacy of the product—for example, the presence of monocytes–reduces T-cell transduction and CAR T-cell expansion *in vitro* (78).

Consolidation with hematopoietic stem cell transplant

Currently, there is no data to support consolidation with hematopoietic stem cell transplant following CAR T-cell therapy. In the ZUMA 1 study, two patients who responded to axi-cel for DLBCL underwent allogeneic stem cell transplant (alloSCT) (14). In the long-term study, the median OS of those who achieved a CR was not reached (15), suggesting that axi-cel was potentially curative as majority of the patients did not receive consolidative transplant. AlloSCT has been used in those who had relapsed after CAR T-cell therapy. The American Society of Transplantation and Cellular Therapy (ASTCT) considers ASCT for consolidation for early-relapse DLBCL patients who achieve a PR or CR following salvage chemotherapy as a category B recommendation (79). They also consider CAR T-cell therapy as an acceptable alternative in the same patient population, also with a category B recommendation (79).

In one multi-center retrospective study, 88 patients underwent alloSCT following failure of CAR T-cell therapy (80) for DLBCL. The follow-up was short, with a median of 15 months, and the 1-year PFS and OS were 45% and 59%, respectively. The 1-year non-relapse mortality was high 22%, and the 1-year relapse/progression rate was 33%.

For MCL, only one patient who had a PR following brexu-cel underwent alloSCT (25); thus, the role of consolidative transplantation following CAR T-cell therapy is unknown. The ASTCT (American Society for Transplantation and Cellular Therapy), CIBMTR (Center for International Blood and Marrow Transplant Research), and EBMT (European Society for Blood and Marrow Transplantation) recommend alloSCT for MCL patients who relapse or progress following CAR T-cell therapy if they achieve CR or PR with subsequent lymphoma-directed therapies (81).

Cost-effectiveness

While CAR T-cell therapies represent a paradigm shifting standard-of-care practice in the treatment of relapsed and refractory lymphomas with meaningful and prolonged remissions for patients, the resources required to manufacture these personalized products are significant, not to mention the burden on the patient. In one study of over 3,900 patients eligible for CAR T-cell therapy, over one-third traveled over an hour to the nearest academic center (82). Several cost-effectiveness analyses have been conducted to better understand the relationship between patient benefit and the economic impact of axi-cel, liso-cel, tisa-cel, and brexu-cel within North America and Europe for patients with R/R aggressive B-cell lymphoma (83–90).

The first study to look at the cost-effectiveness of CAR T-cell therapy used a decision analytic Markov model and assumed that, at 40% 5-year PFS, axi-cel increased the life expectancy by 8.2 years at $129,000/quality-adjusted life years (QALY) gained (87). However, at 30% 5-year PFS, axi-cel increased the life expectancy by 6.4 years at $159,000/QALY gained. The 5-year ZUMA 1 study showed a 5-year PFS of 31% (15). For tisa-cel, assuming 35% 5-year PFS, life expectancy would be increased by 4.6 years at $168,000/QALY gained, while the numbers were 3.4 years gained at $233,000/QALY gained assuming a 25% 5-year PFS. The authors determined that the prices of axi-cel and tisa-cel would need to be reduced to $250,000 and $200,000, respectively, or payment only for patients who achieve CR. However, at the time of analysis, fewer SOC options were available to R/R DLBCL patients. A later study did not find second-line CAR T-cell therapy to be cost-effective in DLBCL patients (88) at a willingness-to-pay threshold of $200,000/QALY. However, two other cost analyses did find CAR T-cell therapy to be cost-effective in the second-line setting (89, 90) at a willingness-to-pay threshold of $150,000 in both studies as these studies took into account less effective and newer but more expensive and indefinite salvage treatment options.

In contract, numerous studies have shown brexu-cel to be a cost-effective alternative to standard-of-care practice due to its benefit in health-related quality-of-life and incremental survival. There is no established standard-of-care therapy in the treatment of relapsed or refractory MCL following the use of a BTK inhibitor. Options include lenalidomide, bortezomib, venetoclax, other BTK inhibitors, and bendamustine-containing chemo-immunotherapy regimens. Accepted comparisons for survival in patients with relapsed or refractory MCL who progressed on BTK inhibition include the retrospective SCOLAR-2 study conducted in Europe and a large 2016 retrospective study by Martin et al. (5, 6).

In the cost-effectiveness analysis for brexu-cel in patients with relapsed/refractory MCL conducted in the United States, the population inputs and health state utilities were derived from the ZUMA-2 trial. The model assumed that patients whose disease had not progressed after 5 years experienced long-term remissions. In the analysis, the median survival was 9.71 years *versus* 2.13 years, estimated expected life years (LY) were 8.99 years vs. 4.47 years, and QALY were 7.39 years vs. 3.65 years for brexu-cel *versus* standard of care. The total cost for brexu-cel was $693,832 USD *versus* $574,263 USD for standard of care. The brexu-cel *versus* standard-of-care cost per QALY was $31 985 (83). The substantial LY and QALY benefit supports brexu-cel as a cost-effective therapy. The benefit was sustained in the cost-effectiveness analyses conducted in Canada, England, and Italy despite the total cost of brexu-cel and especially with the standard of care being significantly lower (84–86)—for example, in the cost-effectiveness analysis conducted in England, whose benchmark for standard of care was the SCHOLAR-2 study, the total cost of brexu-cel *versus* SOC was £385,765 *versus* £48,645. The brexu-cel versus SOC cost per QALY remained comparable with the findings in the US at £67,713 (85). These findings support the continued development of CAR T cell and other cellular therapies for patients with relapsed and refractory MCL.

Comparison of CAR T-cell products

While there is only one CAR T-cell product for MCL currently, there are three for large B-cell lymphomas. The choice of product is chosen by the cellular therapy specialist and considers the impact of various factors such as manufacturing time, toxicities, and efficacy as well as patient-related factors such as co-morbidities, age, and tumor burden. While axi-cel is associated with a higher incidence and a higher grade of CRS and ICANS, the manufacturing time is significantly shorter and the manufacturing success rate is higher than that of liso-cel and tisa-cel (14, 17, 20). This may be a good option for the young, healthy patients with a high tumor burden and refractory disease where time is of essence with the caveat that toxicities may be high, whereas older, frailer patients with multiple co-morbidities with a lower tumor burden may benefit from liso-cel or tisa-cel due to their lower toxicity profile with the option of outpatient administration but at the cost of longer manufacturing time and increased chance of receiving a non-conforming product. While non-conforming products have been shown to have similar efficacy to lisa-cel in the TRANSCEND NHL study (20), patients often have to enroll in an expanded access protocol to receive their CAR T cells, thus further delaying the time between leukapheresis and infusion.

Relapses after CAR T-cell therapy

Resistance to CAR T-cell therapies includes loss of CD19 antigen, new mutations or post-translational modifications in CD19, defective manufacturing of T cells, insufficient T cell expansion, changes to the cytokine milieu or functioning of CD4/CD8, upregulation of negative regulatory receptors, interaction between the tumor microenvironment on T-cell expansion, and impaired death receptor signaling (91, 92). Genomic profiling can uncover these mechanisms and develop strategies to mitigate them —for example, single-cell RNA sequencing and multiplex cytokine profiling on serial peripheral blood samples of patients treated with brexu-cel who eventually relapsed showed that the proportion of T cells, particularly cytotoxic T cells (CTLs), decreased. While TIGIT, LAG3, and CD96 were the most common checkpoint molecules expressed on exhausted CTLs and T cells, in general, only TIGIT significantly increased after relapse. CTLs expanded during remission and contracted at relapse with upregulated TIGIT expression. In addition, tumor cells acquired TIGIT expression (93). Co-targeting TIGIT during CAR T-cell therapy may serve as another avenue to prevent CAR T-cell relapse in MCL. In addition, the receptor tyrosine kinase-like orphan receptor 1 (ROR1) is expressed on MCL cells and has been shown to be particularly elevated in CAR T-cell relapsed MCL cells (94, 95). *In vitro*, an antibody–drug conjugate of ROR1 conjugated to monomethyl auristatin E, known as VLS-101, has induced tumor regression in MCL models of CAR T cell, ibrutinib, and venetoclax resistance (96). A phase 1 study of VLS-101 demonstrated safety and durable responses in patients with MCL, including those who have received prior BTK inhibitors and cellular therapies (97).

Future directions

While the current CAR T-cell products have revolutionized the treatment of aggressive B-cell lymphomas, improvement of the current landscape is already occurring. A phase 2 trial of axi-cel in high-risk large B-cell lymphoma patients who failed to achieve a Deauville score of 3 or better after two cycles of frontline chemoimmunotherapy has shown remarkable results of 78% CR (ORR of 89%), with median EFS and PFS not reached (98). Third-generation CAR T-cell products have two co-stimulatory domains containing CD28 and 4–1BB, which have been shown to improve efficacy *in vitro* and in animal models *in vivo*, with human trials being underway (99). Additionally, bispecific CAR T cells (targeted against both CD19 and CD20) have also been made to counteract the loss of CD19 expression in some B-cell lymphoproliferative disorders (100). CRISPR/Cas9 technology is also being used to enhance the effectiveness of CAR T-cell therapy by modifying T cells to improve their persistence and efficacy by disrupting genes associated with T cell exhaustion (101). Finally, allogeneic CAR T-cell products from healthy donors offer the most excitement as these counteract the need for leukapheresis and long wait time for manufacturing and potential for re-treatment if necessary. The phase 1 study of anti-CD19 allogeneic CAR T-cell products of the ALLO-501 and ALLO-501A ALPHA studies administered in patients with large B-cell lymphoma with two failed lines of treatment demonstrated a promising ORR of 67% with CR of 58% (102).

Conclusion

The success of CAR T-cell therapy in the treatment of patients with aggressive B-cell lymphomas is practice-changing and provides a needed, durable therapeutic option for many patients who historically would have had dismal outcomes. While work remains to be done to optimize the effectiveness and toxicity management of this novel therapeutic approach and better incorporate it into the most effective sequence of therapy, especially with the advent of bispecific antibodies with milder toxicity profiles, there is no doubt that cellular therapies have changed the paradigm with which aggressive B-cell lymphoma patients are treated.

Author contributions

BH: Conceptualization, Supervision, Writing – original draft, Writing – review & editing. VK: Writing – original draft, Writing – review & editing. MP: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Conflict of interest

BH participated on advisory board for Janssen Biotech and Pharmacyclics. Research funding to institution from BMS, Genentech/Roche, and Beigene. MP has received research funding and honorarium from BMS, Cellectar, Ceramedix, Juno, Kite MustangBio, Garuda Therapeutics, Novartis, Pluto Immunotherapeutics, Rheos, Seres Therapeutics, Smart Immune, Thymofox, Synthekine. Received other from June and Seres.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Crump M, Neelapu SS, Farooq U, Van Den Neste E, Kuruvilla J, Westin J, et al. Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. *Blood*. (2017) 130:1800–8. doi: 10.1182/blood-2017-03-769620

2. Cheah CY, Chihara D, Romaguera JE, Fowler NH, Seymour JF, Hagemeister FB, et al. Patients with mantle cell lymphoma failing ibrutinib are unlikely to respond to salvage chemotherapy and have poor outcomes. *Ann Oncol*. (2015) 26:1175–9. doi: 10.1093/annonc/mdv111

3. Epperla N, Hamadani M, Cashen AF, Ahn KW, Oak E, Kanate AS, et al. Predictive factors and outcomes for ibrutinib therapy in relapsed/refractory mantle cell lymphoma-a "real world" study. *Hematol Oncol*. (2017) 35:528–35. doi: 10.1002/hon.2380

4. Jain P, Kanagal-Shamanna R, Zhang S, Ahmed M, Ghorab A, Zhang L, et al. Long-term outcomes and mutation profiling of patients with mantle cell lymphoma (MCL) who discontinued ibrutinib. *Br J Haematol*. (2018) 183:578–87. doi: 10.1111/bjh.15567

5. Martin P, Maddocks K, Leonard JP, Ruan J, Goy A, Wagner-Johnston N, et al. Postibrutinib outcomes in patients with mantle cell lymphoma. *Blood*. (2016) 127:1559–63. doi: 10.1182/blood-2015-10-673145

6. Hess G, Dreyling M, Oberic L, Gine E, Zinzani PL, Linton K, et al. Real-world experience among patients with relapsed/refractory mantle cell lymphoma after Bruton tyrosine kinase inhibitor failure in Europe: The SCHOLAR-2 retrospective chart review study. *Br J Haematol*. (2023) 202:749–59. doi: 10.1111/bjh.18519

7. Jensen MC, Popplewell L, Cooper LJ, DiGiusto D, Kalos M, Ostberg JR, et al. Antitransgene rejection responses contribute to attenuated persistence of adoptively transferred CD20/CD19-specific chimeric antigen receptor redirected T cells in humans. *Biol Blood Marrow Transplant*. (2010) 16:1245–56. doi: 10.1016/j.bbmt.2010.03.014

8. Savoldo B, Ramos CA, Liu E, Mims MP, Keating MJ, Carrum G, et al. CD28 costimulation improves expansion and persistence of chimeric antigen receptor-modified T cells in lymphoma patients. *J Clin Invest*. (2011) 121:1822–6. doi: 10.1172/JCI46110

9. Salter AI, Ivey RG, Kennedy JJ, Voillet V, Rajan A, Alderman EJ, et al. Phosphoproteomic analysis of chimeric antigen receptor signaling reveals kinetic and quantitative differences that affect cell function. *Sci Signal*. (2018) 11. doi: 10.1126/scisignal.aat6753

10. Davila ML, Riviere I, Wang X, Bartido S, Park J, Curran K, et al. Efficacy and toxicity management of 19-28z CAR T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med*. (2014) 6:224ra25. doi: 10.1126/scitranslmed.3008226

11. Zhao Z, Condomines M, van der Stegen SJC, Perna F, Kloss CC, Gunset G, et al. Structural design of engineered costimulation determines tumor rejection kinetics and persistence of CAR T cells. *Cancer Cell*. (2015) 28:415–28. doi: 10.1016/j.ccell.2015.09.004

12. Turtle CJ, Hanafi LA, Berger C, Hudecek M, Pender B, Robinson E, 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) 8:355ra116. doi: 10.1126/scitranslmed.aaf8621

13. Klebanoff CA, Khong HT, Antony PA, Palmer DC, Restifo NP. Sinks, suppressors and antigen presenters: how lymphodepletion enhances T cell-mediated tumor immunotherapy. *Trends Immunol*. (2005) 26:111–7. doi: 10.1016/j.it.2004.12.003

14. 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) 377:2531–44. doi: 10.1056/NEJMoa1707447

15. Neelapu SS, Jacobson CA, Ghobadi A, Miklos DB, Lekakis LJ, Oluwole OO, et al. Five-year follow-up of ZUMA-1 supports the curative potential of axicabtagene ciloleucel in refractory large B-cell lymphoma. *Blood*. (2023) 141:2307–15.

16. 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) 386:640–54. doi: 10.1056/NEJMoa2116133

17. 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) 380:45–56. doi: 10.1056/NEJMoa1804980

18. Schuster SJ, Tam CS, Borchmann P, Worel N, McGuirk JP, Holte H. Long-term clinical outcomes of tisagenlecleucel in patients with relapsed or refractory aggressive B-cell lymphomas (JULIET): a multicentre, open-label, single-arm, phase 2 study. *Lancet Oncol. The*. (2021) 22:1403. doi: 10.1016/S1470-2045(21)00375-2

19. 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) 386:629–39. doi: 10.1056/NEJMoa2116596

20. 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) 396:839–52.

21. Abramson JS, Palomba ML, Gordon LI, Lunning M, Wang M, Arnason J, 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):404–16. doi: 10.1182/blood.2023020854

22. 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) 399:2294–308.

23. 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) 23:1066–77.

24. Wang M, Siddiqi T, Gordon L, Kamdar M, Lunning M, Hirayama A, et al. (Liso-cel) in r/r mcl: primary analysis results from the mcl cohort of the single-arm, multicenter, seamless design transcend nhl 001 study. *Hematol Oncol*. (2023) 41:875–7. doi: 10.1002/hon.3196_LBA3

25. 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) 382:1331–42. doi: 10.1056/NEJMoa1914347

26. 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) 41:555–67. doi: 10.1200/JCO.21.02370

27. 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) 4:4898–911. doi: 10.1182/bloodadvances.2020002394

28. Ramsborg C, Guptill P, Weber C, Christin B, Larson R, Lewis K, et al. JCAR017 is a defined composition CAR T cell product with product and process controls that deliver precise doses of CD4 and CD8 CAR T cell to patients with NHL. *Blood*. (2017) 130:4471.

29. Jain P, Wang M. Blastoid mantle cell lymphoma. *Hematol Oncol Clin North Am*. (2020) 34:941–56. doi: 10.1016/j.hoc.2020.06.009

30. Eskelund CW, Dahl C, Hansen JW, Westman M, Kolstad A, Pedersen LB, et al. TP53 mutations identify younger mantle cell lymphoma patients who do not benefit from intensive chemoimmunotherapy. *Blood*. (2017) 130:1903–10. doi: 10.1182/blood-2017-04-779736

31. Jain P, Wang Y, Locke FL, Munoz J, Beitinjaneh A, Frank MJ, et al. Brexucabtagene autoleucel for relapsed/refractory mantle cell lymphoma: Real-world experience from the United States lymphoma CAR T consortium. *Am Soc Clin Oncol*. (2022) 40(16 supplement). doi: 10.1200/JCO.2022.40.16_suppl.e19583

32. Iacoboni G, Rejeski K, Villacampa G, van Doesum JA, Chiappella A, Bonifazi F, et al. Real-world evidence of brexucabtagene autoleucel for the treatment of relapsed or refractory mantle cell lymphoma. *Blood Adv*. (2022) 6:3606–10. doi: 10.1182/bloodadvances.2021006922

33. 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) 41:2594–606. doi: 10.1200/JCO.22.01797

34. Siddiqi T, Wang X, Blanchard MS, Wagner JR, Popplewell LL, Budde LE, et al. CD19-directed CAR T-cell therapy for treatment of primary CNS lymphoma. *Blood Adv*. (2021) 5:4059–63. doi: 10.1182/bloodadvances.2020004106

35. Alcantara M, Houillier C, Blonski M, Rubio M-T, Willems L, Rascalou AW, et al. CAR T-cell therapy in primary central nervous system lymphoma: the clinical experience of the French LOC network. *Blood*. (2022) 139:792–6. doi: 10.1182/blood.2021012932

36. Karschnia P, Blobner J, Teske N, Schöberl F, Fitzinger E, Dreyling M, et al. CAR T-cells for CNS lymphoma: driving into new terrain? *Cancers (Basel)*. (2021) 13.

37. Ahmed G, Hamadani M, Shah NN. CAR T-cell therapy for secondary CNS DLBCL. *Blood Adv*. (2021) 5:5626–30.

38. Frigault MJ, Dietrich J, Martinez-Lage M, Leick M, Choi BD, DeFilipp Z, et al. Tisagenlecleucel CAR T-cell therapy in secondary CNS lymphoma. *Blood*. (2019) 134:860–6.

39. Cook MR, Dorris CS, Makambi KH, Luo Y, Munshi PN, Donato M, et al. Toxicity and efficacy of CAR T-cell therapy in primary and secondary CNS lymphoma: a meta-analysis of 128 patients. *Blood Adv*. (2023) 7:32–9.

40. Epperla N, Feng L, Shah NN, Fitzgerald L, Shah H, Stephens DM, et al. Outcomes of patients with secondary central nervous system lymphoma following CAR T-cell therapy: a multicenter cohort study. *J Hematol Oncol*. (2023) 16:111.

41. Vu K, Frank MJ. CAR T-cell therapy for mantle cell lymphoma with central nervous system relapse. *Blood Adv*. (2023) 7:375–8. doi: 10.1182/bloodadvances.2022008031

42. Caillet A, Houillier C, Sourdeau E, Gazzano M, Uzunov M, Friser V, et al. Successful treatment by CAR T-cells in multi-refractory mantle cell lymphoma with

central nervous system involvement. *Ann Hematol*. (2023) 102:3295. doi: 10.1007/s00277-023-05408-x

43. Khurana A, Dalland JC, Young JR, Inwards DJ, Paludo J. Brexucabtagene autoleucel therapy induces complete remission in a primary refractory blastoid mantle cell lymphoma with neurolymphomatosis. *Am J Hematol*. (2021) 96. doi: 10.1002/ajh.26233

44. Palomba M, Siddiqi T, Gordon L, Kamdar M, Lunning M, Hirayama A, et al. Lisocabtagene maraleucel (liso-cel) in patients (Pt) with R/R MCL: subgroup analyses in pts with high-risk disease features from the MCL cohort of the TRANSCEND NHL 001. *Transplant Cell Ther*. (2024) 30. doi: 10.1016/j.jtct.2023.12.037

45. Lee DW, Santomasso BD, Locke FL, Ghobadi A, Turtle CJ, Brudno JN, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. *Biol Blood Marrow Transplant*. (2019) 25:625–38. doi: 10.1016/j.bbmt.2018.12.758

46. Hay KA, Hanafi LA, Li D, Gust J, Liles WC, Wurfel MM, et al. Kinetics and biomarkers of severe cytokine release syndrome after CD19 chimeric antigen receptor-modified T-cell therapy. *Blood*. (2017) 130:2295–306. doi: 10.1182/blood-2017-06-793141

47. Morris EC, Neelapu SS, Giavridis T, Sadelain M. Cytokine release syndrome and associated neurotoxicity in cancer immunotherapy. *Nat Rev Immunol*. (2022) 22:85–96. doi: 10.1038/s41577-021-00547-6

48. Le RQ, Li L, Yuan W, Shord SS, Nie L, Habtemariam BA, et al. FDA approval summary: tocilizumab for treatment of chimeric antigen receptor T cell-induced severe or life-threatening cytokine release syndrome. *Oncologist*. (2018) 23:943–7. doi: 10.1634/theoncologist.2018-0028

49. van Rhee F, Fayad L, Voorhees P, Furman R, Lonial S, Borghaei H, et al. Siltuximab, a novel anti-interleukin-6 monoclonal antibody, for Castleman's disease. *J Clin Oncol*. (2010) 28:3701–8. doi: 10.1200/JCO.2009.27.2377

50. Mahmoudjafari Z, Hawks KG, Hsieh AA, Plesca D, Gatwood KS, Culos KA. American society for blood and marrow transplantation pharmacy special interest group survey on chimeric antigen receptor T cell therapy administrative, logistic, and toxicity management practices in the United States. *Biol Blood Marrow Transplant*. (2019) 25:26–33. doi: 10.1016/j.bbmt.2018.09.024

51. Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, et al. Current concepts in the diagnosis and management of cytokine release syndrome. *Blood*. (2014) 124:188–95. doi: 10.1182/blood-2014-05-552729

52. Gabay C, Lamacchia C, Palmer G. IL-1 pathways in inflammation and human diseases. *Nat Rev Rheumatol*. (2010) 6:232–41. doi: 10.1038/nrrheum.2010.4

53. Prahalad S, Bove KE, Dickens D, Lovell DJ, Grom AA. Etanercept in the treatment of macrophage activation syndrome. *J Rheumatol*. (2001) 28:2120–4.

54. Flammiger A, Fiedler W, Bacher U, Bokemeyer C, Schneider M, Binder M. Critical imbalance of TNF-alpha and soluble TNF receptor 1 in a patient with macrophage activation syndrome: potential implications for diagnostics and treatment. *Acta Haematol*. (2012) 128:69–72. doi: 10.1159/000338179

55. Park JH, Nath K, Devlin SM, Sauter CS, Palomba ML, Shah G, et al. CD19 CAR T-cell therapy and prophylactic anakinra in relapsed or refractory lymphoma: phase 2 trial interim results. *Nat Med*. (2023) 29:1710–7. doi: 10.1038/s41591-023-02404-6

56. Norelli M, Camisa B, Barbiera G, Falcone L, Purevdorj A, Genua M, et al. Monocyte-derived IL-1 and IL-6 are differentially required for cytokine-release syndrome and neurotoxicity due to CAR T cells. *Nat Med*. (2018) 24:739–48. doi: 10.1038/s41591-018-0036-4

57. Santomasso BD, Park JH, Salloum D, Riviere I, Flynn J, Mead E, et al. Clinical and biological correlates of neurotoxicity associated with CAR T-cell therapy in patients with B-cell acute lymphoblastic leukemia. *Cancer Discov*. (2018) 8:958–71. doi: 10.1158/2159-8290.CD-17-1319

58. Giavridis T, van der Stegen SJC, Eyquem J, Hamieh M, Piersigilli A, Sadelain M. CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. *Nat Med*. (2018) 24:731–8. doi: 10.1038/s41591-018-0041-7

59. Gutierrez EG, Banks WA, Kastin AJ. Blood-borne interleukin-1 receptor antagonist crosses the blood-brain barrier. *J Neuroimmunol*. (1994) 55:153–60. doi: 10.1016/0165-5728(94)90005-1

60. Galea J, Ogungbenro K, Hulme S, Greenhalgh A, Aarons L, Scarth S, et al. Intravenous anakinra can achieve experimentally effective concentrations in the central nervous system within a therapeutic time window: results of a dose-ranging study. *J Cereb Blood Flow Metab*. (2011) 31:439–47. doi: 10.1038/jcbfm.2010.103

61. Jain T, Knezevic A, Pennisi M, Chen Y, Ruiz JD, Purdon TJ, et al. Hematopoietic recovery in patients receiving chimeric antigen receptor T-cell therapy for hematologic Malignancies. *Blood Adv*. (2020) 4:3776–87. doi: 10.1182/bloodadvances.2020002509

62. Rejeski K, Perez A, Iacoboni G, Penack O, Bücklein V, Jentzsch L, 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. doi: 10.1136/jitc-2021-004475

63. 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) 98:1699–710. doi: 10.1002/ajh.27056

64. 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) 138:2499–513. doi: 10.1182/blood.2020010543

65. Fried S, Avigdor A, Bielorai B, Meir A, Besser MJ, Schachter J, et al. Early and late hematologic toxicity following CD19 CAR-T cells. *Bone Marrow Transplantat*. (2019) 54:1643–50. doi: 10.1038/s41409-019-0487-3

66. Rejeski K, Perez A, Iacoboni G, Blumenberg V, Bücklein VL, Völkl 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:eadg3919. doi: 10.1126/sciadv.adg3919

67. Rejeski K, Subklewe M, Aljurf M, Bachy E, Balduzzi A, Barba P, et al. Immune effector cell–associated hematotoxicity: EHA/EBMT consensus grading and best practice recommendations. *Blood*. (2023) 142:865–77. doi: 10.1182/blood.2023020578

68. Hill JA, Seo SK. How I prevent infections in patients receiving CD19-targeted chimeric antigen receptor T cells for B-cell Malignancies. *Blood*. (2020) 136:925–35. doi: 10.1182/blood.2019004000

69. Hines MR, Knight TE, McNerney KO, Leick MB, Jain T, Ahmed S, et al. Immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome. *Transplant Cell Therapy Off Publ Am Soc Transplant Cell Ther*. (2023) 29:438. doi: 10.1016/j.jtct.2023.03.006

70. Verdun N, Marks P. Secondary cancers after chimeric antigen receptor T-cell therapy. *N Engl J Med*. (2024) 390:584–6. doi: 10.1056/NEJMp2400209

71. McCulloch R. Post-BTK inhibitor mantle cell lymphoma: When is CAR-T not the answer? *Br J Haematol*. (2023) 202:718–9. doi: 10.1111/bjh.18868

72. 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) 42:205–17. doi: 10.1200/JCO.23.01097

73. Pinnix CC, Gunther JR, Dabaja BS, Strati P, Fang P, Hawkins MC, et al. Bridging therapy prior to axicabtagene ciloleucel for relapsed/refractory large B-cell lymphoma. *Blood Adv*. (2020) 4:2871–83. doi: 10.1182/bloodadvances.2020001837

74. Sim AJ, Jain MD, Figura NB, Chavez JC, Shah BD, Khimani F, et al. Radiation therapy as a bridging strategy for CAR T cell therapy with axicabtagene ciloleucel in diffuse large B-cell lymphoma. *Int J Radiat Oncol Biol Phys*. (2019) 105:1012–21. doi: 10.1016/j.ijrobp.2019.05.065

75. DeSelm C, Palomba ML, Yahalom J, Hamieh M, Eyquem J, Rajasekhar VK, et al. Low-dose radiation conditioning enables CAR T cells to mitigate antigen escape. *Mol Ther*. (2018) 26:2542–52. doi: 10.1016/j.ymthe.2018.09.008

76. Lai JZ, Zhu YY, Ruan M, Chen L, Zhang QY. Local irradiation sensitized tumors to adoptive T cell therapy *via* enhancing the cross-priming, homing, and cytotoxicity of antigen-specific CD8 T cells. *Front Immunol*. (2019) 10:2857. doi: 10.3389/fimmu.2019.02857

77. Baguet C, Larghero J, Mebarki M. Early predictive factors of failure in autologous CAR T-cell manufacturing and/or efficacy in hematologic Malignancies. *Blood Adv*. (2024) 8:337–42. doi: 10.1182/bloodadvances.2023011992

78. Wang X, Borquez-Ojeda O, Stefanski J, Du F, Qu J, Chaudhari 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) 22:377–87. doi: 10.1016/j.omtm.2021.06.014

79. Epperla N, Kumar A, Abutalib SA, Awan FT, Chen YB, Gopal AK, et al. ASTCT clinical practice recommendations for transplantation and cellular therapies in diffuse large B cell lymphoma. *Transplant Cell Ther*. (2023) 29:548–55. doi: 10.1016/j.jtct.2023.06.012

80. Zurko J, Ramdial J, Shadman M, Ahmed S, Szabo A, Iovino L, et al. Allogeneic transplant following CAR T-cell therapy for large B-cell lymphoma. *Haematologica*. (2023) 108:98–109. doi: 10.3324/haematol.2022.281242

81. Munshi PN, Hamadani M, Kumar A, Dreger P, Friedberg JW, Dreyling M, et al. ASTCT, CIBMTR, and EBMT clinical practice recommendations for transplant and cellular therapies in mantle cell lymphoma. *Bone Marrow Transplant*. (2021) 56:2911–21. doi: 10.1038/s41409-021-01288-9

82. Snyder S, Chung KC, Jun MP, Gitlin M. Access to chimeric antigen receptor T cell therapy for diffuse large B cell lymphoma. *Adv Ther*. (2021) 38:4659–74. doi: 10.1007/s12325-021-01838-z

83. Simons CL, Malone D, Wang M, Maglinte GA, Inocencio T, Wade SW, et al. Cost-effectiveness for KTE-X19 CAR T therapy for adult patients with relapsed/refractory mantle cell lymphoma in the United States. *J Med Econ*. (2021) 24:421–31. doi: 10.1080/13696998.2021.1894158

84. Ball G, Lemieux C, Cameron D, Seftel MD. Cost-Effectiveness of Brexucabtagene Autoleucel versus Best Supportive Care for the Treatment of Relapsed/Refractory Mantle Cell Lymphoma following Treatment with a Bruton's Tyrosine Kinase Inhibitor in Canada. *Curr Oncol*. (2022) 29:2021–45. doi: 10.3390/curroncol29030164

85. Petersohn S, Salles G, Wang M, Wu J, Wade SW, Simons CL, et al. Cost-effectiveness analysis of KTE-X19 CAR T therapy versus real-world standard of care in patients with relapsed/refractory mantle cell lymphoma post BTKi in England. *J Med Econ*. (2022) 25:730–40. doi: 10.1080/13696998.2022.2079317

86. Marchetti M, Visco C. Cost-Effectiveness of brexucabtagene autoleucel for relapsed/refractory mantle cell lymphoma. *Leuk Lymphoma*. (2023) 64:1442–50. doi: 10.1080/10428194.2023.2215888

87. Lin JK, Muffly LS, Spinner MA, Barnes JI, Owens DK, Goldhaber-Fiebert JD. Cost effectiveness of chimeric antigen receptor T-cell therapy in multiply relapsed or refractory adult large B-cell lymphoma. *J Clin Oncol*. (2019) 37:2105–19. doi: 10.1200/JCO.18.02079

88. Kelkar AH, Cliff ERS, Jacobson CA, Abel GA, Dijk SW, Krijkamp EM, et al. Second-line chimeric antigen receptor T-cell therapy in diffuse large B-cell lymphoma : A cost-effectiveness analysis. *Ann Intern Med*. (2023) 176:1625–37. doi: 10.7326/M22-2276

89. Choe JH, Abdel-Azim H, Padula WV, Abou-El-Enein M. Cost-effectiveness of axicabtagene ciloleucel and tisagenlecleucel as second-line or later therapy in relapsed or refractory diffuse large B-cell lymphoma. *JAMA Netw Open*. (2022) 5:e2245956. doi: 10.1001/jamanetworkopen.2022.45956

90. Kambhampati S, Saumoy M, Schneider Y, Serrao S, Solaimani P, Budde LE, et al. Cost-effectiveness of second-line axicabtagene ciloleucel in relapsed refractory diffuse large B-cell lymphoma. *Blood*. (2022) 140:2024–36. doi: 10.1182/blood.2022016747

91. Jain P, Nastoupil L, Westin J, Lee HJ, Navsaria L, Steiner RE, et al. Outcomes and management of patients with mantle cell lymphoma after progression on brexucabtagene autoleucel therapy. *Br J Haematol*. (2021) 192:e38–42. doi: 10.1111/bjh.17197

92. Huang Z, Chavda VP, Bezbaruah R, Dhamne H, Yang DH, Zhao HB. CAR T-Cell therapy for the management of mantle cell lymphoma. *Mol Canc*. (2023) 22:67. doi: 10.1186/s12943-023-01755-5

93. Jiang VC, Hao D, Jain P, Li Y, Cai Q, Yao Y, et al. TIGIT is the central player in T-cell suppression associated with CAR T-cell relapse in mantle cell lymphoma. *Mol Canc*. (2022) 21:185. doi: 10.1186/s12943-022-01655-0

94. Zhang S, Chen L, Wang-Rodriguez J, Zhang L, Cui B, Frankel W, et al. The onco-embryonic antigen ROR1 is expressed by a variety of human cancers. *Am J Pathol*. (2012) 181:1903–10. doi: 10.1016/j.ajpath.2012.08.024

95. Zhang Q, Wang HY, Liu X, Nunez-Cruz S, Jillab M, Melnikov O, et al. Cutting edge: ROR1/CD19 receptor complex promotes growth of mantle cell lymphoma cells independently of the B cell receptor-BTK signaling pathway. *J Immunol*. (2019) 203:2043–8. doi: 10.4049/jimmunol.1801327

96. Jiang VC, Liu Y, Jordan A, McIntosh J, Li Y, Che Y, et al. The antibody drug conjugate VLS-101 targeting ROR1 is effective in CAR T-resistant mantle cell lymphoma. *J Hematol Oncol*. (2021) 14:132. doi: 10.1186/s13045-021-01143-w

97. Wang M, Barrientos JC, Furman RR, Mei M, Barr PM, Choi MY, et al. VLS-101, a ROR1-targeting antibody-drug conjugate, demonstrates a predictable safety profile and clinical efficacy in patients with heavily pretreated mantle cell lymphoma and diffuse large B-cell lymphoma. *Blood*. (2020) 136:13–4. doi: 10.1182/blood-2020-139468

98. Neelapu SS, Dickinson M, Munoz J, Ulrickson ML, Thieblemont C, Oluwole OO, et al. Axicabtagene ciloleucel as first-line therapy in high-risk large B-cell lymphoma: the phase 2 ZUMA-12 trial. *Nat Med*. (2022) 28:735–42. doi: 10.1038/s41591-022-01731-4

99. George P, Dasyam N, Giunti G, Mester B, Bauer E, Andrews B, et al. Third-generation anti-CD19 chimeric antigen receptor T-cells incorporating a TLR2 domain for relapsed or refractory B-cell lymphoma: a phase I clinical trial protocol (ENABLE). *BMJ Open*. (2020) 10:e034629. doi: 10.1136/bmjopen-2019-034629

100. Zah E, Lin MY, Silva-Benedict A, Jensen MC, Chen YY. T cells expressing CD19/CD20 bispecific chimeric antigen receptors prevent antigen escape by Malignant B cells. *Cancer Immunol Res*. (2016) 4:498–508. doi: 10.1158/2326-6066.CIR-15-0231

101. Wei W, Chen ZN, Wang K. CRISPR/cas9: A powerful strategy to improve CAR-T cell persistence. *Int J Mol Sci*. (2023) 24. doi: 10.3390/ijms241512317

102. Locke F, Lekakis L, Eradat H, Munoz J, Tees M, de Vos S, et al. Phase 1 results with anti-CD19 allogeneic CAR T ALLO-501/501A in relapsed/refractory large B-cell lymphoma (r/r LBCL). *J Clin Oncol*. (2023) 41. doi: 10.1200/JCO.2023.41.16_suppl.2517

frontiers | Frontiers in Oncology

TYPE Mini Review
PUBLISHED 03 July 2024
DOI 10.3389/fonc.2024.1398078

OPEN ACCESS

EDITED BY
Jose-Maria Ribera,
Germans Trias i Pujol Health Science Research Institute (IGTP), Spain

REVIEWED BY
Yifan Pang,
Levine Cancer Institute, United States

*CORRESPONDENCE
Zainab Shahid
shahidz@mskcc.org

RECEIVED 08 March 2024
ACCEPTED 04 June 2024
PUBLISHED 03 July 2024

CITATION
Arya S and Shahid Z (2024) Overview of infectious complications among CAR T- cell therapy recipients.
Front. Oncol. 14:1398078.
doi: 10.3389/fonc.2024.1398078

Overview of infectious complications among CAR T- cell therapy recipients

Swarn Arya[1] and Zainab Shahid[1,2]*

[1]Infectious Disease Service, Memorial Sloan Kettering Cancer Center, New York, NY, United States, [2]Department of Medicine, Weill Cornell School of Medicine, New York, NY, United States

Chimeric antigen receptor-modified T cell (CAR T-cell) therapy has revolutionized the management of hematological malignancies. In addition to impressive malignancy-related outcomes, CAR T-cell therapy has significant toxicity-related adverse events, including cytokine release syndrome (CRS), immune effector cell associated neurotoxicity syndrome (ICANS), immune effector cell-associated hematotoxicity (ICAHT), and opportunistic infections. Different CAR T-cell targets have different epidemiology and risk factors for infection, and these targets result in different long-term immunodeficiency states due to their distinct on-target and off- tumor effects. These effects are exacerbated by the use of multimodal immunosuppression in the management of CRS and ICANS. The most effective course of action for managing infectious complications involves determining screening, prophylactic, and monitoring strategies and understanding the role of immunoglobulin replacement and re-vaccination strategies. This involves considering the nature of prior immunomodulating therapies, underlying malignancy, the CAR T-cell target, and the development and management of related adverse events. In conclusion, we now have an increasing understanding of infection management for CAR T-cell recipients. As additional effector cells and CAR T-cell targets become available, infection management strategies will continue to evolve.

KEYWORDS

infectious complications, chimeric antigen receptor T-cell therapy, infection management, immunoglobulin replacement therapy, vaccinations

1 Introduction

The chimeric antigen receptor-modified T cell (CAR T-cell) therapy field has rapidly expanded since the US Food and Drug Administration (FDA) first approved CD19-targeted CAR T-cells for patients with relapsed refractory B lymphoid malignancies in 2017 (1, 2). There are 6 FDA-approved CAR-T products (four CD19 CAR T-cell products and two B cell maturation [BCMA] CAR T-cells) for hematological malignancies; several are under investigation for other malignancies (1).

Clinical trials have demonstrated encouraging outcomes regarding hematological malignancy-related outcomes, albeit accompanied by infectious complications and toxicity-related adverse events (3). The burden of infection among CAR T-cell therapy recipients remains a critical consideration in managing these patients. Most patients are at high infection risk due to their underlying malignancies, prior lines of cancer-directed treatment, and pre-CAR T-cell lymphodepletion (2, 4, 5). Furthermore, the intensive lymphodepleting chemotherapy given before CAR-T infusion exacerbates immune system suppression, heightening susceptibility to opportunistic infections. Post infusion, immune dysregulation can lead to cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), and hemophagocytic lymphohistiocytosis (HLH) -like syndrome; their treatments further predispose to infection (6–8). The post-CAR T-cell period is marked by varying degrees of lymphopenia, B-cell depletion, and hypogammaglobulinemia, which may influence long-term susceptibility to infection (5).

Prophylactic and monitoring measures based on an understanding of infection epidemiology are part of the efforts to lessen the burden of infections among CAR-T recipients. Due to the absence of comprehensive data, prophylactic and management strategies are based on consensus guidelines largely extrapolated from the post-hematopoietic stem cell transplant (HSCT) population (4, 9), and approaches vary widely by institution. This review aims to comprehensively analyze the epidemiology and risk factors associated with infections following CAR T-cell therapy in patients with hematological malignancy. It explores strategies for managing infectious risks associated with treatment-related toxicities and offers suggestions for screening, prophylaxis, immunoglobulin replacement therapy (IgRT), and vaccinations. Further research is needed in all these domains to expand our knowledge and optimize clinical practice.

2 Epidemiology of infections

The incidence of infection in patients receiving CAR T-cell therapy varies widely across prospective and retrospective trials, likely reflecting differences amongst patient populations, CAR T-cell related factors, definitions of infection, and follow-up duration. Epidemiology varies with CAR T-cell target and with time since cell infusion (day 0) due to the chronological evolution of infectious risk factors (4). For this review, epidemiology will be discussed in the context of two broad time points: those occurring in the early period, between days 0 and 30, and those occurring in the late period, beyond day 30. Overall, across the largest observational studies of infectious risk, infections were reported in 19–69% of patients after CD19-directed CAR T-cell therapy (2, 5), and in 42%-69% of patients after BCMA-directed CAR T-cell therapy; life-threatening infections are infrequently reported (2). A recent meta-analysis reported the pooled incidence of infection-related mortality as 1% (95% CI 0.01–0.02) and comparable amongst hematological malignancies (MM, ALL, NHL) (10).

2.1 Early infections: day 0 to day 30

Cohort studies evaluating infectious complications after CD19-directed CAR T-cell therapy report a higher incidence of infections within the first month and a subsequent decrease in the following months (11–20). The largest study of infectious complications after CD19-directed CAR T-cell therapy to date followed 133 patients with various malignancies (ALL, CLL, NHL) for up to 3 months after infusion; infection density was reported at 1.19 infections for every 100 days at risk within the first 28 days after cell infusion, and 0.67 between days 29 and 90 (21). The frequency of serious infection (grade 3 or higher) varies between 5–32% across the largest trials (2). Most documented early infections are bacterial, including both bacteremias and site infections; clostridium difficile colitis has specifically been identified in multiple trials (12, 13). The second most common infection is viral infections, of which respiratory viral infections account for the majority12-14. Hill et al. reported that infections, if present before lymphodepletion, can progress after CAR T-cell infusion (bacterial sinusitis, invasive fungal sinusitis, perirectal abscess) (11).

Patterns of early infection after BCMA-targeted CAR T-cell therapy differ somewhat from those encountered after CD19-directed therapy, likely reflecting differences in infectious risk attributable to patients' baseline malignancies. Infectious risk is reported to be highest in the first 30–100 days and decreases thereafter (22, 23). Most infections are mild to moderate in severity and involve the respiratory tract. When a microbiologic diagnosis is made, bacterial and viral pathogens are identified, but viral etiologies are slightly more common (22, 23). Severe infections are infrequently reported; by one report, most serious infections and bacterial bloodstream infections occurred in the first 28 days after BCMA CAR-T infusion (23).

2.2 Late infections (beyond day 30)

CD19-directed CAR T-cell therapy results in the depletion of endogenous B-cells and hypogammaglobulinemia, which lasts for an unclear duration and may impact long-term risk for infectious complications (19, 24). Overall infection density decreased over time by one report; the incidence decreased from 11.7 infections per 1000 person-days in the first 30 days to 2.3 between days 31 and 90, and incidence continued to decrease over time (16). Cordeiro et al. reported infection density beyond day 90 was 0.55 infections/100 days at risk, or 2.08 per patient-year (25). The most common infections reported are of the respiratory tract; in some reports, viral etiologies predominate (13, 16, 21); in other reports, bacterial etiologies remain important causes of infection (12, 25). Bacteremias (21)and other bacterial site infections, particularly those of the urinary tract (12), also continue to be reported. In another cohort of 60 diffuse large b-cell lymphoma (DLBCL) patients, 37% of patients who developed bacterial infections after day 30 had documented bacterial infections between days 0 and 30 after CD19-directed therapy (12). Most infections are mild-moderate in severity and managed in the outpatient setting (12, 13, 16, 25).

The cumulative incidence of infection also declines over time among patients with multiple myeloma treated with BMCA-directed CAR-T therapy (23, 26). Bacterial infections continue to be reported between days 31 and 100, but viral infections predominate after that, mostly mild to moderate in severity (23, 26, 27). Amongst bacterial infections, site infections, specifically pneumonia and sinusitis, were most frequently reported in the late period by two reports (27, 28). The epidemiology of less common infections and risk factors are discussed later in the article.

Key points:

- -The risk of infection is higher in the early risk period compared to the late risk period.
- -Bacterial infections are relatively common during the neutropenic phase, then viral infections are more common. BCMA CAR-T recipients have a comparable incidence of viral infections to bacterial infections in the early risk period.
- -Data on long-term infection incidence and risk is still evolving.

3 Factors associated with infectious risk

Factors influencing infection risk in CAR T-cell therapy recipients can relate to the host at baseline or the intervention; only a few known factors are modifiable. We will examine infectious risk in the context of two broad categories: pre-CAR T-cell infusion and CAR-T/post-CAR T-cell infusion. The post-CAR-T period can have early and late risk factors. Data comes from small, single-center experiences with heterogeneous cohorts and varying patterns of prophylaxis and management, which limits cross-trial comparisons. However, several distinct patterns do emerge.

3.1 Pre-CAR-T risk factors

CAR T-cell therapy recipients often are heavily treated and can have varying degrees of pre-existing cytopenia, decreased bone marrow reserve, and hypogammaglobulinemia. The burden of pretreatment (>3 prior lines) has been identified as a risk factor for infection regardless of baseline malignancy and CAR T-cell target (15, 17, 21, 26). History of allogeneic HCT was identified as a risk factor for early bacterial and viral infections in a cohort of 84 pediatric and young adult patients with R/R ALL (14). Impaired baseline performance status is associated with increased infectious risk after CAR T-cell therapy (12, 26). Finally, a history of infection 30–100 days before lymphodepletion has been associated with increased infectious risk post in the early period after cell therapy in multiple studies (12, 15, 26); Hill et al. reported that severe early infections present prior to lymphodepletion progressed after CAR T-cell infusion (11). Bridging chemotherapy, which is at times given for disease control during the CAR-T manufacturing period, was identified as a risk factor for severe infection before day 30 amongst 85 adults receiving CD19-targeted CAR T-cell therapy for DLBCL (16). This relationship was also noted by Kambhampati et al. in a cohort of 56 adults with MM undergoing BCMA-directed CAR T-cell therapy (26).

Underlying malignancy type has shown to play a role: a recent systematic review of 41 studies with 3199 patients receiving CAR-T therapy for hematological malignancy identified multiple myeloma patients as those at the highest risk for bacterial and viral infections as compared to those with ALL or NHL (29). Mikkilineni et al., in a retrospective analysis of 162 children and adults with a variety of malignancies treated with CAR T-cells directed against a variety of targets (CD19, CD22, GD2, BCMA), also identified those with multiple myeloma as the group with the highest risk for infection (15). Among CD19-directed CAR T-cell therapy, Hill et al. identified diagnosis of ALL as a risk factor for infectious complication within 90 days among 133 patients (ALL, NHL, CLL) (11).

Baseline hypogammaglobulinemia (IgG<400mg/dL) has been reported amongst CD19- and BCMA-directed CAR T-cell therapy recipients (11, 23–26). However, the impact of this characteristic on overall infectious risk is unclear. While some reports detect an association between baseline hypogammaglobulinemia and infectious risk after CD19-directed CAR T-cell therapy (12, 24), one large study did not (11). Moreover, baseline hypogammaglobulinemia has been reported in up to 88% of patients before receiving BCMA-directed therapy (23), and this has not been found to correlate with post-CAR-T infectious risk consistently (26).

Baseline neutropenia (absolute neutrophil count, ANC<500cell/mm^3) has been shown to increase infectious risk in the early period after CAR T-cell therapy (11, 15, 18, 30). Recently, the CAR-HEMATOTOX (HT) score model evaluated baseline marrow reserve (platelets, hemoglobin, ANC) and baseline inflammatory markers (ferritin level and CRP) as predictors of post-CAR-T prolonged neutropenia and clinical outcomes, including infection, where high HT score was found to be associated with high risk of severe infection (30–32) Table 1. This tool might become helpful in identifying patients at high risk for post-CAR-T complications, including infections.

TABLE 1 CAR-HEMATOTOX (HT) Score Model for Pre CAR-T Risk Assessment (31).

Baseline Features	0 Points	1 Point	2 Points
Platelet Count	> 175,000/µl	75,000–175,000/µL	< 75,000/mL
Absolute Neutrophil Count (ANC)	> 1200/µl	< 1200/mL	–
Hemoglobin	> 9.0 g/dL	< 9.0 g/dL	–
C-reactive protein (CRP)	< 3.0 mg/dL	> 3.0 mg/dL	–
Ferritin	< 650 ng/mL	650 – 2000 ng/mL	> 2000 ng/mL

Low: 0–1 High ≥ 2.

Key points:

- -Prior lines of therapy, history of allogeneic HCT, underlying malignancy, performance status, baseline hypogammaglobulinemia, pancytopenia, and inflammatory markers have been associated with increased baseline before CAR-T.
- -Baseline risk factor assessment is important to identify high-risk patients before CAR-T infusion.

3.2 CAR-T/Post CAR-T related risk factors

Lymphodepletion (LD) before CAR T-cell infusion attenuates the immune response to CAR T-cells, allowing for robust engraftment and anti-tumor effect (33, 34). Cyclophosphamide with fludarabine has been associated with lower infectious risk than other lymphodepletion regimens (11, 24). Notably, early infections most often occur at times of neutropenia (13), which may be pre-existing or brought on by bridging chemotherapy and/or LD prior to CAR T-cell infusion.

A high CAR T-cell dose (2 x 10^7 cells/kg) is associated with an increased risk for infection (11). This may be due to the reported relationship between higher CAR T-cell dose and CRS development and severity. This study identified an increased hazard for infection with each increase in CRS severity category (11). Multiple other studies have demonstrated this relationship between CRS severity and infectious risk (13, 24, 35). Park et al. identified CRS of grade 3 or higher as an independent risk factor for infection (adjusted hazard ratio 2.67, p = .05), particularly with bloodstream infection (13). Still, this relationship has not been replicated in other studies (12).

Post CAR T-cell therapy, the use of steroids and tocilizumab in the management of CRS is not consistently shown to be associated with increased infection density. Hill et al. did not identify treatment with corticosteroids as a risk factor for infectious complications, and there was insufficient evidence to ascertain any correlation between tocilizumab duration or dose and risk for infectious complications (11). However, other studies have found that CRS, tocilizumab use, and corticosteroid use were associated with increased infectious risk after BCMA-directed CAR T-cell therapy (23) and in the first 30 days after CD19-directed CAR T-cell therapy (16). It is observed that most infections occur after the onset of CRS and do not appear to precipitate or exacerbate it (11). ICANS grade >2 has been identified as a risk factor for infection after CD19-directed therapy in multiple studies (11, 12, 16). The mechanisms by which CRS and ICANS may predispose to infection are unclear; pre-clinical studies have shown that chronic CAR signaling may induce early exhaustion of T cells, but there is currently no evidence to suggest that this results in clinically significant immune dysfunction (36). Also, severe CRS is associated with hematological toxicity, which can indirectly increase infection risk (37). While high-dose systemic corticosteroids are well known to predispose to infection (12, 15, 16, 19, 30), the effect of limited doses of tocilizumab and/or anakinra on overall infectious risk is less clear (38). Long-term treatment with tocilizumab has been shown to increase susceptibility to opportunistic infections in rheumatoid arthritis patients treated with these agents (39).

Hematological toxicity, also known as Immune Effector Cell-Associated Hematotoxicity (ICAHT), is recognized as an important toxicity attributable to CAR T-cell therapy regardless of target (40). Cytopenia persists beyond the immediate post-CAR-T phase, and count recovery often follows a nonlinear trajectory–intermittent recovery is often followed by subsequent dips (40). Patients can develop severe bone marrow aplasia, often refractory to growth factor support. ICAHT is divided into early (day 0–30) and late (day +30) based on the depth and duration of neutropenia (40). ICAHT has come to be recognized as a novel toxicity category of CAR T-cell therapy (40). A real-world experience applying the grading system to a cohort of 549 patients treated with BCMA- or CD19-directed CAR T-cells for refractory B-cell malignancies (MM, DLBCL, MCL) found that severe ICAHT was associated with a higher rate of severe infections and inferior survival outcomes (41). Among ICAHT, late neutropenia has been reported in multiple trials involving BCMA-directed and CD19-directed CAR T-cell therapy and can occur in a biphasic pattern with an intermediate recovery period (25, 26, 42, 43). In multiple studies, prolonged neutropenia due either to CAR-T-related factors or persistent disease has been shown to increase the risk for late infections (11, 26). CD4 lymphopenia (CD4 < 200 cell/mm^3) is also frequently reported, lasting beyond day 30 (42) and up to 1 year (16) after CD19-directed CAR T-cell therapy and up to 9–12 months after BCMA-directed therapy (26). However, the impact of lymphopenia on overall infectious risk still needs further exploration. One study in a cohort that received CD19-directed therapy for DLBCL detected no relationship between CD4 and CD8 count at 30 days and infectious risk over 1 year (27). However, another study did detect a trend toward increased infectious risk with post-CAR-T lymphopenia after BCMA-directed therapy (22).

Hypogammaglobulinemia (IgG, <400mg/dL) affects 16–40% of patients before CAR T-cell therapy, and levels may decrease further after cell infusion and remain low for months or even years (11, 16, 20, 26). Amongst pediatric and young adult populations, CD19-directed therapy is associated with prolonged B-cell aplasia in up to two-thirds of patients and can persist for up to 5 years (44, 45). In a real-world experience, as many as half of patients received immunoglobulin replacement therapy (IGRT) for IgG<400 mg/dL in the post-CAR T-cell period (46). The reported severity and duration of hypogammaglobulinemia differs amongst adult populations receiving CD19-directed therapy; in one study, about half of patients had IgG >400mg/dL at the 1-year time point (26) after BCMA-directed CAR T-cell therapy, IgG<300 mg/dL in 70% of patients between 30–90 days and in 41% of patients after 1 year. Hill et al. reported that among 39 patients, 22 (56%) had a total IgG concentration <400 mg/dL at any time post CD19 CAR-T, and the cumulative incidence of an IgG concentration <400 mg/dL by 3 and 12 months post–CD19-CARTx was 36% and 60%, respectively (47). The significance of prolonged hypogammaglobulinemia on infectious

risk remains unclear and may vary with CAR-T target. BCMA and CD19 are expressed on normal B cells at different stages of differentiation; CD19 is expressed on B cells at earlier stages and is lacking from long-lived plasma cells, which maintain stable concentrations of antigen-specific antibodies (48). Targeting CD19, therefore, leads to B-cell aplasia and hypogammaglobulinemia, but pathogen-specific IgG levels may be maintained. This was illustrated by the persistence of seroprotective levels of measles antibody independent of total immunoglobulin level after CD19-directed CAR T-cell therapy in one study (47). In agreement with this finding, evidence suggests that infectious risk may not correlate with lower IgG levels in this population (20, 47). In contrast, BCMA is expressed on plasma cells, so targeting this would be expected to lead to more severe hypogammaglobulinemia with a decline in pathogen-specific antibodies. Loss of immunoglobulin diversity and pathogen-specific immunity after BCMA-directed CAR T-cell therapy has been demonstrated in two cohort studies to date (22, 49). Kambhampati et al. also found a trend toward more infections during times of profound hypogammaglobulinemia after BCMA-directed therapy (22).

None of the risk variables listed above has an established attributable risk. The interaction and cumulative risk of the aforementioned variables may impact the patient's overall infection risk. Thus, it is important to evaluate each situation carefully to optimize screening and preventive measures.

Key points:

- Risk factors associated with and after CAR-T infections include the type of lymphodepletion regimen, the dose of CAR T-cells, CRS post-infusion, use of systemic steroids, ICAHT, and hypogammaglobulinemia.
- Management of ICAHT and hypogammaglobulinemia management may modify infection risk after CAR-T infusion; prospective trials are needed to support this approach.

3.3 Multidrug-resistant organisms and antibiotic utilization

Antibiotic utilization post-CAR-T infection remains high (50), given that the most common infections in the early post-CAR-T period are bacterial, and most IEC-associated toxicities can present with fevers. The epidemiology of MDRO infections among CAR-T recipients is unknown; Yang J et al. showed poor 1-year clinical outcomes associated with Carbapenem-resistant organism infections among these patients (51). The utility of MDRO screening to assess MDRO colonization [as studied among HCT recipients (52)] and its impact on clinical outcomes still needs further investigation for CAR-T recipients. Similarly, antibiotic-associated microbiome dysbiosis has been associated with poor response to CAR-T therapy and increased toxicity (53, 54). These findings highlight the unmet need to study MDROs and antimicrobial stewardship in these complex settings.

3.4 Fungal infections and their risk factors

Despite multifactorial immune suppression, both mold and non-mold infections are infrequently reported as complications of CAR T-cell therapy (11, 55). Based upon cohort studies, epidemiology varies with CAR-T target, possibly due to unique pre- and post-CAR T-cell period features.

A review of published studies in 2021 by Garner et al. among CD-19-directed CAR T-cell recipients reported 1–10% incidence of yeast and 0–7% incidence of mold infections; most fungal occurred within the first 30 days and often represented breakthrough yeast infections in patients receiving fluconazole or echinocandin prophylaxis (56). Earlier, Hill et al. reported a 3% incidence of fungal infections between days 0 and 28 amongst 133 patients who all received fluconazole prophylaxis during the period of neutropenia after CD19-directed CAR T-cell therapy; all patients with fungal infections were reported to have been treated for CRS or ICANS with tocilizumab and/or corticosteroids (11). Incidence of fungal infections declined between days 28 and 90; late fungal infections were noted to have occurred in patients who had undergone prior allogeneic HSCT. Invasive mold infections were documented in both early and late periods but remain rare (11). This pattern of early-period fungemia while on echinocandin prophylaxis has been described by Park et al., and others have also found low-frequency invasive mold infections in both early and late periods (12, 13). More data is needed, but according to these results, it seems that a higher net burden of immunosuppression—development of CRS/ICANS (11, 16), HLH (35), treatment with tocilizumab or corticosteroids (11), other immunomodulating agents, and higher burden of prior treatment (>5 prior lines of therapy) (16), and history of HSCT (11)correlates with risk for fungal infection after CAR T-cell therapy.

While the low incidence of fungal infections reported in studies suggests the efficacy of antifungal prophylaxis (11–13), one large study reported a similarly low 2.9% incidence of invasive fungal infections at 1-year follow-up amongst 280 CD19 CAR T-cell patients with NHL who did not receive any antifungal prophylaxis (35). That cohort was also reported to have a high (41%) prevalence of severe delayed neutropenia, which may have been expected to increase susceptibility to fungal infection. Five of eight fungal infections reported occurred before day 100, including non-mold and mold infections. Of the three invasive mold infections reported, all were diagnosed by day 100 (35). Two of these infections occurred in patients with CLL and had received ibrutinib (35), which is an independent risk factor for mold infection (57, 58). Garner et al., in their review of invasive fungal disease, also noted that 73% of invasive mold infections occurred in patients with B-ALL or CLL (56). Studies to date have not been sufficiently powered to detect differences in risk for mold infection by different malignancy types. However, the underlying immune deficits associated with CLL and B-ALL and treatment with Bruton's tyrosine kinase (BTK) inhibitors likely impact infectious risk in these patients (59, 60).

The epidemiology of fungal infections after BCMA-directed CAR T-cell therapy is less well-characterized. The overall incidence

of fungal infection at 6 months of follow-up amongst patients who received some form of antifungal prophylaxis during their period of neutropenia has been reported at 4% (49) and 6% (22). Cumulative incidence declines with distance from the date of infusion (49), although one report recorded fungal infections, including invasive mold infections, occurred beyond 30 days after CAR T-cell infusion (11). Other reports also identified invasive mold infections in the early period (27, 49). High-grade CRS[2516] and severe prolonged neutropenia (49) are possible risk factors for mold infections in the early and late periods, respectively.

3.5 Pneumocystis *jirovecii* pneumonia and its risk factors

PJP has been reported in patients beyond 3 months after CD19-directed CAR T-cell infusion and typically occurs after PJP prophylaxis has been discontinued (12, 35) or when prescribed prophylaxis has not been appropriately taken (35). According to one report, most cases of PJP occurred in patients with CD4 <200 cells/mm (35). A recent report of a real-world research network database showed that among 1107 Cd-19 CAR-T patients and 280 BCMA CAR-T patients, the incidence of PJP pneumonia was 1.7% and 1.4%, respectively. Patients who developed PJP had a higher likelihood of prior dexamethasone usage (65% versus 43%, p=0.02) and a reduced duration of trimethoprim/sulfamethoxazole (TMP/SMX) prophylaxis (median 9 weeks [range 1 to 45] versus 19 weeks [range 1 to 106], p=0.002. There was no difference in overall survival among patients with and without PJP (median 518 days vs not-reached, HR 1.61, 95%CI 0.91 to 2.88) (61). PJP incidence remains low likely related to widespread use of prophylaxis for a reported 3–6 months starting after neutrophil recovery and/or low rates of sustained CD4 lymphopenia among BCMA CAR-T, though further data is needed (22, 49).

3.6 Cytomegalovirus infection and its risk factors

The epidemiology of CMV infection after CAR T-cell therapy is poorly understood due to inconsistencies in routine surveillance practices. One prospective trial of 72 adult CMV seropositive patients receiving CD19-, CD20-, or BCMA-targeted CAR T-cell therapy identified a 27% (95% CI 16.8–38.2) cumulative incidence of CMV viremia (62). No end-organ disease was observed in that cohort, although 5 patients received preemptive therapy. BCMA-directed CAR T-cell therapy and corticosteroid use for >3 days were significantly associated with CMV reactivation (62). Another study reported a 10% incidence of CMV viremia among 61 BCMA CAR-T recipients in the first 6 months after cell infusion; end-organ involvement was uncommon but was reported in 3 cases, including gastrointestinal and possible lung involvement (63).

In one recent study, CMV accounted for 11% of all documented viral infections in the first year after CD19-directed therapy for DLBCL and was one of the most common viral infections in the study period (64). ICANS grade 3 or 4, CRS grade 3 or 4, anakinra use for treating CRS/ICANS, and higher cumulative doses of steroids within the first 30 days after cell infusion were all associated with CMV reactivation in this report.

In one large study by Marquez-Algaba et al. among 95 CMV-seropositive patients receiving CD19 CAR T-cell therapy for aggressive B cell lymphoma, 42(44%) patients had at least one positive serum CMV viral PCR; only 7 patients received preemptive antiviral treatment, and no CMV end-organ disease was reported (65). Dexamethasone treatment was the sole independent risk factor associated with CMV viremia > 1000 IU/mL in the study (65). In another cohort, amongst 133 patients with various B cell malignancies receiving CD19-directed therapy, there was one case of CMV pneumonia that occurred between days 29 and 90 in a patient with B-ALL (11). A recent meta-analysis identified an increased incidence of CMV reactivation in NHL patients; there were 39 cases in 949 patients, most of which occurred in the late period (10). Fareed et al. reported among 230 CD-19 CAR-T recipients, 10% developed clinically significant CMV infection. CMV infection was observed more among the female gender, with low ANC and monocyte count at day 30, grade 2 or higher CRS or ICANS requiring higher doses of steroids with higher mortality (66). These results demonstrate that CMV can cause disease in specific high-risk groups after CAR T-cell therapy. However, the significance of CMV viremia in the absence of end-organ disease still needs to be further explored.

3.7 Herpes simplex virus and herpes zoster virus infection and its risk factors

Reports of (HSV) and (VZV) reactivations after CAR T-cell therapy are infrequent, perhaps owing to the widespread use of antiviral prophylaxis. Reactivations have been reported after both CD19 and BCMA-directed therapies after discontinuing prophylaxis or in the setting of nonadherence with the prophylactic regimen (11, 13, 24, 28). However, delayed reactivations have also been reported despite the appropriate use of prophylaxis. There were two cases (3% incidence) of herpes zoster reactivation while on acyclovir prophylaxis in one study; both cases occurred after day 30 post-CAR T-cell infusion in patients who had received CD19 CAR T cells for DLBCL (11). A recent meta-analysis detected an increased signal for HSV/VZV reactivations in the late period in those with NHL (10). End-organ disease is not often reported to our knowledge; there was one reported case of fatal HSV pneumonia after BCMA-directed therapy in the setting of severe CRS and acyclovir resistance (67).

3.8 Human herpes virus 6 infection and its risk factors

Multiple cases of HHV-6 encephalitis in CAR-T recipients have been reported in the literature (19, 68–72). In one recent study, HHV-6 accounted for 8% of all documented viral infections in the first year after CD19-directed therapy for DLBCL (64). Several reported cases occurred in the context of mental status changes that initially responded to steroids but then relapsed and lasted beyond

the typical time course of ICANS, prompting further infectious workup (68, 69). There has also been one case report of fatal HHV-6 myelitis following CD19-targeted CAR T-cell therapy in a patient who developed CRS and ICANS that initially responded to corticosteroids with subsequent development of ascending flaccid paralysis (73). Younger age, more prior lines of therapy, including allogeneic HSCT, and receipt of systemic corticosteroids may also increase susceptibility and lower the threshold for investigation (69, 71). Recently, Lareau et al. reported that HHV-6 can be reactivated among cultured T cells; implications and significance of this finding still need to be determined (74).

3.9 Adenovirus infections and its risk factors

There are no studies on the incidence of adenovirus infection in patients treated with CAR T-cells. Logue et al. reported one case of adenovirus viremia in a patient with DLBCL within 30 days of receiving CD19-directed CAR T-cell therapy. Still, the clinical significance of this finding was unclear (16). There are case reports of hemorrhagic cystitis cases associated with adenovirus infection (75). Further study is needed to elucidate the epidemiology and manifestations of adenovirus infection in the post-CAR T-cell period.

3.10 Polyomavirus (BKv) infection and its risk factors

There are anecdotal reports of BK virus infections and hemorrhagic cystitis after CD19-directed CAR T-cell therapy (11, 13). There are no reports of such infections associated with BCMA-directed CAR T-cell therapy. Case reports of late development of progressive multifocal leukoencephalopathy associated with JV virus have also been described (76, 77). Given the lack of conclusive data on the risk and impact of reactivation, the index of suspicion should be high in the context of hemorrhagic cystitis or atypical neurological symptoms with multifocal demyelination.

4 Prevention of infections

Preventive strategies can also be divided into two-time points, pre-CAR-T, and post-CAR-T, keeping the risk modifiable risk factors in mind. Screening and prophylaxis strategies are determined before CAR T-cell therapy. In contrast, post- CAR-T strategies are focused on reducing infection risk by infection surveillance when needed, ongoing chemoprophylaxis, immunoglobulin replacement therapy (IGRT), and vaccination administration.

4.1 Baseline screening pre CAR T-cell therapy

Infection screening before CAR T-cell therapy is an important risk assessment component. HIV, HBV, and HCV serologic testing with reflex nucleic acid testing is recommended for all patients (4). False positive HIV nucleic acid amplification tests (NAAT) may be seen in CAR-T recipients when the CAR-T product was generated using lentiviral vectors, owing to the use of conserved regions of HIV-1 (78). Products prepared with murine gamma-retroviral vectors are not thought to carry the same risk. In the case of a true false positive test, confirmatory fourth-generation HIV-1/2 antibody and p24 antigen testing returns negative, and HIV-1 RNA may be detectable at a low level, possibly due to circulating cell-free DNA (78). No further intervention or monitoring is required in these cases, and CAR T-cell therapy may proceed as planned (78). Patients with HIV infection were excluded from clinical trials, so the safety of CAR T-cell therapy in the HIV-positive population is poorly understood (79). There are, however, published reports of successful treatment of HIV-infected patients with DLBCL with CD19-targeted CAR T-cell therapy (80, 81).

Patients with HCV viremia (chronic HCV infection) should be considered for antiviral treatment if compatible with liver function and overall clinical situation (82). If liver test abnormalities develop during treatment, then the potential role of HCV and other hepatotropic viruses should be assessed. Notably, hepatitis E has been reported to cause chronic disease in immunocompromised patients (83). Prophylaxis and surveillance strategies in the case of positive hepatitis B serologies are discussed in detail in the next section. Serologic screening for HSV1/2 and VZV is recommended in those not already receiving antiviral prophylaxis; this is also important for future consideration of VZV vaccination (4). Screening for Mycobacterium tuberculosis (MTB) should be considered in patients with risk factors for exposure (84). Tocilizumab use is independently associated with an increased risk for MTB infections in rheumatoid arthritis patients [91]; however, the impact of the short-term dosing utilized in CRS management has not yet been reported. Baseline toxoplasma serologies may be considered on a case-by-case basis (4, 9). Finally, screening for antibodies to Strongyloides stercoralis or empiric treatment with ivermectin should be considered in patients with a history of time spent in tropical or subtropical regions, given the risk for reactivation due to high-dose corticosteroids and/or tocilizumab (4, 9).

A complete history and physical exam should be performed to evaluate for active infections before lymphodepletion. If the evaluation concerns an infection, a more directed workup should be performed as indicated by the clinical situation.

4.2 Prophylaxis and monitoring post CAR T-Cell infusion:

Based on the current literature, the recommendations below represent our opinion; further prospective studies are needed to optimize these practices.

The role of antibacterial prophylaxis in this population is unclear; fluoroquinolones are often employed, but practice patterns vary widely across institutions. A large retrospective analysis of patients with DLBCL receiving CD19 CAR T-cells identified a significant reduction of severe bacterial infections with fluoroquinolone prophylaxis in patients who were categorized as CAR-HEMATOTOX^{high} but not in those who were CAR-HEMATOTOX^{low} at baseline (41). A risk-adapted approach may

be the optimal strategy, but more studies are needed, and institutional guidelines vary. Multiple studies have reported early bacterial infections with gram-negative organisms with acquired or intrinsic fluoroquinolone resistance, regardless of fluoroquinolone prophylaxis patterns (11, 13). It is unclear how much antibacterial prophylaxis strategies should be adjusted to account for this. We suggest using fluoroquinolone prophylaxis when ANC<500 cells/mm^3. Optimal infection prophylaxis strategies for those with severe ICAHT require further study. There may be a role in closely monitoring patients with high CAR-HEMATOTOX scores before lymphodepletion or among those who develop severe ICAHT post-CAR T-cell therapy (41).

The role of routine antifungal prophylaxis is also debated, given the low reported incidences of non-mold infections with or without prophylaxis (5, 11, 13, 16, 35, 49, 85). Until further data are available, we recommend fluconazole prophylaxis during periods of neutropenia in all CAR T-cell recipients. Anti-mold prophylaxis should be considered in select high-risk patients for invasive mold infection. We would recommend the use of a mold-active azole in those with a history of invasive fungal infection (IFI), ANC < 500 cell/mm3 for >21 days present prior to CAR T-cell therapy or developing after infusion, and treatment with high-dose steroids and for longer duration. Duration of anti-mold prophylaxis should be determined on a case-by-case basis; in the presence of multiple risk factors, extending prophylaxis beyond ANC recovery (ANC > 500 cell/mm^3 for 3 consecutive days) may be appropriate.

PJP prophylaxis has also been widely adopted, though opinions on the optimal duration of prophylaxis remain mixed. Given reports of PJP infections diagnosed beyond 3–6 months after cell infusion in the context of prolonged CD4 lymphopenia and prophylaxis discontinuation or incomplete adherence (5, 12, 35), we would suggest starting prophylaxis upon initiation of lymphodepleting chemotherapy and continuing for at least 6 months or until CD4 count >200 cell/mm^3. HSV/VZV prophylaxis with acyclovir or valacyclovir has been widely adopted and recommended.

Entecavir is recommended when HBsAg is positive and/or when HBV DNA is detectable before receiving CAR T therapy and should be continued for at least 6–12 months from infusion due to the risk of reactivation following B cell depletion (4, 9). If anti-HBc is positive but surface antigen and DNA are negative, antiviral prophylaxis OR lab monitoring with liver function tests and HBV DNA every 1–3 months can be performed. Those with anti-HBc+ and anti-HBs+ likely have a lower risk of reactivation. A more conservative approach may be appropriate in multiple myeloma patients given their more severe humoral immunodeficiency, including pathogen-specific immunoglobulin deficiency, which is depleted after BCMA CAR T-cell therapy (49). Table 2 summarizes recommendations for antimicrobial prophylaxis.

TABLE 2 Proposed Antimicrobial Prophylaxis for CAR-T Patients (4, 86–90).

	Agent	Alternative agent (s)	Comment
Antibacterial	Levofloxacin		Start when ANC < 500 and continue until neutrophil recovery (ANC >500 for at least 3 days)
Antifungal	Fluconazole	Micafungin	Start when ANC<500 and continue until neutrophil recovery (ANC >500 for at least 3 days)
Anti-mold	Voriconazole	Posaconazole	Consider in those at high risk for mold infection: ANC<500 for >21 days, treatment with prednisone >20mg for >2 weeks or equivalent, history of IFI, history of allogeneic HSCT; duration determined case by case
Anti-PJP	Trimethoprilm/ Sulfamethoxazole (TMP/SMX)	Inhaled pentamidine OR dapsone OR atovaquone	Start with lymphodepleting chemotherapy and continue for at least 6 months post- CAR T infusion or until CD4 count >200 cell/mm^3
Antiviral	Acyclovir	Valacyclovir	Start with lymphodepleting chemotherapy and continue for 6–12 months or until CD4 >200 cell/mm^3

4.3 CMV monitoring, prophylaxis, and pre-emptive therapy post CAR-T infusion

The role of CMV monitoring remains a topic of debate. Still, there are reports of both CMV viremia and end-organ disease occurring after CD19- and BCMA-directed CAR T-cell therapy (11, 28, 65, 91). We suggest checking baseline CMV serostatus and serum PCR in all patients proceeding with CAR T-cell therapy. Those with a negative CMV assessment before CAR T-cell infusion and who do not require systemic steroids for related complications would likely not benefit from regular monitoring.

Those with a positive CMV assessment and/or those who require >3 days of high-dose systemic steroids and have received BCMA-directed therapy may benefit from weekly CMV monitoring during the first 6 weeks after CAR T-cell therapy. This recommendation is based upon limited data on the kinetics of CMV reactivation after CAR T-cell therapy (62), the duration of monitoring should be adjusted on a case-by-case basis. There is insufficient data to suggest prophylactic strategies in this setting. The optimal role and threshold for pre-emptive therapy also remain unclear and must be balanced against the risks of therapy-related toxicity, including further bone marrow suppression.

Allogeneic CAR T-cell therapy (allo CAR-T) is an active research area and may carry unique risk factors for CMV and other viral reactivations. Chemotherapy ahead of allo CAR-T, or lymphodepletion in preparation, may require novel strategies such as using alemtuzumab to mitigate the risk of GVHD and promote

engraftment and expansion of the infused cells (92, 93). The resultant deeper and more prolonged lymphodepletion would pose an important risk factor for viral reactivation (93).

5 CRS, ICANS, and fever after CAR-T infusion

CAR T-cell infusion can result in CRS, manifesting as fever, capillary leak, and end-organ dysfunction (94–96). The typical time frame for CRS presentation is 2–7 days after CAR T-cell infusion, but it may occur within hours or up to 10–15 days after infusion (97). ASTCT consensus grading ranges from grade 1 to 5, reflecting a spectrum of presentations from fever and constitutional symptoms to critical illness requiring invasive monitoring, pressors, and ventilatory support (97). ICANS is regarded as a separate clinical entity characterized by encephalopathy that can be progressive, language disturbances, motor weakness, seizures, and cerebral edema (3, 95, 96). ICANS typically present later than CRS, with a typical time to onset of 4 to 19 days after receiving CAR T-cell infusion (98). While the two entities do not always occur in the same host, severe ICANS is unlikely to be seen without severe CRS (95, 96).

Both CRS and ICANS can mimic infections. Severe CRS may present as septic shock requiring invasive monitoring and is managed with high doses of steroids; critical illness and steroids can cause neurologic symptoms (3, 95–97, 99), which may be difficult to distinguish from ICANS. Moreover, managing CRS and ICANS involves multimodal systemic immunosuppression, which can mask the clinical presentation of infection. Corticosteroids and anti-IL-6 therapy with tocilizumab remain cornerstones of CRS management, while corticosteroids are the treatment of choice for ICANS with the addition of anti-IL-1 therapy with anakinra in refractory cases (3, 94–96). More recently, there has been a paradigm shift towards pre-emptive use of these modalities to prevent progression to higher-grade manifestations (98, 100).

Given the ambiguity of the clinical presentation, it is crucial to initiate broad-spectrum antibiotics promptly and to investigate reversible infectious causes as part of the initial assessment. In practice, broad-spectrum antibiotics are generally administered in the setting of CRS after CAR-T infusion in the setting of febrile neutropenia, hemodynamic instability, and hypoxia (4, 8). Empiric coverage should consider the patient's history of prophylaxis, and local antibiogram and infection work-up should be initiated. Infectious disease consultation should be considered early, especially when the clinical picture is complex. There is increasing awareness of the adverse effects of broad-spectrum antimicrobials, including microbiome dysbiosis, the emergence of multidrug resistance organisms, and Clostridium difficile infection (101, 102). To mitigate these risks, we advocate for diligent de-escalation strategies. Consideration should be given to stopping broad-spectrum antibiotics in patients with neutropenia who have been afebrile for 72 hours and remain without clinical or microbiologic source of infection (103–105).

6 Emergent hemophagocytic lymphohistiocytosis-like toxicities and infections

Immune effector cell-associated HLH-like syndrome (IEC-HS) has been described post-CD-19 directed CAR-T infusion, with severe and fulminant cases occurring in <1% of patients (6). Earlier reports characterized IEC-HS progressing from cases of severe CRS, but recently, there has been increasing recognition of delayed HLH-like toxicities (7). IEC-HS is also becoming more apparent after BCMA CAR T-cell therapy (105). The definition of IEC-HS is outside the scope of this paper; it is important to recognize that the clinical presentation can mimic infection and that infection can co-occur with this entity. We recommend initiating appropriate antimicrobials while infectious work-up is underway. Treatment of IEC-HS involves extensive immunosuppression, thus increasing overall infection risk. Table 3 highlights the immunosuppressive agents and the infections associated with them. As treatment strategies for CRS, ICANS, and IEC-HS evolve, it is imperative to keep the infectious risks attributed to these agents in mind and consider adjusting the prophylaxis strategy to account for their use.

7 Post-infusion hypogammaglobulinemia and IgRT

Although prophylactic IgG has received regulatory approval in specific immunocompromised groups, less extensive data indicates similar effectiveness in CAR T-cell recipients[5510]. IgRT has primarily been established as beneficial in preventing serious bacterial infections; there is less evidence to support its use in the prevention of viral infections, which are more frequently reported as late complications of CAR T-cell therapy (48). In fact, studies to date have failed to reliably show an association between hypogammaglobulinemia and infectious risk after CAR T-cell therapy regardless of targe (47, 48), and there is similarly mixed data on the efficacy of IgRT in these populations (22).

However, based on the pathophysiology of humoral immunodeficiency after CAR T-cell infusion, there is consensus that IgG levels should be monitored both pre-CAR T-cell infusion and monthly for at least 3 months after infusion (4, 5). A threshold IgG level of 400 mg/dL is frequently used to initiate IGRT in adults (4, 5). Adverse events are infrequently reported after IVIG infusion but do include mild and occasionally severe infusion reactions, and delayed toxicities including thrombosis that may manifest as ischemic stroke or myocardial infarction, renal failure, and transient hemolytic anemia (48, 114). IVIG is also associated with significant costs and accessibility issues, so use should be judicious and guided by a multidisciplinary team effort (48).

Given mixed data on the impact of hypogammaglobulinemia on infectious risk after CD19-directed CAR T-cell therapy, as well as the preservation of pathogen-specific antibodies detected after CD19-

TABLE 3 Immunosuppressive Agents Used for Treatment Of CRS/ICANS/HLH and their Associated Infection.

Immunosuppressive Therapy	Associated Infection Risk	Prophylaxis Strategy Considerations
Steroids (dexamethasone, methylprednisolone)	Well-known association with fungal infections, viral reactivations, and PJP, which is also noted in some cohorts after CAR T-cell therapy (11, 16, 22, 35, 49, 65)	Mold active prophylaxis HSV/VZV prophylaxis if seropositive Weekly monitoring for CMV reactivation and strong consideration of pre-emptive therapy
IL-1 Receptor antagonist (anakinra)	Well tolerated with extended treatment in the rheumatoid arthritis population (106); no specific association with infectious risk in limited experience after CAR-T (107) In combination with steroids, the risk of infection may be higher (106)	If being administered with steroids, above considerations apply
IL-6 receptor antagonist (tocilizumab, siltuximab)	Safety profile post CAR-T infusion is unclear; in one report, use was associated with infections and death (108). In other populations, it has been associated with tuberculosis (TB), other mycobacterial infections, and fungal infections (39)	Mold active prophylaxis HSV prophylaxis if seropositive CMV preemptive therapy Weekly monitoring for viral reactivation Bacterial prophylaxis when ANC<500
JAK1/2 inhibitor (ruxolitinib)	Safety in CAR T-cell population unclear; associated with higher rates of VZV infection and hepatitis B reactivation (109, 110) in hematological malignancy population; also reports of disseminated TB, cryptococcal infection, toxoplasmosis, CMV disease, mold infections (109, 111).	Mold-active prophylaxis (with attention to drug-drug interactions between ruxolitiib and azoles) PJP prophylaxis VZV prophylaxis if seropositive Weekly monitoring for CMV reactivation among those on multiple and strong consideration of pre-emptive therapy HBV prophylaxis if HBSAg+ and/or HBV DNA PCR is detectable Bacterial prophylaxis when ANC<500
Chemotherapy (etoposide)	Bacterial infections with neutropenia	Attention to bacterial prophylaxis when ANC<500
Anti-IFN-gamma monoclonal antibody (emapalumab)	Viral reactivations, fungal infections, TB reactivation and other mycobacterial infections have been reported in other populations (112, 113)	Fungal prophylaxis on case by case basis PJP prophylaxis VZV prophylaxis if seropositive Weekly monitoring for CMV reactivation and consideration of pre-emptive therapy

directed therapy, we would suggest a tailored approach to IgRT in this subset of CAR-T recipients. It may be appropriate to reserve IgRT for patients with recurrent and/or severe infections and IgG < 400 mg/dL before cell infusion and in the first 3–6 months after CD19-targeted therapy. In those with IgG levels between 400 mg/dL and 600 mg/dL, IGRT may also be considered for severe or recurrent infections. If there are recurrent infections in normal serum immunoglobulin levels, testing for functional humoral immune dysfunction, e.g., vaccine response, may be helpful (4, 114). A more conservative approach may be appropriate in BCMA CAR T-cell recipients given their more profound depletion of pathogen-specific immunity (22, 49); IgRT may be considered in those with IgG< 400 mg/dL, even without evidence of recurrent infection with caution (48). In these patients, distinguishing between normal IgG and paraprotein with serum protein electrophoresis is important. Prospective trials are needed to optimize these strategies.

8 Vaccinations for CAR T-cell recipients

B-cell aplasia and hypogammaglobulinemia can increase susceptibility to infection by vaccine-preventable encapsulated bacteria such as *Haemophilus influenzae* type B, *Neisseria meningitidis*, and *Streptococcus pneumoniae*. Appropriate vaccination has the potential to prevent infections, decrease their severity, mitigate the need for IgRT, and improve survival and quality of life (4).

Walti et al. identified decreased seroprotection levels against *S. pneumoniae and H. influenzae* type B after CD19-directed therapy (115). In this same study, levels of IgG against measles, tetanus toxin, Epstein-Barr virus, varicella-zoster virus, and herpes simplex virus remained detectable despite decreased total serum IgG and B-cell aplasia (115). Among those who attained CR, the proportion of participants with seroprotective IgG titers to vaccine-preventable infections was comparable to population-based seroprevalence data without re-vaccination (115). In contrast, a small cross-sectional study suggested that BCMA CAR T-cell recipients are less likely to have seroprotective IgG titers to vaccine-preventable infections (49). It remains unclear whether this reflects CAR T-cell therapy's effects or indicates baseline humoral immunodeficiency; in that same study, measles-specific IgG was present in only 16% of patients before cell infusion (49).

The optimal timing of vaccination after CAR T-cell therapy is unclear, as the timing of cellular and humoral immunity reconstitution after CAR T-cell therapy can vary widely (2, 9). However, a recent study reported that neither B-cell aplasia nor

hypogammaglobulinemia reduced influenza vaccine immunogenicity after CAR T-cell therapy (116), suggesting that strictly following these markers to assess for immune reconstitution may create unnecessary delays in vaccination. Another study noted recovery of seroprotective measles IgG level by 114 days after CD19 CAR T-cell therapy without vaccination and coinciding with CD19+ B-cell recovery (47), suggesting that immune recovery may obviate the need for aggressive re-vaccination strategies in select groups. Optimal strategies may need to be considered based on the CAR-T target.

Vaccination against influenza during flu season should be considered at least two weeks before lymphodepletion; additional vaccination before cell infusion is likely of low utility given the impending severe immunosuppression. Clinical practice for revaccination currently follows protocols used in HCT recipients, though the need for revaccination for all previously completed vaccine series remains unclear (4, 48, 117, 118). In general, for patients who are in remission and not planned to receive further T-cell and/or B-cell depleting therapies, killed/inactivated vaccinations should be considered starting at least 6 months after CAR T-cell infusion, and live and adjuvant vaccines should be considered at least 1 year after cell infusion. IgRT may interfere with the efficacy of live vaccines, so these should generally be delayed for at least 9 months after the most recent IgRT (4, 48). This recommendation is based on the guidelines for vaccination of immunocompromised hosts (118, 119) and the kinetics of immune reconstitution after CAR T-cell therapy (11, 120, 121). SARs-CoV-2 vaccination can be started after 90 days given (122).

Based on the epidemiology of infections after CAR T-cell therapy and known effects of B-cell aplasia and hypogammaglobulinemia, key vaccines to consider include annual influenza, *Streptococcus pneumoniae*, *Haemophilus influenzae type b*, *Corynebacterium diphtheriae* and *Clostridium tetani* toxins, *Bordetella pertussis*, and hepatitis A and B viruses. For patients 50 years old who are seropositive for VZV or have a history of shingles, recombinant zoster vaccine (Shingrix) should also be considered. Conjugated vaccines should be used, when possible, given higher response rates in immunocompromised patients (123). Measuring vaccine responses may be helpful to assess the utility of additional vaccination on a case-by-case basis (4).

For respiratory viruses, COVID-19 and RSV vaccines (age-dependent indication) should also be considered in addition to the influenza vaccine (124, 125). Data regarding the durability of seroprotection provided by pre-infusion COVID vaccination and the immunogenicity of mRNA-based COVID vaccines after CAR T-cell therapy are mixed (126). We suggest following the CDC's guidance on COVID-19 vaccines for moderately and severely immunocompromised people (127). The efficacy of RSV vaccines in CAR-T recipients is unknown. Table 4 highlights the vaccine recommendations for CAR-T recipients.

9 Future directions and knowledge gaps

The field of CAR T-cell therapy is continuously evolving, with the development of newer targets, combination therapies, newer CAR-T designs, and off-the-shelf products [121], and important gaps remain in our understanding of short and long-term infectious complications. As new CAR T-cell targets are introduced to the market for hematological malignancies as well as for solid tumors, and potentially for use in autoimmune diseases and suppression of rejection in organ transplant (82), transparency and completeness of reporting about infectious complications of these therapies will remain critical as we seek to devise strategies to mitigate risks (128). An example is the development of allogeneic (off-the-shelf) CAR T-cell therapy; these products come with the risk of rejection and development of graft versus host disease (GVHD) and may require unique pre-infusion LD and post-infusion immunosuppression strategies (129). Allogeneic T cell-based products have entered Phase I and II clinical trials, and different effector cell types, such

TABLE 4 Vaccines For CAR T-cell Therapy Recipients.

	Killed/inactivated vaccines	Live and non-live adjuvant vaccines
Eligibility	6 months post-CAR-T 2 months since last IGRT	1-year post-CAR-T
Contraindications	• IGRT within the past 2 months • Receiving T-cell or B-cell directed immunosuppressive therapy. • Receipt of anti-CD20 or anti-CD19 in the prior 6 months • Actively receiving chemotherapy	• Received anti-CD19 or anti-CD20 therapy within the past 6 months. • 1 year post CAR T-cell therapy • 2 years post autologous or allogeneic HCT • <1 y off of all systemic immunosuppressive therapy • < 8 months after the last dose of IGRT • Absolute CD4 count < 200 cells/mm^3 • Absolute CD19+ or CD20+ B cell count < 20 cells/mm^3 • Actively receiving chemotherapy
Vaccinations to consider	Influenza Covid-19 Pneumococcal conjugate Pneumococcal polysaccharide Diphtheria, tetanus, and acellular pertussis (DTaP) Hepatitis A virus Hepatitis B virus	Varicella Zoster Virus

*Those who have undergone prior HCT without completing all post-transplant re-vaccinations should restart the whole vaccination series once they meet the eligibility criteria described above and all HCT-related criteria. Antibody responses can be checked if possible before and after starting the vaccine series to guide clinical decision-making; if there is no response to vaccination despite meeting eligibility criteria, then further vaccinations can be attempted once there is immune reconstitution (IgA > 6mgdL + CD19 or CD20 B cell count > 20cells/mm^3 + CD4 count > 200 cells/mm^3).

*Those post-HCT who have completed their post-HCT vaccination series OR those who have never undergone HCT should also be fully vaccinated once they meet the eligibility criteria described above. Antibody titers can be monitored before and after vaccination to guide subsequent steps in the vaccine series. This allows for preserved immunity and the ability to generate a boosted response with a single dose of a given vaccine.

*Antibody response can be determined by checking serum IgG titers to S pneumoniae (23 serotypes), tetanus toxoid, hepatitis A virus, and Hepatitis B virus surface antigen.

*For non–S pneumoniae vaccines, a response is defined as at least a twofold increase in IgG from prevaccination to 1 to 2 months postvaccination or achieving a seroprotective IgG level at 1 to 2 months postvaccination. For the S pneumoniae vaccine (Prevnar 15 or 20) response is defined as at least a twofold increase in IgG from prevaccination to 1-month postvaccination, achieving an IgG ≥ 1.3 ug/mL for ≥50% of the Prevnar serotypes, or as defined by the testing laboratory.

as NK cells and macrophages, are also under investigation (129). Dual-targeted CAR T-cells, for example, targeting CD19 and CD22 for treating R/R aggressive B-cell lymphomas, are also in development (130). CAR-T therapy for Acute Myeloid Leukemia can lead to myeloablation (131). Understanding infection incidence and risk associated with these novel treatments is vital to developing mitigating strategies.

The infection burden of DNA viruses post CAR T-cell therapy is still evolving (63, 74, 132, 133), and might vary among CAR T-cell targets. Prospective studies are important to understand CAR T-cell recipients' specific pathogen and infection burden. Similarly, long-term infection data are lacking. Extended research is necessary to evaluate the frequency of late-onset infections and the influence of persistent immune dysfunction on the risk of infections among individuals treated with CAR T-cell therapy.

Microbiome dysbiosis among CAR T-cell recipients is associated with poor clinical outcomes, including CAR-T toxicities and response to therapy (53). Similarly, a non-antibiotic-disrupted gut microbiome is associated with improved clinical response to CD-19 CAR T-cell therapy (134). This highlights the importance of developing strategies for early identification of infection mimickers post CAR T-cell and prompt de-escalation of broad-spectrum antimicrobials when appropriate.

In conclusion, infections remain an important concern among CAR T-cell recipients, and our understanding of infection dynamics among different settings is still evolving. Addressing these knowledge gaps is imperative to improving patient outcomes. Additionally, ongoing research efforts should prioritize the establishment of standardized guidelines for monitoring infections, implementing prophylactic measures, and administering treatments that cater to the unique requirements of CAR T-cell recipients. This approach will ultimately enhance our patients' overall quality of care and prognosis.

Author contributions

ZS: Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing. SA: Data curation, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors express their deep appreciation to the Leon Levine Foundation and the Kerry and Simone Vickar Family Foundation for their support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Chen YJ, Abila B, Mostafa Kamel Y. CAR-T: what is next? *Cancers (Basel).* (2023) 15(3):663. doi: 10.3390/cancers15030663

2. Kampouri E, Little JS, Rejeski K, Manuel O, Hammond SP, Hill JA. Infections after chimeric antigen receptor (CAR)-T-cell therapy for hematologic Malignancies. *Transpl Infect Dis.* (2023) 25:e14157. doi: 10.1111/tid.14157

3. Neelapu SS, Tummala S, Kebriaei P, Wierda W, Gutierrez C, Locke FL, et al. Chimeric antigen receptor T-cell therapy - assessment and management of toxicities. *Nat Rev Clin Oncol.* (2018) 15(1):47–62. doi: 10.1038/nrclinonc.2017.148

4. Hill JA, Seo SK. How I prevent infections in patients receiving CD19-targeted chimeric antigen receptor T cells for B-cell Malignancies. *Blood.* (2020) 136(8):925–35. doi: 10.1182/blood.2019004000

5. Wudhikarn K, Perales MA. Infectious complications, immune reconstitution, and infection prophylaxis after CD19 chimeric antigen receptor T-cell therapy. *Bone Marrow Transplantation..* (2022) 57:1477–88. doi: 10.1038/s41409-022-01756-w

6. Hines MR, Knight TE, McNerney KO, Leick MB, Jain T, Ahmed S, et al. Immune effector cell-associated hemophagocytic lymphohistiocytosis-like syndrome. *Transplant Cell Ther.* (2023) 29:438.e1–438.e16. doi: 10.1016/j.jtct.2023.03.006

7. Hashmi H, Bachmeier C, Chavez JC, Song J, Hussaini M, Krivenko G, et al. Haemophagocytic lymphohistiocytosis has variable time to onset following CD19 chimeric antigen receptor T cell therapy. *Br J Haematol.* (2019) 187(2):e35–8. doi: 10.1111/bjh.16155

8. Jain MD, Smith M, Shah NN. How I treat refractory CRS and ICANS after CAR T-cell therapy. *Blood.* (2023) 141:2430–42. doi: 10.1182/blood.2022017414

9. Los-Arcos I, Iacoboni G, Aguilar-Guisado M, Alsina-Manrique L, Díaz de Heredia C, Fortuny-Guasch C, et al. Recommendations for screening, monitoring, prevention, and prophylaxis of infections in adult and pediatric patients receiving CAR T-cell therapy: a position paper. *Infection.* (2021) 49(2):215–31. doi: 10.1007/s15010–020-01521–5

10. Reynolds GK, Sim B, Spelman T, Thomas A, Longhitano A, Anderson MA, et al. Infections in haematology patients treated with CAR-T therapies: A systematic review and meta-analysis. *Crit Rev Oncol Hematol.* (2023) 192:104134. doi: 10.1016/j.critrevonc.2023.104134

11. Hill JA, Li D, Hay KA, Green ML, Cherian S, Chen X, et al. Infectious complications of CD19-targeted chimeric antigen receptor-modified T-cell immunotherapy. *Blood.* (2018) 131(1):121–30. doi: 10.1182/blood-2017–07-793760

12. Wudhikarn K, Palomba ML, Pennisi M, Garcia-Recio M, Flynn JR, Devlin SM, et al. Infection during the first year in patients treated with CD19 CAR T-cells for diffuse large B cell lymphoma. *Blood Cancer J.* (2020) 10(8):79. doi: 10.1038/s41408–020-00346–7

13. Park JH, Romero FA, Taur Y, Sadelain M, Brentjens RJ, Hohl TM, et al. Cytokine release syndrome grade as a predictive marker for infections in patients with relapsed or refractory b-cell acute lymphoblastic leukemia treated with chimeric antigen receptor T cells. *Clin Infect Diseases..* (2018) 67:533–40. doi: 10.1093/cid/ciy152

14. Vora SB, Waghmare A, Englund JA, Hill JA, Gardner R. Infectious complications following CD19 CAR T cell immunotherapy for children and young adults with refractory ALL. *Biol Blood Marrow Transplantation..* (2019) 25:S355–6. doi: 10.1016/j.bbmt.2018.12.575

15. Mikkilineni L, Yates B, Steinberg SM, Shahani SA, Molina JC, Palmore T, et al. Infectious complications of CAR T-cell therapy across novel antigen targets in the first 30 days. *Blood Advances*. (2021) 5(23):5312–22. doi: 10.1182/bloodadvances.2021004896

16. Logue JM, Zucchetti E, Bachmeier CA, Krivenko GS, Larson V, Ninh D, et al. Immune reconstitution and associated infections following axicabtagene ciloleucel in relapsed or refractory large B-cell lymphoma. *Haematologica*. (2021) 106(4):978–86. doi: 10.3324/haematol.2019.238634

17. Zhu F, Hu Y, Guoqing W, Huang H, Wu W. Incidence and risk factors associated with infection after chimeric antigen receptor T cell therapy for relapsed/refractory B-cell Malignancies. *Blood*. (2019) 134. doi: 10.1182/blood-2019–129649

18. Zhu F, Wei G, Liu Y, Zhou H, Wu W, Yang L, et al. Incidence and risk factors associated with infection after chimeric antigen receptor T cell therapy for relapsed/refractory B-cell Malignancies. *Cell Transplant*. (2021) 30:9636897211025503. doi: 10.1177/09636897211025503

19. Baird JH, Epstein DJ, Tamaresis JS, Ehlinger Z, Spiegel JY, Craig J, et al. Immune reconstitution and infectious complications following axicabtagene ciloleucel therapy for large B-cell lymphoma. *Blood Advances*. (2021) 5(1):143–55. doi: 10.1182/bloodadvances.2020002732

20. Luo H, Wang N, Huang L, Zhou X, Jin J, Li C, et al. Inflammatory signatures for quick diagnosis of life-threatening infection during the CAR T-cell therapy. *J ImmunoTherapy Cancer*. (2019) 7(1):271. doi: 10.1186/s40425-019-0767-x

21. Hill J, Li D, Hay K, Green ML, Riddell S, Maloney D, et al. Infectious complications of CD19-targeted chimeric antigen receptor-modified T cell immunotherapy. *Open Forum Infect Dis*. (2017) 4:S698–9. doi: 10.1093/ofid/ofx163.1875

22. Kambhampati S, Sheng Y, Huang CY, Bylsma S, Lo M, Kennedy V, et al. Infectious complications in patients with relapsed refractory multiple myeloma after BCMA CAR T-cell therapy. *Blood Adv*. (2022) 6(7):2045–54. doi: 10.1182/bloodadvances.2020004079

23. Josyula S, Pont MJ, Dasgupta S, Song X, Thomas S, Pepper G, et al. Pathogen-Specific Humoral Immunity and Infections in B Cell Maturation Antigen-Directed Chimeric Antigen Receptor T Cell Therapy Recipients with Multiple Myeloma23. *Transplant Cell Ther*. (2021) 28(6):304.e1–304.e9. doi: 10.1016/j.jtct.2022.03.005

24. Vora SB, Waghmare A, Englund JA, Qu P, Gardner RA, Hill JA. Infectious complications following CD19 chimeric antigen receptor t-cell therapy for children, adolescents, and young adults. *Open Forum Infect Diseases*. (2020) 7:ofaa121. doi: 10.1093/OFID/OFAA121

25. Cordeiro A, Bezerra ED, Hirayama AV, Hill JA, Wu QV, Voutsinas J, et al. Late events after treatment with CD19-targeted chimeric antigen receptor modified T cells. *Biol Blood Marrow Transplant*. (2020) 26(1):26–33. doi: 10.1016/j.bbmt.2019.08.003

26. Kambhampati S, Fakhri B, Sheng Y, Huang C-Y, Byslma S, Natsuhara K, et al. Infectious complications of BCMA-targeted and CD19-targeted chimeric antigen receptor T-cell immunotherapy. *Blood*. (2020) 136:4–5. doi: 10.1182/blood-2020–138940

27. Logue JM, Peres LC, Hashmi H, Colin-Leitzinger CM, Shrewsbury AM, Hosoya H, et al. Early cytopenias and infections after standard of care idecabtagene vicleucel in relapsed or refractory multiple myeloma. *Blood Advances*. (2022) 6(24):6109–19. doi: 10.1182/bloodadvances.2022008320

28. Wang Y, Li C, Xia J, Li P, Cao J, Pan B, et al. Humoral immune reconstitution after anti-BCMA CAR T-cell therapy in relapsed/refractory multiple myeloma. *Blood Adv*. (2021) 5(23):5290–9. doi: 10.1182/bloodadvances.2021004603

29. Reynolds G, Hall VG, Teh BW. Vaccine schedule recommendations and updates for patients with hematologic Malignancy post-hematopoietic cell transplant or CAR T-cell therapy. *Transplant Infect Dis*. (2023) 25:e14109. doi: 10.1111/tid.14109

30. Rejeski K, Perez A, Iacoboni G, Penack O, Bücklein V, Jentzsch L, 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

31. 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. doi: 10.1182/blood.2020010543

32. 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. doi: 10.1002/ajh.27056

33. Turtle CJ, Hanafi LA, Berger C, Hudecek M, Pender B, Robinson E, 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

34. 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. doi: 10.1172/jci85309

35. Little JS, Aleissa MM, Beluch K, Gonzalez-Bocco IH, Marty FM, Manne-Goehler J, et al. Low incidence of invasive fungal disease following CD19 chimeric antigen receptor T-cell therapy for non-Hodgkin lymphoma. *Blood Advances*. (2022) 6(16):4821–30. doi: 10.1182/bloodadvances.2022007474

36. 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. doi: 10.1038/nm.3838

37. Juluri KR, Wu QV, Voutsinas J, Hou J, Hirayama AV, Mullane E, et al. Severe cytokine release syndrome is associated with hematologic toxicity following CD19 CAR T-cell therapy. *Blood Adv*. (2022) 6(7):2055–68. doi: 10.1182/bloodadvances.2020004142

38. Frigault MJ, Nikiforow S, Mansour MK, Hu ZH, Horowitz MM, Riches ML, et al. Tocilizumab not associated with increased infection risk after CAR T-cell therapy: implications for COVID-19? *Blood*. (2020) 136(1):137–9. doi: 10.1182/blood.2020006216

39. Schiff MH, Kremer JM, Jahreis A, Vernon E, Isaacs JD, van Vollenhoven RF. Integrated safety in tocilizumab clinical trials. *Arthritis Res Ther*. (2011) 13(5):R141. doi: 10.1186/ar3455

40. Rejeski K, Subklewe M, Aljurf M, Bachy E, Balduzzi A, Barba P, et al. Immune effector cell-associated hematotoxicity: EHA/EBMT consensus grading and best practice recommendations. *Blood*. (2023) 142(10):865–77. doi: 10.1182/blood.2023020578

41. Rejeski K, Wang Y, Hansen DK, Iacoboni G, Bachy E, Bansal R, et al. Applying the EHA/EBMT grading for ICAHT after CAR-T: comparative incidence and association with infections and mortality. *Blood Adv*. (2024) 8(8):1857–68. doi: 10.1182/bloodadvances.2023011767

42. Little JS, Hammond SP, Jacobson C, Aleissa MM. Bacterial infections in the setting of late neutropenia following CD19 chimeric antigen receptor T-cell (CAR-T) therapy for non-hodgkin lymphoma (NHL). *Open Forum Infect Dis*. (2022) 9. doi: 10.1093/ofid/ofac492.1719

43. Fried S, Avigdor A, Bielorai B, Meir A, Besser MJ, Schachter J, et al. Early and late hematologic toxicity following CD19 CAR-T cells. *Bone Marrow Transplant*. (2019) 54(10):1643–50. doi: 10.1038/s41409–019-0487–3

44. Levine JE, Grupp SA, Pulsipher MA, Dietz AC, Rives S, Myers GD, et al. Pooled safety analysis of tisagenlecleucel in children and young adults with B cell acute lymphoblastic leukemia. *J Immunother Cancer*. (2021) 9(8):e002287. doi: 10.1136/jitc-2020–002287

45. Arnold DE, Maude SL, Callahan CA, DiNofia AM, Grupp SA, Heimall JR. Subcutaneous immunoglobulin replacement following CD19-specific chimeric antigen receptor T-cell therapy for B-cell acute lymphoblastic leukemia in pediatric patients. *Pediatr Blood Cancer*. (2020) 67:e28092. doi: 10.1002/pbc.28092

46. Pasquini MC, Hu ZH, Curran K, Laetsch T, Locke F, Rouce R, et al. Real-world evidence of tisagenlecleucel for pediatric acute lymphoblastic leukemia and non-Hodgkin lymphoma. *Blood Adv*. (2020) 4(21):5414–24. doi: 10.1182/bloodadvances.2020003092

47. Hill JA, Krantz EM, Hay KA, Dasgupta S, Stevens-Ayers T, Bender Ignacio RA, et al. Durable preservation of antiviral antibodies after CD19-directed chimeric antigen receptor T-cell immunotherapy. *Blood Advances*. (2019) 3(22):3590–601. doi: 10.1182/bloodadvances.2019000717

48. Kampouri E, Walti CS, Gauthier J, Hill JA. Managing hypogammaglobulinemia in patients treated with CAR-T-cell therapy: key points for clinicians. *Expert Rev Hematol*. (2022) 15:305–20. doi: 10.1080/17474086.2022.2063833

49. Josyula S, Pont MJ, Dasgupta S, Song X, Thomas S, Pepper G, et al. Pathogen-specific humoral immunity and infections in B cell maturation antigen-directed chimeric antigen receptor T cell therapy recipients with multiple myeloma. *Transplant Cell Ther*. (2022) 28(6):304.e1–9. doi: 10.1016/j.jtct.2022.03.005

50. Nagel J, Boeser KD, Hale C, Harris A, Jain R, Postelnick M, et al. 1015. A multicenter evaluation of antimicrobial utilization in hospitalized bone marrow transplant (BMT) and T-cell therapy (CAR-T) patients, and the impact on rates of C. difficile infection (CDI). *Open Forum Infect Diseases*. (2023) 10. doi: 10.1093/ofid/ofad500.046

51. Yang J, Hu H, Zhu X, Zou S, Song J, Liu D, et al. CRO infection and the Use of MRSA-active Medication for Prophylaxis affect the Prognosis of Patients with Hematological Malignancies after CAR-T Infusion. *Int J Antimicrob Agents*. (2023) 62:106874. doi: 10.1016/j.ijantimicag.2023.106874

52. Scheich S, Lindner S, Koenig R, Reinheimer C, Wichelhaus TA, Hogardt M, et al. Clinical impact of colonization with multidrug-resistant organisms on outcome after allogeneic stem cell transplantation in patients with acute myeloid leukemia. *Cancer*. (2018) 124(2):286–96. doi: 10.1002/cncr.31045

53. Smith M, Dai A, Ghilardi G, Amelsberg KV, Devlin SM, Pajarillo R, et al. Gut microbiome correlates of response and toxicity following anti-CD19 CAR T cell therapy. *Nat Med*. (2022) 28(4):713–23. doi: 10.1038/s41591–022-01702–9

54. Abid MB, Shah NN, Maatman TC, Hari PN. Gut microbiome and CAR-T therapy. *Exp Hematol Oncol*. (2019) 8:31. doi: 10.1186/s40164–019-0155–8

55. Haidar G, Dorritie K, Farah R, Bogdanovich T, Nguyen MH, Samanta P. Invasive mold infections after chimeric antigen receptor-modified T-cell therapy: A case series, review of the literature, and implications for prophylaxis. *Clin Infect Dis*. (2020) 71:672–6. doi: 10.1093/cid/ciz1127

56. Garner W, Samanta P, Haidar G. Invasive fungal infections after anti-cd19 chimeric antigen receptor-modified t-cell therapy: State of the evidence and future directions. *J Fungi*. (2021) 7:1–15. doi: 10.3390/jof7020156

57. Chamilos G, Lionakis MS, Kontoyiannis DP. Call for action: invasive fungal infections associated with ibrutinib and other small molecule kinase inhibitors

targeting immune signaling pathways. *Clin Infect Dis*. (2018) 66:140–8. doi: 10.1093/cid/cix687

58. Little JS, Weiss ZF, Hammond SP. Invasive fungal infections and targeted therapies in hematological Malignancies. *J Fungi (Basel)*. (2021) 7. doi: 10.3390/jof7121058

59. Ghez D, Calleja A, Protin C, Baron M, Ledoux MP, Damaj G, et al. Early-onset invasive aspergillosis and other fungal infections in patients treated with ibrutinib. *Blood*. (2018) 131(17):1955–9. doi: 10.1182/blood-2017–11-818286

60. Tillman BF, Pauff JM, Satyanarayana G, Talbott M, Warner JL. Systematic review of infectious events with the Bruton tyrosine kinase inhibitor ibrutinib in the treatment of hematologic Malignancies. *Eur J Haematol*. (2018) 100:325–34. doi: 10.1111/ejh.13020

61. Wang J, Daunov M, Cooper BW. Incidence and outcomes of pneumocystis pneumonia following chimeric antigen receptor T-cell therapy: A real-world analysis. *Blood*. (2023) 142:6897–7. doi: 10.1182/blood-2023–190785

62. Kampouri E, Ibrahimi SS, Xie H, Wong ER, Hecht JB, Sekhon MK, et al. CMV reactivation and CMV-specific cell-mediated immunity after chimeric antigen receptor T-cell therapy. *Clin Infect Dis*. (2023) 78(4):1022–32. doi: 10.1093/cid/ciad708

63. Chen G, Herr M, Nowak J, Ho C, Almyroudis N, Attwood K, et al. Cytomegalovirus reactivation after CD19 CAR T-cell therapy is clinically significant. *Haematologica*. (2023) 108(2):615–20. doi: 10.3324/haematol.2022.281719

64. Khawaja F, Sassine J, Handley G, Iyer SP, Ramdial J, Ahmed S, et al. Herpesviruses infections in CAR T cell recipients. *Transplantation and Cellular Therapy*. (2022) 28:S381–S382. doi: 10.1016/S2666-6367(22)00649-2

65. Márquez-Algaba E, Iacoboni G, Pernas B, Esperalba J, Los Arcos I, Navarro V, et al. Impact of cytomegalovirus replication in patients with aggressive B cell lymphoma treated with chimeric antigen receptor T cell therapy. *Transplant Cell Ther*. (2022) 28 (12):851. doi: 10.1016/j.jtct.2022.09.007

66. Khawaja F, Prakash R, Sassine J, Handley G, VanWieren T, Angelidakis G, et al. Cytomegalovirus (CMV) Reactivation within in the First Year after Chimeric Antigen Receptor (CAR) T Cell Therapy: Experience from the First Two Years at a Major Cancer Center. *Blood*. (2022) 140:7533–5. doi: 10.1182/blood-2022–167908

67. Heldman MR, Ma J, Gauthier J, O'Hara RA, Cowan AJ, Yoke LM, et al. CMV and HSV pneumonia after immunosuppressive agents for treatment of cytokine release syndrome due to chimeric antigen receptor-modified T (CAR-T)-cell immunotherapy. *J Immunother*. (2021) 44(9):351–4. doi: 10.1097/CJI.0000000000000388

68. Spanjaart AM, van der Valk FM, van Rooijen G, Brouwer MC, Kersten MJ. Confused about confusion. *N Engl J Med*. (2022) 386:80–7. doi: 10.1056/NEJMcps2114818

69. Rebechi MT, Bork JT, Riedel DJ. HHV-6 encephalitis after chimeric antigen receptor T-cell therapy (CAR-T): 2 case reports and a brief review of the literature. *Open Forum Infect Dis*. (2021) 8:ofab470. doi: 10.1093/ofid/ofab470

70. Locke FL, Ghobadi A, Jacobson CA, Miklos DB, Lekakis LJ, Oluwole OO, 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

71. 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. doi: 10.1056/NEJMoa1709866

72. Shah M, Kuhnl A, Shields G, Sudhanva M, Metaxa V, Wong S, et al. Human herpesvirus 6 encephalitis following axicabtagene ciloleucel treatment for refractory diffuse large B cell lymphoma. *Hemasphere*. (2021) 5(3):e535. doi: 10.1097/hs9.0000000000000535

73. Handley G, Khawaja F, Kondapi DS, Lee HJ, Kaufman GP, Neelapu SS, et al. Human herpesvirus 6 myelitis after chimeric antigen receptor T-cell therapy. *Int J Infect Dis*. (2021) 112:327–9. doi: 10.1016/j.ijid.2021.09.061

74. Lareau CA, Yin Y, Maurer K, Sandor KD, Daniel B, Yagnik G, et al. Latent human herpesvirus 6 is reactivated in CAR T cells. *Nature*. (2023) 623(7987):608–15. doi: 10.1038/s41586–023-06704–2

75. Khan AM, Ajmal Z, Tuz Zahra F, Ramani A, Zackon I. Hemorrhagic cystitis secondary to adenovirus and BK virus infection in a diffuse large B-cell lymphoma patient with recent CAR T-cell therapy. *Case Rep Hematol*. (2020) 2020:6621967. doi: 10.1155/2020/6621967

76. Ahrendsen JT, Sehgal K, Sarangi S, Uhlmann EJ, Varma H, Arnason J, et al. Progressive multifocal leukoencephalopathy after chimeric antigen receptor T-cell therapy for recurrent non-hodgkin lymphoma. *J Hematol*. (2021) 10:212–6. doi: 10.14740/jh903

77. Mackenzie S, Shafat M, Roddy H, Hyare H, Neill L, Marzolini MAV, et al. Pembrolizumab for the treatment of progressive multifocal leukoencephalopathy following anti-CD19 CAR-T therapy: a case report. *EJHaem*. (2021) 2(4):848–53. doi: 10.1002/jha2.274

78. Villalba JA, Maus MV, Frigault MJ, Zaffini R, Gandhi RT, Rosenberg ES, et al. False-positive human immunodeficiency virus test results in patients receiving lentivirus-based chimeric antigen receptor T-cell therapy: case report, review of the literature, and proposed recommendations. *J Infect Dis*. (2022) 225:1933–6. doi: 10.1093/infdis/jiab605

79. Hayden PJ, Sirait T, Koster L, Snowden JA, Yakoub-Agha I. An international survey on the management of patients receiving CAR T-cell therapy for haematological Malignancies on behalf of the Chronic Malignancies Working Party of EBMT. *Curr Res Trans Med*. (2019) 67:79–88. doi: 10.1016/j.retram.2019.05.002

80. Abramson JS, Irwin KE, Frigault MJ, Dietrich J, McGree B, Jordan JT, et al. Successful anti-CD19 CAR T-cell therapy in HIV-infected patients with refractory high-grade B-cell lymphoma. *Cancer*. (2019) 125(21):3692–8. doi: 10.1002/cncr.32411

81. Abbasi A, Peeke S, Shah N, Mustafa J, Khatun F, Lombardo A, et al. Axicabtagene ciloleucel CD19 CAR-T cell therapy results in high rates of systemic and neurologic remissions in ten patients with refractory large B cell lymphoma including two with HIV and viral hepatitis. *J Hematol Oncol*. (2020) 13(1):1. doi: 10.1186/s13045-019-0838-y

82. EASL. Recommendations on treatment of hepatitis C 2018. *J Hepatol*. (2018) 69:461–511. doi: 10.1016/j.jhep.2018.03.026

83. Mallet V, van Bömmel F, Doerig C, Pischke S, Hermine O, Locasciulli A, et al. Management of viral hepatitis in patients with haematological Malignancy and in patients undergoing haemopoietic stem cell transplantation: recommendations of the 5th European Conference on Infections in Leukaemia (ECIL-5). *Lancet Infect Dis*. (2016) 16(5):606–17. doi: 10.1016/s1473–3099(16)00118–3

84. Available online at: https://wwwnc.cdc.gov/travel/yellowbook/2024/infections-diseases/tuberculosis#epi.

85. Balmaceda N, Aziz M, Chandrasekar VT, McClune B, Kambhampati S, Shune L, et al. Infection risks in multiple myeloma: a systematic review and meta-analysis of randomized trials from 2015 to 2019. *BMC Cancer*. (2021) 21(1):730. doi: 10.1186/s12885-021-08451-x

86. Hayden PJ, Roddie C, Bader P, Basak GW, Bonig H, Bonini C, et al. Management of adults and children receiving CAR T-cell therapy: 2021 best practice recommendations of the European Society for Blood and Marrow Transplantation (EBMT) and the Joint Accreditation Committee of ISCT and EBMT (JACIE) and the European Haematology Association (EHA). *Ann Oncol*. (2022) 33(3):259–75. doi: 10.1016/j.annonc.2021.12.003

87. Wudhikarn K, Perales MA. Infectious complications, immune reconstitution, and prophylaxis after CD19 chimeric antigen receptor T-cell therapy. *Bone Marrow Transplant*. (2022) 57:1477–88. doi: 10.1038/s41409–022-01756-w

88. Los-Arcos I, Iacoboni G, Aguilar-Guisado M. Recommendations for screening, monitoring, prevention and prophylaxis of infections in adult and pediatric patients receiving CAR T-cell therapy: a position paper. *Infection*. (2021) 49:215–31. doi: 10.1007/s15010–020-01521–5

89. Stewart AG, Henden AS. Infectious complications of CAR T-cell therapy: a clinical update. *Ther Adv Infect Dis*. (2021) 8(1):1–22. doi: 10.1177/20499361211036773

90. Kampouri E, Little JS, Rejeski K, Manuel O, Hammond SP, Hill JA. Infections after chimeric antigen receptor (CAR)-T-cell therapy for hematologic malignancies. *Transpl Infect Dis*. (2023) 25(Suppl 1):S1–S17. doi: 10.1111/tid.141576

91. Zu C, Xu Y, Wang Y, Zhang M, Zhao H, Fang X, et al. Cytomegalovirus retinitis and retinal detachment following chimeric antigen receptor T cell therapy for relapsed/refractory multiple myeloma. *Curr Oncol*. (2022) 29(2):490–6. doi: 10.3390/curroncol29020044

92. Qasim W. Allogeneic CAR T cell therapies for leukemia. *Am J Hematol*. (2019) 94:S50–s54. doi: 10.1002/ajh.25399

93. Depil S, Duchateau P, Grupp SA, Mufti G, Poirot L. 'Off-the-shelf' allogeneic CAR T cells: development and challenges. *Nat Rev Drug Discovery*. (2020) 19:185–99. doi: 10.1038/s41573–019-0051–2

94. Brudno JN, Kochenderfer JN. Toxicities of chimeric antigen receptor T cells: recognition and management. *Blood*. (2016) 127:3321–30. doi: 10.1182/blood-2016–04-703751

95. Penack O, Koenecke C. Complications after CD19+ CAR T-cell therapy. *Cancers*. (2020) 12:3445. doi: 10.3390/cancers12113445

96. Sheth VS, Gauthier J. Taming the beast: CRS and ICANS after CAR T-cell therapy for ALL. *Bone Marrow Transplant*. (2021) 56:552–66. doi: 10.1038/s41409–020-01134–4

97. Lee DW, Santomasso BD, Locke FL, Ghobadi A, Turtle CJ, Brudno JN, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. *Biol Blood Marrow Transplant*. (2019) 25 (4):625–38. doi: 10.1016/j.bbmt.2018.12.758

98. Gardner RA, Ceppi F, Rivers J, Annesley C, Summers C, Taraseviciute A, et al. Preemptive mitigation of CD19 CAR T-cell cytokine release syndrome without attenuation of antileukemic efficacy. *Blood*. (2019) 134(24):2149–58. doi: 10.1182/blood.2019001463

99. Thompson JA, Schneider BJ, Brahmer J, Achufusi A, Armand P, Berkenstock MK, et al. Management of immunotherapy-related toxicities, version 1.2022, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw*. (2022) 20(4):387–405. doi: 10.6004/jnccn.2022.0020

100. Banerjee R, Marsal J, Huang CY, Lo M, Kambhampati S, Kennedy VE, et al. Early time-to-tocilizumab after B cell maturation antigen-directed chimeric antigen receptor T cell therapy in myeloma. *Transplant Cell Ther*. (2021) 27(6):477. doi: 10.1016/j.jtct.2021.03.004

101. Robilotti E, Holubar M, Seo SK, Deresinski S. Feasibility and applicability of antimicrobial stewardship in immunocompromised patients. *Curr Opin Infect Dis*. (2017) 30:346–53. doi: 10.1097/qco.0000000000000380

102. Puerta-Alcalde P, Cardozo C, Marco F, Suárez-Lledó M, Moreno E, Morata L, et al. Changing epidemiology of bloodstream infection in a 25-years hematopoietic stem cell transplant program: current challenges and pitfalls on empiric antibiotic treatment impacting outcomes. *Bone Marrow Transplant.* (2020) 55:603–12. doi: 10.1038/s41409–019-0701–3

103. Shahid Z, Epstein DJ. Noninfectious causes of fever in hematologic Malignancies. Are antibiotics still indicated? *Curr Opin Infect Dis.* (2023) 36:209–17. doi: 10.1097/qco.0000000000000940

104. Rearigh L, Stohs E, Freifeld A, Zimmer A. De-escalation of empiric broad spectrum antibiotics in hematopoietic stem cell transplant recipients with febrile neutropenia. *Ann Hematol.* (2020) 99:1917–24. doi: 10.1007/s00277–020-04132–0

105. Gustinetti G, Raiola AM, Varaldo R, Galaverna F, Gualandi F, Del Bono V, et al. De-escalation and discontinuation of empirical antibiotic treatment in a cohort of allogeneic hematopoietic stem cell transplantation recipients during the pre-engraftment period. *Biol Blood Marrow Transplant.* (2018) 24(8):1721–6. doi: 10.1016/j.bbmt.2018.03.018

106. Fleischmann RM, Tesser J, Schiff MH, Schechtman J, Burmester GR, Bennett R, et al. Safety of extended treatment with anakinra in patients with rheumatoid arthritis. *Ann Rheum Dis.* (2006) 65(8):1006–12. doi: 10.1136/ard.2005.048371

107. Wehrli M, Gallagher K, Chen YB, Leick MB, McAfee SL, El-Jawahri AR, et al. Single-center experience using anakinra for steroid-refractory immune effector cell-associated neurotoxicity syndrome (ICANS). *J Immunother Cancer.* (2022) 10(1): e003847. doi: 10.1136/jitc-2021–003847

108. Kim JY, Kim M, Park JK, Lee EB, Park JW, Hong J. Limited efficacy of tocilizumab in adult patients with secondary hemophagocytic lymphohistiocytosis: a retrospective cohort study. *Orphanet J Rare Dis.* (2022) 17:363. doi: 10.1186/s13023–022-02516–1

109. Lussana F, Cattaneo M, Rambaldi A, Squizzato A. Ruxolitinib-associated infections: A systematic review and meta-analysis. *Am J Hematol.* (2018) 93:339–47. doi: 10.1002/ajh.24976

110. Gill H, Leung GMK, Seto WK, Kwong YL. Risk of viral reactivation in patients with occult hepatitis B virus infection during ruxolitinib treatment. *Ann Hematol.* (2019) 98:215–8. doi: 10.1007/s00277–018-3405–7

111. Sylvine P, Thomas S, Pirayeh E. Infections associated with ruxolitinib: study in the French Pharmacovigilance database. *Ann Hematol.* (2018) 97:913–4. doi: 10.1007/s00277–018-3242–8

112. Dorman SE, Picard C, Lammas D, Heyne K, van Dissel JT, Baretto R, et al. Clinical features of dominant and recessive interferon gamma receptor 1 deficiencies. *Lancet.* (2004) 364(9451):2113–21. doi: 10.1016/s0140–6736(04)17552–1

113. Locatelli F, Jordan MB, Allen C, Cesaro S, Rizzari C, Rao A, et al. Emapalumab in children with primary hemophagocytic lymphohistiocytosis. *N Engl J Med.* (2020) 382(19):1811–22. doi: 10.1056/NEJMoa1911326

114. Hill JA, Giralt S, Torgerson TR, Lazarus HM. CAR-T – and a side order of IgG, to go? – Immunoglobulin replacement in patients receiving CAR-T cell therapy. *Blood Rev.* (2019) 38. doi: 10.1016/j.blre.2019.100596

115. Walti CS, Krantz EM, Maalouf J, Boonyaratanakornkit J, Keane-Candib J, Joncas-Schronce L, et al. Antibodies against vaccine-preventable infections after CAR-T cell therapy for B cell Malignancies. *JCI Insight.* (2021) 6(11):e146743. doi: 10.1172/jci.insight.146743

116. Walti CS, Loes AN, Shuey K, Krantz EM, Boonyaratanakornkit J, Keane-Candib J, et al. Humoral immunogenicity of the seasonal influenza vaccine before and after CAR-T-cell therapy: A prospective observational study. *J ImmunoTherapy Cancer.* (2021) 9(10):e003428. doi: 10.1136/jitc-2021–003428

117. Hill JA, Giralt S, Torgerson TR, Lazarus HM. CAR-T - and a side order of IgG, to go? - Immunoglobulin replacement in patients receiving CAR-T cell therapy. *Blood Rev.* (2019) 38:100596. doi: 10.1016/j.blre.2019.100596

118. Mikulska M, Cesaro S, de Lavallade H, Di Blasi R, Einarsdottir S, Gallo G, et al. Vaccination of patients with haematological Malignancies who did not have transplantations: guidelines from the 2017 European Conference on Infections in Leukaemia (ECIL 7). *Lancet Infect Dis.* (2019) 19(6):e188–99. doi: 10.1016/s1473–3099(18)30601–7

119. Cordonnier C, Einarsdottir S, Cesaro S, Di Blasi R, Mikulska M, Rieger C, et al. Vaccination of haemopoietic stem cell transplant recipients: guidelines of the 2017 European Conference on Infections in Leukaemia (ECIL 7). *Lancet Infect Dis.* (2019) 19(6):e200–12. doi: 10.1016/s1473–3099(18)30600–5

120. 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. doi: 10.1056/NEJMoa2116133

121. Schuster SJ, Svoboda J, Chong EA, Nasta SD, Mato AR, Anak Ö, et al. Chimeric antigen receptor T cells in refractory B-cell lymphomas. *N Engl J Med.* (2017) 377 (26):2545–54. doi: 10.1056/NEJMoa1708566

122. Hill JA, Martens MJ, Young JH, Bhavsar K, Kou J, Chen M, et al. SARS-CoV-2 vaccination in the first year after hematopoietic cell transplant or chimeric antigen receptor T cell therapy: A prospective, multicenter, observational study (BMT CTN 2101). *Clin Infect Dis.* (2024) 27:ciae291. doi: 10.1093/cid/ciae291.

123. Antin JH, Guinan EC, Avigan D, Soiffer RJ, Joyce RM, Martin VJ, et al. Protective antibody responses to pneumococcal conjugate vaccine after autologous hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant..* (2005) 11:213–22. doi: 10.1016/j.bbmt.2004.12.330

124. Melgar M, Britton A, Roper LE, Talbot HK, Long SS, Kotton CN, et al. Use of respiratory syncytial virus vaccines in older adults: recommendations of the advisory committee on immunization practices — United states, 2023. *MMWR Morb Mortal Wkly Rep.* (2023) 72(29):793–801. doi: 10.15585/mmwr.mm7229a4

125. Khawaja F, Papanicolaou G, Dadwal S, Pergam SA, Wingard JR, Boghdadly ZE, et al. Frequently asked questions on coronavirus disease 2019 vaccination for hematopoietic cell transplantation and chimeric antigen receptor T-cell recipients from the american society for transplantation and cellular therapy and the american society of hematology. *Transplant Cell Ther.* (2023) 29(1):10–8. doi: 10.1016/j.jtct.2022.10.010

126. Uyemura BS, Abid MA, Suelzer E, Abid MB. Efficacy of SARS-CoV-2 primary and booster vaccine doses in CAR-T recipients – targeting the target antigen. *Bone Marrow Transplant.* (2022) 57:1727–31. doi: 10.1038/s41409–022-01795–3

127. Available online at: https://www.cdc.gov/coronavirus/2019-ncov/vaccines/recommendations/immuno.html#:~:text=Learn%20more.&text=CDC%20recommends%20the%202023%E2%80%932024,2024%20updated%20COVID%2D19%20vaccine.

128. Teh BW, Mikulska M, Averbuch D, de la Camara R, Hirsch HH, Akova M, et al. Consensus position statement on advancing the standardised reporting of infection events in immunocompromised patients. *Lancet Infect Dis.* (2024) 24(1):e59–68. doi: 10.1016/s1473–3099(23)00377–8

129. Larson RC, Maus MV. Recent advances and discoveries in the mechanisms and functions of CAR T cells. *Nat Rev Cancer..* (2021) 21:145–61. doi: 10.1038/s41568-020-00323-z

130. Wei G, Zhang Y, Zhao H, Wang Y, Liu Y, Liang B, et al. CD19/CD22 dual-targeted CAR T-cell therapy for relapsed/refractory aggressive B-cell lymphoma: A safety and efficacy study. *Cancer Immunol Res.* (2021) 9(9):1061–70. doi: 10.1158/2326–6066.Cir-20–0675

131. Mardiana S, Gill S. CAR T cells for acute myeloid leukemia: state of the art and future directions. *Front Oncol.* (2020) 10:697. doi: 10.3389/fonc.2020.00697

132. Kunte AS, Gagelmann N, Badbaran A, Berger C, Massoud R, Klyuchnikov E, et al. Viral reactivation and immune reconstitution after CAR-T cell treatment in patients with hematologic Malignancies. *Blood.* (2022) 140:7531–2. doi: 10.1182/blood-2022–168667

133. Wang D, Mao X, Que Y, Xu M, Cheng Y, Huang L, et al. Viral infection/reactivation during long-term follow-up in multiple myeloma patients with anti-BCMA CAR therapy. *Blood Cancer J.* (2021) 11(10):168. doi: 10.1038/s41408–021-00563–8

134. Stein-Thoeringer CK, Saini NY, Zamir E, Blumenberg V, Schubert ML, Mor U, et al. A non-antibiotic-disrupted gut microbiome is associated with clinical responses to CD19-CAR-T cell cancer immunotherapy. *Nat Med.* (2023) 29(4):906–16. doi: 10.1038/s41591–023-02234–6

frontiers | Frontiers in Oncology

TYPE Mini Review
PUBLISHED 09 July 2024
DOI 10.3389/fonc.2024.1433432

OPEN ACCESS

EDITED BY
Edward Copelan,
Atrium Healthcare, United States

REVIEWED BY
Simrit Parmar,
University of Texas MD Anderson Cancer Center, United States

*CORRESPONDENCE
Aleksandr Lazaryan
Aleksandr.Lazaryan@moffitt.org

RECEIVED 15 May 2024
ACCEPTED 13 June 2024
PUBLISHED 09 July 2024

CITATION
Mohty R and Lazaryan A (2024) "Off-The-Shelf" allogeneic chimeric antigen receptor T-cell therapy for B-cell malignancies: current clinical evidence and challenges. *Front. Oncol.* 14:1433432. doi: 10.3389/fonc.2024.1433432

"Off-The-Shelf" allogeneic chimeric antigen receptor T-cell therapy for B-cell malignancies: current clinical evidence and challenges

Razan Mohty[1] and Aleksandr Lazaryan[2]*

[1]Division of Hematology Oncology, Department of Medicine, O'Neal Comprehensive Cancer Center, University of Alabama at Birmingham Heersink School of Medicine, Birmingham, AL, United States, [2]Department of Blood and Marrow Transplantation and Cellular Immunotherapy, Moffitt Cancer Center, Tampa, FL, United States

Chimeric antigen receptor T-cell therapy (CAR T) has revolutionized the treatment landscape for hematologic malignancies, notably B-cell non-Hodgkin lymphoma (B-NHL) and B-cell acute lymphoblastic leukemia (B-ALL). While autologous CAR T products have shown remarkable efficacy, their complex logistics, lengthy manufacturing process, and high costs impede widespread accessibility and pose therapeutic challenge especially for patients in rapid need for therapy. "Off-the-shelf" allogeneic CAR T-cell therapy (alloCAR T) has emerged as a promising alternative therapy, albeit experimental to date. AlloCARTs are derived from healthy donors, manufactured by batches and stored, making them available off-the-shelf which lowers financial burden. Various gene editing techniques have been employed to mitigate graft-versus-host disease (GVHD) and host-versus-graft (HvG) to enhance alloCAR T persistence. In this review, we summarize available manufacturing techniques, current evidence, and discuss challenges faced with the use of alloCAR Ts.

KEYWORDS

CAR (chimeric antigen receptor) T cells, allogeneic CAR T therapy, B-cell malignances, B-cell ALL, B-cell NHL, gene editing

Introduction

Chimeric antigen receptor T-cell therapy (CAR T) has revolutionized the treatment of many hematologic malignancies, particularly B-cell non-Hodgkin lymphoma (B-NHL) and B-cell acute lymphoblastic leukemia (B-ALL). Four CAR T products have been FDA-approved for commercial use in patients with various B-NHLs and B-ALL and all of them are autologous as they are derived from the patient's apheresed CD3+ T-cells. While currently approved CAR Ts have been very effective in potentially curing up to 35–40% of patients, many

limitations are associated with their use. Due to their autologous nature, commercial CAR Ts require complex logistics and pose a significant regulatory burden on the treating institution and manufacturing companies. They require leukapheresis and manufacturing for each patient, similar to the production of individualized treatment. As a consequence of this manufacturing complexity, the cost of autologous CAR T production averages $373,000 to $475,000 per product (1). This, in turn, leads to increased costs for patients, the healthcare system, and society at large, eventually affecting access to this life-saving therapy for many patients.

A relatively long wait time has also been reported during the manufacturing of autologous CAR T-cells, with an average vein-to-vein time of 2–6 weeks, depending on the product used (1, 2). This may pose a therapeutic challenge in delivering CAR T-cell therapy to patients with rapidly progressive diseases who need timely treatment. Another limitation of using autologous CAR T cells is related to challenges in separating regular T-cells from circulating malignant cells during CAR T-cell manufacturing (3). This is particularly relevant for hematologic malignancies with a high likelihood of circulating disease and has led to CD19-negative selection during the manufacturing of brexucabtagene autoleucel (brexu-cel). Potential transduction of malignant cells was demonstrated by Ruella et al. in the case report of a patient with B-ALL treated with CD19-directed CAR T cells who relapsed 9 months later and was found to have CAR-transduced leukemic blasts (4). Other issues with autologous CAR T manufacturing are related to interpatient variability and often impaired quality and quantity of residual T-cells after prior line(s) of lymphotoxic therapy. Previous treatments and the tumor microenvironment have both been shown to affect T-cell fitness, thereby limiting CAR T-cell expansion *in vitro* and *in vivo* (5). This, in turn, contributes to the risk of manufacturing failure (5). Furthermore, given the correlation between CAR T-cell dose and response to treatment, any compromise to the number and quality of CAR T-cells in the final product may lead to inferior outcomes (6). Hence, healthy donor "off-the-shelf" allogeneic CAR T-cell therapy (alloCAR T) has emerged as a major alternative to overcome most of the aforementioned limitations (7). AlloCAR Ts can be derived from peripheral blood T-cells or NK cells of healthy donors, umbilical cord blood (UCB), or induced pluripotent stem cells (iPSCs) (7). Multiple batches of cryopreserved T-cells can be obtained from the same healthy donor, resulting in faster manufacturing of more accessible "off-the-shelf" CAR T products with potentially reduced financial burden to institutions and payers of this otherwise costly therapy. Furthermore, faster access to readily available alloCAR Ts allows easier re-dosing of the product if needed. This review summarizes current evidence for "off-the-shelf" alloCAR T therapy and discusses its therapeutic potential and challenges.

Manufacturing of alloCAR Ts and gene editing techniques

AlloCAR T-cells from a single manufacturing batch have the potential to benefit multiple patients. The manufacturing of alloCAR Ts involves the use of immunologically intact and otherwise fit healthy-donor T-cells obtained via leukapheresis and subsequently cryopreserved (8). Various technologies have evolved around alloCAR T manufacturing, primarily focusing on immunologic incompatibilities between the recipient (patient) and healthy donor, with inherent risks of graft-versus-host disease (GVHD) and host-versus-graft (HvG), which can substantially limit the persistence and efficacy of alloCAR Ts. Generation of the CAR construct on the surface of alloCAR Ts involves established techniques such as viral vector-mediated transgenesis or gene knock-in editing for permanent insertion of recombinant DNA coding for a CAR and possibly additional genes (e.g., a suicide gene or a co-stimulatory receptor). This can be followed by the knockout of αβ T-cell receptor (*TCR*), β2-microglobulin (*β2M*), and *CD52* genes (8). The alloCAR Ts are subsequently cultured and expanded in the presence of cytokines. The remaining non-disrupted αβ TCR-positive cells are typically removed by negative selection using anti-αβ TCR antibodies. The final product is cryopreserved and shipped to the clinic for infusion whenever needed (Figure 1) (8).

Both gene- and non-gene editing technologies have been used to decrease the risk of GVHD as well as HvG, with the latter ultimately responsible for alloCAR T rejection following the immune reconstitution of the patient (7). Several methods have been developed to mitigate GVHD risk, including commonly targeted gene editing of the T-cell receptor constant α chain (*TRAC*) together with β_2M gene knockout leading to disrupted α and/or β TCR and HLA MHC class I expression, respectively (8, 9). Other methodologies to mitigate those risks are based on the use of virus-specific memory T-cells, non-αβ T cells (e.g., γδ T cells), iPSCs, and donor-derived allogeneic T-cells in stem cell transplant recipients. Of note, knockout out of the HLA class I molecules could render CAR Ts more prone to be targeted by NK cells due to "missing self-signal" (8). Some studies have shown that knocking in HLA-E can prevent NK-cell-mediated elimination of those CAR Ts (8). Finally, the use of anti-CD52 monoclonal antibodies and *CD52* knockout within CAR Ts aim at decreasing the risk of HvG (10).

Several commonly used gene editing techniques include clustered regularly interspaced short palindromic repeats (CRISPR)/Cas systems, transcription activator-like effector nuclease (TALEN), zinc finger nuclease (ZFN), and ARCUS, among others (11–14) (Precision BioSciences I)[1]. These techniques share a common goal of creating specific DNA double-stranded breaks at pre-specified sites through chimeric nucleases. Gene repair mechanisms lead to gene inactivation (knock-out) or gene insertion (knock-in) through available exogenous DNA repair templates. They differ in their flexibility and efficacy in targeting the preselected DNA sites and the number of off-target cleavages.

Clinical trials in B-NHL and B-ALL

Allogeneic CAR Ts targeting CD19 antigen

CD19 is a transmembrane protein which is highly regulated and expressed throughout B-cell development (15). It is ubiquitously

1 Precision BioSciences I. A precise platform for clinical gene editing. Biopharma Dealmakers (Biopharm Deal). Available at: https://www.nature.com/articles/d43747-022-00219-x.

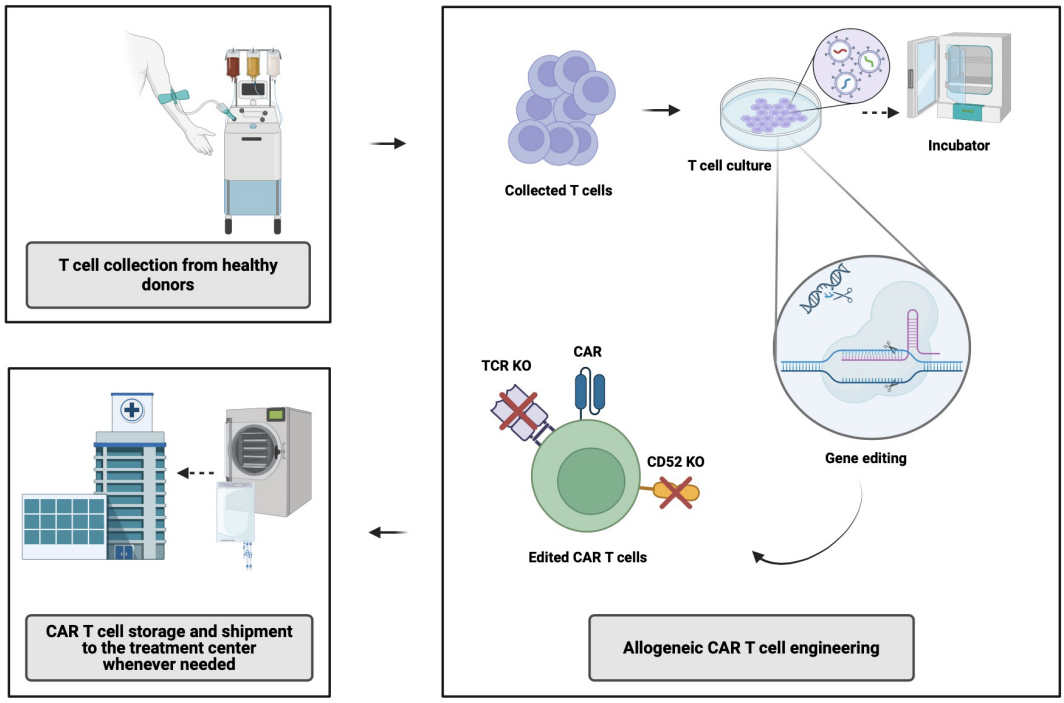


FIGURE 1
Allogeneic CAR T-cell manufacturing process. CAR, chimeric antigen receptor; TCR, T-cell receptor; KO, knock out. Created with BioRender.com.

expressed across most B-cell malignancies, making it a successful target for many immunotherapies (15), including all approved autologous CAR T product. CD19 has also become the target of several alloCAR Ts which are currently under investigation (Table 1).

The ALPHA trial (NCT03939026), a phase 1, open-label, multicenter trial, evaluated the use of anti-CD19 alloCAR T (ALLO-501), which is TALEN gene-edited to disrupt *TRAC* and to knockout CD52 gene to decrease the risk of GVHD and GvH (22). The CD52 gene knock-out within alloCAR Ts also allowed the use of the anti-CD52 monoclonal antibody (ALLO-647) for better lymphodepletion along with standard fludarabine (30 mg/m^2 per day) and cyclophosphamide (500 mg/m^2 per day) given for 3 days. A total of 47 patients with large B-cell lymphoma (LBCL) and follicular lymphoma (FL) were enrolled, and 46 were treated (22). The study consisted of 2 cohorts: 39 patients received single infusion and 7 patients with at least stable disease at day 28 received another infusion of ALLO-647 and ALLO-501 (consolidation cohort). Twenty percent of the patients had received and failed prior autologous CAR T. No dose-limiting toxicity was reported. Only 1 patient developed grade ≥3 cytokine release syndrome (CRS), and no patient developed grade≥ 3 immune effector cell-associated neurotoxicity syndrome (ICANS). The objective response rate (ORR) and complete response (CR) rates were 75% and 50%, respectively, in the intention-to-treat population (22). The subsequent ALPHA2 trial enrolled patients with relapsed/refractory (R/R) LBCL who received a slightly modified product (aka ALLO-501A or cemacabtagene ansegedleucel [cema-cel]), which had the rituximab kill switch removed from ALLO-501 construct (23). In ALPHA2, CAR Ts were infused in a single (N=7) or repeated schedule (i.e., consolidation cohort, N=21) (24). No GVHD was reported. Only 3 and 6 patients developed low grade CRS and ICANS, respectively. Cytopenias were the most common adverse events in the entire cohort (57%). The ORR and CR rates were 48% and 28% among 28 evaluable patients, respectively. Longest CR durability was 15 months. After a median follow-up of 7.1 months from the single infusion in both ALPHA and ALPHA2 trials no dose-limiting toxicity (DLT), grade≥3 CRS/ICANS, or GVHD were reported in patients with LBCL treated with optimized lymphodepleting regimen. Among 12 evaluable patients, the ORR and CR rates were 66.7% and 58.3%, respectively, with a median duration of response of 23.1 months (25). Only five infusion reactions were reported following ALLO-647 (all grade 3) (15). Altogether, these data have supported the safety and efficacy of ALLO-501/ALLO-501A,however, longer follow-up is needed to evaluate the durability of these responses. The subsequent ALPHA3 trial will assess the use of cema-cel (ALLO-501A) as a consolidation therapy in patients with LBCL who have a minimal residual disease (MRD) detected after the first line of treatment. The MRD will be evaluated via investigational assay measuring circulating tumor (ct) DNA (PhasED-Seq^{TM}) (26).

The phase 1 Carbon trial evaluated CD19-directed alloCAR Ts (CTX110) in patients with R/R LBCL (27). CTX110 manufacturing involved the use of CRISPR/Cas-9 technology to disrupt *TRAC* and β_2M genes (28). Patients who received prior autologous CAR Ts were excluded. The ORR and CR rates in patients who received dose level 3 (≥300 x 10^6 CAR T cells; N=27) were 67% (18/27) and 47% (11/27), respectively. No GVHD was reported. The CRS and ICANS rates were 56% (18/32; all grade≤2) and 9.4% (3/32; 2 grade≥3), respectively. There were 7 serious adverse events attributed to CTX110, with 4 patients developing grade≥3 infections, including 1 fatal HHV6 infection (27). Despite its promising efficacy, CTX110

TABLE 1 Summary of major clinical trials utilizing allogeneic CAR T-cells in B-cell malignancies (final and/or interim data).

Ref/NCT	Trial phase	Disease	N	Manufacturing technique	Lympho depletion	Response	CRS	ICANS	GVHD	Other toxicity
CD19-directed CAR T cells										
ALPHA trial NCT03939026	Phase 1	LBCL FL	46	ALLO-501 *TRAC*, *CD52* loci editing, rituximab kill switch TALEN	FC, anti-CD52	ORR=75% CR=50%	Gr1–2 = 21.7% Gr3+=2%	None	None	Gr3+ infections=23.9%
ALPHA2 trial NCT04416984	Phase 1	LBCL	28	ALLO-501A *TRAC*, *CD52* loci editing TALEN	FC, anti-CD52	ORR=48% CR=28%	Gr1–2 = 11%	Gr1–2 = 21%	None	Cytopenia=57%
CARBON trial NCT04035434	Phase 1	LBCL	27	CTX110 CRISPR/Cas9	FC				None	
Shah et al. (16) NCT03666000	Phase 1/2a	B-NHL	13	PBCAR0191 *TRAC* locus editing ARCUS	FC	ORR=85% CR=62%	None	Gr3+=6%	None	Gr3+ neutropenia=15% Gr3+ infections=31%
		B-ALL	15		FC	CR/CRi=60%	Gr1–2 = 60% No Gr3+	Gr3+=6.7%	None	Gr3+ infections=40%
Shahid et al. (17) NCT01430390	Phase 1	B-NHL B-ALL	16	19–28 CAR EBV-CTLs	FC	NR	None	None	37%	NR
Xiao et al. (18) NCT05453669	Phase 1	B-NHL	15	RJMty19 Double-negative T- cells	FC	DL≥3: ORR=40%	Gr1–2 = 13% No Gr3+	None	None	None
Mehta et al. (19) NCT04629729	Phase 1	B-NHL B-ALL	12 (LBCL)	FT819–101 CRISPR/Cas *TRAC*/*TCR* editing 1XX signaling domain iPSC	FC	NR	Gr1–2 = 25% No Gr3+	None	None	None
ANTLER trial NCT04637763	Phase 1	B-NHL	5	cb-010: CRISPR *TRAC*/*PD-1* editing	FC	ORR=100% CR=80%	Grade 1 = 20%	Grade 3 = 20%	None	Gr3+ cytopenia and hypogamma globulinemia=50%
CALM trial NCT02746952	Phase 1	B-ALL	25	UCART19: TALEN *TRAC*/*CD52* editing	FC, anti-CD52	CR/CRi=100% MRD neg=48%	Gr1–2 = 56% Gr3+=24%	Gr1 = 24% Cr3+=4%	Gr1 = 8%	Gr4 cytopenia=16% Gr3 TLS=8%

(Continued)

10.3389/fonc.2024.1433432

TABLE 1 Continued

Ref/NCT	Trial phase	Disease	N	Manufacturing technique	Lympho depletion	Response	CRS	ICANS	GVHD	Other toxicity
CD20-directed CAR T cells										
Neelapu et al. (20) NCT04735471	Phase 1	B-NHL	9	ADI-001 γδ CAR T cells No gene editing	FC	ORR and CR=78%	Gr1 = 11%	Gr3 = 11%	None	NR
CD22-directed CAR T-cells										
BALLI-01 NCT04150497	Phase 1	B-ALL	18	UCART22 TALEN editing for *TRAC*/*CD52* editing	FC, anti-CD52	CR/CRi=33% MLFS=5.6%	Gr1–2 = 61%	None	Gr2 = 5%	Gr3+=72%
Dual directed CAR T cells										
NCT05105867	Phase 1	B-ALL	4	Truucar GC502 CD19/CD7 Technology NR *TRAC*/*CD7* editing	FC	CR/CRi=75%	Gr2 = 50% Gr3 = 50%	None	None	Gr3 + cytopenia=100%
Hu et al. (21) NCT05350787	Phase 2	B-ALL	7	ThisCART19A CD19 TCRαβ/CD3 No gene editing	FC, etoposide	CR/CRi=100% MRD neg=100%	Gr3+=25%	Gr3+=37.5%	NR	Fatal CRS/ infection=14%

Ref, reference; NCT, ClinicalTrials.gov ID; Nb of pts, Number of patients; CRS, cytokine release syndrome; ICANS, Immune effector cell-associated neurotoxicity syndrome; ORR, Overall response rate; CR, complete response; MRD, minimal residual disease; FC, fludarabine, cyclophosphamide; NHL, non-Hodgkin lymphoma; B-ALL, B-cell acute lymphoblastic lymphoma; LBCL, large B-cell lymphoma; FL, follicular lymphoma; Gr3+, ≥grade 3; NR, not reported; TRAC, T-cell receptor α constant; TLS, tumor lysis syndrome; TALEN, transcription activator-like effector nuclease; PD-1, programmed-cell death; DL, dose level; MLFS, morphologic leukemia free state; iPSC, induced pluripotent stem cell; EBV, Epstein Barr Virus; CTL, cytotoxic lymphocytes.
*Percentages were computed whenever only numeric data were published.

10.3389/fonc.2024.1433432

appeared to be associated with substantial toxicity. Thus, future studies will need to focus on preventing such alloCAR T-related toxicities by possibly incorporating prophylactic measures for patients at risk.

In the phase 1 ANTLER trial, the CD19-directed alloCAR T product (CB-010) was manufactured using CRISPR/Cas9 to knockout *TRAC* and *PD-1* genes to limit both GVHD and CAR T-cell exhaustion (29). Six patients with B-NHL were treated, and 5 were evaluable for response assessment. All patients responded, with 4 patients achieving CR. One patient developed grade 1 CRS together with grade 3 ICANS attributed to CB-010 but resolved within few days of therapy with tocilizumab and steroids. No GVHD was reported. This promising trial continues to accrue participants with the most recent updates by the manufacturer consistent with 94% ORR (15/16) and 69% CR (11/16) (30). While half of the 10 patients with R/R LBCL appeared to maintain CR at/or beyond 6 months, the final peer-reviewed and audited results of this trial are eagerly awaited to confirm these interim findings (NCT04637763).

Another innovative CD19-directed alloCAR T (PBCAR0191) was evaluated in a phase 1/2a trial for patients with B-cell malignancies (ie. B-NHL and B-ALL) (16). The PBCAR0191 CAR T manufacturing included a) single-step knock-in of the CD19 CAR into the *TRAC* locus via ARCUS gene editing; and b) more intense lymphodepletion (4 days of fludarabine 30 mg/m^2/day and 3 days of cyclophosphamide 1000 mg/m^2/day) to mitigate HvG. The ORR and CR rates in NHL cohort (N=16) were 85% (n=11/13) and 62% (n=8/13), respectively. Only 1 grade 3 ICANS (6%) and no severe CRS occurred. Five patients developed grade≥3 infections and 2 patients had severe neutropenia. The B-ALL cohort included 15 patients (31). The CR or CR with incomplete marrow recovery (CR/CRi) rate was 60% (9/15) with 4 patients bridged to allogeneic hematopoietic stem cell transplantation (alloHCT). Severe CRS and ICANS rates were similar to B-NHL patients. Six patients (%) developed severe infections. No GVHD was reported in any cohort (16, 31). While final trial results are still pending for both B-NHL and B-ALL cohorts, the available preliminary data appear promising with manageable safety and promising efficacy.

The CD19-directed UCART19 alloCAR T was designed by TALEN gene editing to disrupt *TRAC* and *CD52* (32). The UCART19 was evaluated in both pediatric (PALL trial) and adult (CALM trial) patients with B-ALL (32, 33). The combined data of PALL and CALM trials, including 7 children and 14 adult patients, demonstrated CR/CRi rate of 67% (14/21), with 4.1 months of median duration of response. Ten out 14 responders (71%) received subsequent alloHCT. CRS was reported in 91% (19/21) of patients, with only 3 (14%) having grade 3–4 CRS. Neurotoxicity was reported in 8 patients (38%). Six patients (32%) experienced grade 4 cytopenias. Two patients experienced treatment related death due to sepsis and CRS in 1 patient and pulmonary hemorrhage in the setting of prolonged cytopenia (33). The phase 1 CALM trial enrolled 25 patients (age range, 18–64 years). Eighteen patients (72%) received prior alloHCT, and 13 (52%) received cytoreductive therapy before lymphodepletion. Alemtuzumab was administered prior to UCART19, except for 3 patients due to concerns for increased risk of viral infections (32). Two patients developed grade 1 acute GVHD of the skin. CRS was reported in 20 patients (80%), of whom 6 had grade≥3 (24%) CRS. Seven patients (28%) had ICANS (only 1 grade 4) (32). Severe prolonged (day≥42) cytopenias occurred in 8 patients (32%). Moreover, severe (grade≥3) infections occurred in 7 patients including 2 fatal events among the alloHCT recipients. At a median follow-up of 12.8 months, all patients achieved CR/CRi, with 12 patients achieving MRD-negative remission. Of these patients, 9 (75%) received subsequent alloHCT at a median time of 1.7 months after UCART19. The median progression free survival (PFS) and overall survival (OS) were 2.1 and 13.4 months, respectively. Five patients received the second infusion of UCART19 for progressive disease or suboptimal response. All UCART19 batches were found to carry t (1, 14), which was expected following *TRAC* and *CD52* gene editing, however it was not associated with any lymphoproliferation. An allogeneic donor-derived product has been evaluated in 13 children with B-cell precursor ALL (34). The manufacturing of the products involved caspase-9 retrovirus or prodigy-lentivirus. All patients achieved CR MRD-negative in bone marrow at day 14 following alloCAR T infusion. Five of 6 patients with extramedullary involvement achieved CR at day 28Three patients underwent alloHCT. After 11 months of follow up, 8 patients maintained CR. Thus, the results of this trial appeared promising in the absence of increased toxicity or GVHD (34).

The anti-CD19-CD28-CAR, transduced into allogeneic Epstein-Barr Virus (EBV)-specific cytotoxic lymphocytes (19–28 CAR EBV-CTLs), had variable transduction efficiency with a median rate of 29% (range, 7–41%). A total of 16 patients with B-cell malignancies were treated. No CRS or ICANS were reported and 3 patients developed GVHD. Efficacy data were not reported to our knowledge. The OS was 81% at 12 months and 74% at 24 months (17).

The TCR-positive double-negative T-cells (DNT) targeting CD19 have an advantage of exploiting both natural and adaptive immunity including stem-cell-like memory T-cells (18). This DNT product (RJMty19) was evaluated in a phase 1 trial among patients with B-NHL and it was found to be overall safe with no reported grade≥3 toxicity. The ORR was 40% among the first 5 treated patients (18). Another phase 1 trial evaluated iPSC-derived CAR Ts (FT819) with a novel signaling domain (1XX) (19). It was hypothesized that this domain does not lead to CAR T exhaustion. The *TRAC* locus was edited via TCR knock-out. The precise technology used for gene editing was not reported (19), however FT819 was found to be safe based on the data from 12 patients with DLBCL (19).

Preclinical studies evaluating the use of CRISPR-edited CD19 CAR Ts (nU-CAR-T19) for B-cell malignancies showed promising efficacy *in vitro* and in animal models (9).

Allogeneic CAR Ts targeting CD20 antigen

B-cell surface antigen CD20 was the first monoclonal antibody target leading to profound B-lymphocyte depletion. Surface expression of CD20 is present from early stages of B-cell differentiation (precursor B-cell) until the differentiated plasma cells. Hence, anti-CD20 monoclonal antibodies, such as prototypic rituximab, have been an integral part of B-NHL therapy for the past couple of decades (35). Cellular targeting of CD20 with ADI-001, a γδ alloCAR T has the advantage of not

requiring any gene editing since γδ T-cells do not depend on MHC for their potent cytotoxicity (20). The safety and efficacy of ADI-001 were evaluated in a phase 1 trial of patients with R/R B-NHL. Eleven patients were enrolled, and 9 were evaluable. Two patients developed low grade CRS; 1 patient had grade 1 ICANS; and none had GVHD. The ORR and CR rates were 78% (7/9) (20). Studies evaluating the persistence of ADI-001 showed a median of 16,553 copies/μg at around 28 days from infusion (36). An ongoing trial is currently evaluating donor-derived CD20-directed alloCAR T therapy (LUCAR-20S) in R/R B-NHL (NCT04176913).

Allogeneic CAR Ts targeting CD22 antigen

CD22 is another surface antigen expressed by all B-cells maturing from precursor B-cells until mature B-cells (35) and lost at the plasma cells stage. UCART22 is an alloCAR T directed against CD22, with *TRAC* and *CD52* disruption using TALEN gene editing (37). It was evaluated with or without the use of alemtuzumab in the BALL-01 phase 1 trial which enrolled 19 patients with R/R B-ALL (38) but only 8 were treated. Three patients developed CRS (all low grade), whereas no ICANS or GVHD were observed. Three patients achieved CRi at day 28, and 1 patient achieved a morphologic leukemia-free state (38).

Dual-directed allogeneic CAR Ts

The advantages of dual targeting alloCAR Ts include overcoming single target antigen loss/downregulation, increasing tumor specificity and potentially limiting off-tumor effects via an inhibitory CAR (iCAR) design thereby leading to less toxicity (35). iCAR is a smart gated CAR T cell design that led to dual CAR inhibition via the "OR", "NOT", and "AND" logic gate.

TruUCAR GC502 is a CD19/CD7-directed alloCAR T with disrupted *TRAC* and *CD7* loci (to avoid fratricide) which was evaluated in R/R B-ALL (N=4) (39). All patients developed CRS (2 with grade 3 and 2 with grade 2). No ICANS or GVHD were reported. Three patients achieved CR/CRi, whereas the remaining patient achieved PR and subsequently underwent alloHCT on day 39 after alloCAR T (40). Another dual CD19/CD3 alloCAR T (ThisCART19A) was designed via non-gene-editing platform, based on intracellular retention of TCRαβ/CD3 complex. ThisCART19A was evaluated in 8 patients (7 evaluable) with B-ALL (21). Etoposide was added to standard lymphodepletion with fludarabine and cyclophosphamide. CR/CRi MRD negative rate was 100% with 4 patients remaining MRD negative state after the median follow up of 146 days. Severe CRS and ICANS were observed in 25% (2/8) and 37.5% (3/8) of patients, respectively. Another CD19/CD20 allogeneic bispecific CAR EBV CTLs were evaluated in preclinical studies demonstrating safety profile and potent antitumor activity (41). The CD20/CD22 alloCAR Ts with *TRAC* and *CD52* disrupted loci using TALEN technology were evaluated in a preclinical study showing robust activity *in vitro* and in animal models (42).

All major ongoing clinical trials evaluating healthy donor "off-the-shelf" alloCAR Ts in B-NHL and B-ALL are summarized in Table 2.

Limitations of alloCAR Ts and future perspectives

Despite multiple inherent advantages, promising efficacy, and manageable toxicity alloCAR Ts have certain limitations. Due to their allogeneic nature and expression of alpha beta TCR, the risk of GVHD presents a perpetual challenge together with the risk of HvG reaction jeopardizing persistence of alloCAR Ts and thereby their antitumor activity.

In the aforementioned clinical trials, the incidence of GVHD was low and largely limited to grades 1–2 as reported with UCART19 (32) and 19–28CAR EBV CTL (17), while most of other clinical trials did not report any GVHD. This highlights the effectiveness of technologies aiming at mitigating the risk of GVHD during manufacturing of allogeneic "off-the-shelf" CAR Ts. In contrast, the existing strategies to minimize HvG effect and thereby to enhance durability and efficacy of alloCAR Ts have been less successful. The available data on alloCAR T persistence are limited, given that most studies are still in their early stages. In the CALM trial, for example, UCART19 persisted in the blood for a median of 28 days (32). Only 5 patients had UCART19 persisting in the blood beyond day 42. Previous studies in autologous CAR Ts have shown a correlation between CAR T persistence and long-term response (43).

While in theory, GVHD and HvG can be mitigated or reduced through gene editing of *TRAC*, *CD52*, and *HLA-E* loci, together with the use of anti-CD52 monoclonal antibodies to deplete patient T-cells, this approach is contingent upon successful gene editing techniques. The use of anti-CD52 antibodies in various studies required *CD52* knock-out in alloCAR Ts. Although technologies used in gene editing have substantially improved, there is still a need to enhance the manufacturing platform to: a) improve the sensitivity and specificity of gene editing techniques in order to decrease off-target DNA editing; and b) mitigate the HvG effect through exploring novel allogeneic T-cell platforms with improved and more durable efficacy. Several ongoing studies are exploring innovative gene editing techniques focusing on increased specificity to target gene(s). As such, CRISPR/Cas9 technology has revolutionized the treatment of hematologic disease, while CRISPR/Cas12a system has further improved gene editing specificity without off-target effects. The CB-011, an alloCAR T, produced by CRISPR/Cas12a editing, is currently tested in patients with R/R multiple myeloma in the CaMMouflage trial (44). An innovative technology has been reported recently using transformer base editor (tBE), a multiplex editing that can target multiple genes simultaneously without reported off-target gene modification (45). Prime-Assisted Site-Specific Integrase Gene Editing (PASSIGE) is another novel technology demonstrating precision in multiplex editing without off-target effect (46). Upcoming trials will help to determine if this highly precise gene editing would translate into improved clinical outcomes. To mitigate CAR T exhaustion, a novel platform has been investigated in autologous CAR Ts for lymphoma, involving γδ TCR coupled with a costimulatory agent as an alternative to CAR (47). While this approach has not yet been explored in allogeneic T-cell therapies, it could potentially reduce GVHD risk and increase effectiveness of alloCAR Ts.

TABLE 2 Ongoing clinical trials utilizing allogeneic CAR T-cells in B-cell malignancies.

Study/ NCT	Trial phase	Disease	Target	Product manufacturing	Technology	Status of the study
NCT05164042	Phase 1/2	B-ALL	CD19	NR	NR	UNK
NCT06014073	Phase 1/2	B-NHL	CD19	ATHENA CAR-T	CRISPR-Cas9 TRAC and Power3 genes knock out	Recruiting
NCT06256484	Phase 1	B-NHL	CD19	ATA3219	EBV T cells 1XX co-stimulatory domain	Recruiting
NCT04030195	Phase 1/2a	B-NHL CLL/SLL	CD20	PBCAR20A	ARCUS	Completed (not published)
NCT05106946	Phase 1	B-NHL	CD22	ThisCART22	Non-gene edited	Recruiting
NCT05691153	Phase 1	B-NHL	CD19/CD3	ThisCART19A CD19 TCRαβ/CD3	Non-gene edited	Recruiting
NCT06014762	Phase 1	B-cell malignancies	CD19/ CD20	P-CD19CD20-ALLO1	Poseida Therapeutics	Recruiting
NCT05607420	Phase 1/2	B-NHL	CD20/ CD22	UCART20x22	TALEN	Recruiting

B-ALL, B-cell Acute Lymphoblastic Leukemia; NR, not reported; UNK, unknown; B-NHL, B-cell non-Hodgkin lymphoma; CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; EBV, Epstein Barr virus.

Secondary hematologic malignancies such as T-cell lymphoproliferations, have been reported among 22 autologous CAR T recipients by the end of 2023, leading to addition of the boxed warning by the FDA to CAR T-cell therapies. In theory, similar risk may exist with alloCAR Ts produced by gene editing techniques and/or the use of viral vector platforms. In fact, secondary hematologic malignancies have been previously reported following CRISPR edited gene therapy for sickle cell disease due to vector mediated oncogenesis. It remains unclear whether HvG effect observed with alloCAR Ts would also limit rare instances of secondary lymphoproliferation due to unintentional transduction of healthy-donor T-cells. On another hand, the use of novel non-gene editing alloCAR T platforms (e.g. ThisCART) may further reduce the risk of any secondary oncogenesis.

In conclusion, "off-the-shelf" alloCAR Ts have emerged as a promising therapeutic avenue for patients with B-cell malignancies. Early reports have demonstrated robust efficacy and limited toxicity of alloCAR Ts, however their clinical superiority in comparison to autologous CAR Ts remains uncertain. Ongoing studies harnessing novel technologies in manufacturing next generation alloCAR Ts hold promise in limiting further toxicities while improving persistence and efficacy of alloCAR Ts.

Author contributions

RM: Conceptualization, Writing – original draft, Writing – review & editing. AL: Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors express their deep appreciation to the Leon Levine Foundation and the Kerry and Simone Vickar Family Foundation for their support".

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Geethakumari PR, Ramasamy DP, Dholaria B, Berdeja J, Kansagra A. Balancing quality, cost, and access during delivery of newer cellular and immunotherapy treatments. *Curr Hematol Malig Rep*. (2021) 16:345–56. doi: 10.1007/s11899-021-00635-3

2. Kite Receives U.S. FDA Approval of Manufacturing Process Change Resulting in Reduced Median Turnaround Time for Yescarta® CAR T-cell Therapy 2024. Available online at: https://www.gilead.com/news-and-press/press-room/press-releases/2024/1/kite-receives-us-fda-approval-of-manufacturing-process-change-resulting-in-reduced-median-turnaround-time-for-yescarta-car-tcell-therapy.

3. Mikhael J, Fowler J, Shah N. Chimeric antigen receptor T-cell therapies: barriers and solutions to access. *JCO Oncol Pract*. (2022) 18:800–7. doi: 10.1200/OP.22.00315

4. Ruella M, Xu J, Barrett DM, Fraietta JA, Reich TJ, Ambrose DE, et al. Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell. *Nat Med*. (2018) 24:1499–503. doi: 10.1038/s41591-018-0201-9

5. Baguet C, Larghero J, Mebarki M. Early predictive factors of failure in autologous CAR T-cell manufacturing and/or efficacy in hematologic Malignancies. *Blood Adv*. (2024) 8:337–42. doi: 10.1182/bloodadvances.2023011992

6. Rotte A, Frigault MJ, Ansari A, Gliner B, Heery C, Shah B. Dose-response correlation for CAR-T cells: a systematic review of clinical studies. *J Immunother Cancer*. (2022) 10. doi: 10.1136/jitc-2022-005678

7. Aparicio C, Acebal C, Gonzalez-Vallinas M. Current approaches to develop "off-the-shelf" chimeric antigen receptor (CAR)-T cells for cancer treatment: a systematic review. *Exp Hematol Oncol*. (2023) 12:73. doi: 10.1186/s40164-023-00435-w

8. Depil S, Duchateau P, Grupp SA, Mufti G, Poirot L. 'Off-the-shelf' allogeneic CAR T cells: development and challenges. *Nat Rev Drug Discovery*. (2020) 19:185–99. doi: 10.1038/s41573-019-0051-2

9. Chen X, Tan B, Xing H, Zhao X, Ping Y, Zhang Z, et al. Allogeneic CAR-T cells with of HLA-A/B and TRAC disruption exhibit promising antitumor capacity against B cell Malignancies. *Cancer Immunol Immunother*. (2024) 73:13. doi: 10.1007/s00262-023-03586-1

10. Caldwell KJ, Gottschalk S, Talleur AC. Allogeneic CAR cell therapy-more than a pipe dream. *Front Immunol*. (2020) 11:618427. doi: 10.3389/fimmu.2020.618427

11. Torikai H, Reik A, Soldner F, Warren EH, Yuen C, Zhou Y, et al. Toward eliminating HLA class I expression to generate universal cells from allogeneic donors. *Blood*. (2013) 122:1341–9. doi: 10.1182/blood-2013-03-478255

12. Torikai H, Reik A, Liu PQ, Zhou Y, Zhang L, Maiti S, et al. A foundation for universal T-cell based immunotherapy: T cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR. *Blood*. (2012) 119:5697–705. doi: 10.1182/blood-2012-01-405365

13. Poirot L, Philip B, Schiffer-Mannioui C, Le Clerre D, Chion-Sotinel I, Derniame S, et al. Multiplex genome-edited T-cell manufacturing platform for "Off-the-shelf" Adoptive T-cell immunotherapies. *Cancer Res*. (2015) 75:3853–64. doi: 10.1158/0008-5472.CAN-14-3321

14. Liu X, Zhang Y, Cheng C, Cheng AW, Zhang X, Li N, et al. CRISPR-Cas9-mediated multiplex gene editing in CAR-T cells. *Cell Res*. (2017) 27:154–7. doi: 10.1038/cr.2016.142

15. Locke FL, Munoz JL, Tees MT, Lekakis LJ, Eradat HA, de Vos S, et al. ALLO-647 for lymphodepletion in the allogeneic CAR T setting: safety experience with ALLO-501/501A in patients (Pts) with relapsed/refractory (r/r) large B-cell and follicular lymphomas. *Blood*. (2023) 142:2095–. doi: 10.1182/blood-2023-189196

16. Shah BD, Jacobson C, Solomon SR, Jain N, Johnson MC, Vainorius M, et al. Allogeneic CAR-T PBCAR0191 with intensified lymphodepletion is highly active in patients with relapsed/refractory B-cell Malignancies. *Blood*. (2021) 138:302–. doi: 10.1182/blood-2021-150609

17. Shahid S, Flynn GC, Mauguen A, Bieler J, Prockop SE, Scaradavou A, et al. Long term follow-up after treatment with allogeneic off-the-shelf CAR T cell therapy for relapsed or refractory B-cell Malignancies. *Blood*. (2023) 142:3476–. doi: 10.1182/blood-2023-180753

18. Xiao X, Lei W, Jiang H, Qiu X, Li X, Chen P, et al. A phase 1 study of RJMty19: anti-CD19 humanized CAR-engineered allogeneic double negative T cells in adults with B-cell non-hodgkin's lymphoma. *Blood*. (2023) 142:2094–. doi: 10.1182/blood-2023-189149

19. Mehta A, Farooq U, Chen A, McGuirk JP, Ly T, Wong L, et al. Interim phase I clinical data of FT819-101, a study of the first-ever, off-the-shelf, iPSC-derived TCR-less CD19 CAR T-cell therapy for patients with relapsed/refractory B-cell Malignancies. *Blood*. (2022) 140:4577–8. doi: 10.1182/blood-2022-167194

20. Neelapu SS, Stevens DA, Hamadani M, Frank MJ, Holmes H, Jacobovits A, et al. A phase 1 study of ADI-001: anti-CD20 CAR-engineered allogeneic gamma delta1 (γδ) T cells in adults with B-cell Malignancies. *Blood*. (2022) 140:4617–9. doi: 10.1182/blood-2022-157400

21. Hu Y, Wei G, Fu S, Xiao P, Feng J, Zhang M, et al. Intracellular retention of tcrαβ/CD3 to generate novel allogeneic CAR-T cells (ThisCART19A) with enhanced antitumor potency for treating B-ALL. *Blood*. (2023) 142:2111–. doi: 10.1182/blood-2023-189052

22. Neelapu SS, Nath R, Munoz J, Tees M, Miklos DB, Frank MJ, et al. ALPHA study: ALLO-501 produced deep and durable responses in patients with relapsed/refractory non-hodgkin's lymphoma comparable to autologous CAR T. *Blood*. (2021) 138:3878–. doi: 10.1182/blood-2021-146038

23. Lekakis LJ, Locke FL, Tees M, Neelapu SS, Malik SA, Hamadani M, et al. ALPHA2 study: ALLO-501A allogeneic CAR T in LBCL, updated results continue to show encouraging safety and efficacy with consolidation dosing. *Blood*. (2021) 138:649–. doi: 10.1182/blood-2021-146045

24. Lekakis LJ LF, Tees M, Neelapu SS, Malik SA, Hamadani M, et al. ALPHA2 Study: ALLO-501A Allogeneic CAR T in LBCL, Updated Results Continue to Show Encouraging Safety and Efficacy with Consolidation Dosing: Allogene.com. (2021). Available online at: https://allogene.com/wp-content/uploads/2022/01/ALPHA2-Study.pdf.

25. Locke FL, Lekakis LJ, Eradat H, Munoz J, Tees MT, de Vos S, et al. Phase 1 results with anti-CD19 allogeneic CAR T ALLO-501/501A in relapsed/refractory large B-cell lymphoma (r/r LBCL). *J Clin Oncol*. (2023) 41:2517–. doi: 10.1200/JCO.2023.41.16_suppl.2517

26. *Allogene Therapeutics and Foresight Diagnostics Announce Partnership to Develop MRD-based* In-Vitro *Diagnostic for Use in ALPHA3, the First Pivotal Trial for Frontline Consolidation in Large B-Cell Lymphoma*. Allogene Therapeutic: press release (2024).

27. McGuirk JP, Tam CS, Kröger N, Riedell PA, Murthy HS, Ho PJ, et al. CTX110 allogeneic CRISPR-cas9-engineered CAR T cells in patients (Pts) with relapsed or refractory (R/R) large B-cell lymphoma (LBCL): results from the phase 1 dose escalation carbon study. *Blood*. (2022) 140:10303–6. doi: 10.1182/blood-2022-166432

28. McGuirk J, Bachier CR, Bishop MR, Ho PJ, Murthy HS, Dickinson MJ, et al. A phase 1 dose escalation and cohort expansion study of the safety and efficacy of allogeneic CRISPR-Cas9–engineered T cells (CTX110) in patients (Pts) with relapsed or refractory (R/R) B-cell Malignancies (CARBON). *J Clin Oncol*. (2021) 39:TPS7570–TPS. doi: 10.1200/JCO.2021.39.15_suppl.TPS7570

29. Nastoupil LJ, O'Brien S, Holmes HE, Dsouza L, Hart D, Matsuda E, et al. P1455: FIRST-IN-HUMAN TRIAL OF CB-010, A CRISPR-EDITED ALLOGENEIC ANTI-CD19 CAR -T CELL THERAPY WITH A PD-1 KNOCK OUT, IN PATIENTS WITH RELAPSED OR REFRACTORY B CELL NON-HODGKIN LYMPHOMA (ANTLER STUDY). *Hemasphere*. (2022) 6. doi: 10.1097/01.HS9.0000848676.15840.df

30. Caribou Biosciences Reports Positive Clinical Data from Dose Escalation of CB-010 ANTLER Phase 1 Trial in r/r B-NHL (2023). Available online at: https://investor.cariboubio.com/news-releases/news-release-details/caribou-biosciences-reports-positive-clinical-data-dose.

31. Jain N, Kantarjian H, Solomon SR, He F, Sauter CS, Heery CR, et al. Preliminary safety and efficacy of PBCAR0191, an allogeneic 'Off-the-shelf' CD19-directed CAR-T for patients with relapsed/refractory (R/R) CD19+ B-ALL. *Blood*. (2021) 138:650–. doi: 10.1182/blood-2021-153166

32. Benjamin R, Jain N, Maus MV, Boissel N, Graham C, Jozwik A, et al. UCART19, a first-in-class allogeneic anti-CD19 chimeric antigen receptor T-cell therapy for adults with relapsed or refractory B-cell acute lymphoblastic leukaemia (CALM): a phase 1, dose-escalation trial. *Lancet Haematol*. (2022) 9:e833–e43. doi: 10.1016/S2352-3026(22)00245-9

33. Benjamin R, Graham C, Yallop D, Jozwik A, Mirci-Danicar OC, Lucchini G, et al. Genome-edited, donor-derived allogeneic anti-CD19 chimeric antigen receptor T cells in paediatric and adult B-cell acute lymphoblastic leukaemia: results of two phase 1 studies. *Lancet*. (2020) 396:1885–94. doi: 10.1016/S0140-6736(20)32334-5

34. Del Bufalo F, Becilli M, Rosignoli C, De Angelis B, Algeri M, Hanssens L, et al. Allogeneic, donor-derived, second-generation, CD19-directed CAR-T cells for the treatment of pediatric relapsed/refractory BCP-ALL. *Blood*. (2023) 142:146–57. doi: 10.1182/blood.2023020023

35. Vanegas YM, Mohty R, Gadd ME, Luo Y, Aljurf M, Qin H, et al. CAR-T cell therapies for B-cell lymphoid Malignancies: identifying targets beyond CD19. *Hematol Oncol Stem Cell Ther*. (2022) 15:81–93. doi: 10.56875/2589-0646.1026

36. Moreno MA, Kennedy-Wilde J, Wrong AD, Jahchan N, Galimi F, Ye Y, et al. Expansion, persistence and pharmacodynamic profile of ADI-001, a first-in-class allogeneic CD20-targeted CAR gamma delta T cell therapy, in patients with relapsed/refractory aggressive B-cell non-hodgkin's lymphoma. *Blood*. (2023) 142:3478–. doi: 10.1182/blood-2023-181919

37. Jain N, Roboz GJ, Konopleva M, Liu H, Jabbour E, Poirot C, et al. Preliminary Results of Balli-01: A Phase I Study of UCART22 (allogeneic engineered T-cells expressing anti-CD22 Chimeric Antigen Receptor) in Adult Patients with Relapsed or Refractory (R/R) CD22+ B-Cell Acute Lymphoblastic Leukemia (B-ALL). *Blood*. (2020) 136:7–8. doi: 10.1182/blood-2020-138594

38. Boissel N, Chevallier P, Curran K, Schiller G, Liu H, Larson R, et al. P1408: UPDATED RESULTS OF THE PHASE I BALLI-01 TRIAL OF UCART22, AN ANTI-CD22 ALLOGENEIC CAR-T CELL PRODUCT, IN PATIENTS WITH RELAPSED OR REFRACTORY (R/R) CD22+ B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL). *HemaSphere*. (2023) 7. doi: 10.1097/01.HS9.0000972520.32337.3f

39. Ge J, Yang C, Sun J, Chen J, Qiu S, Yin W, et al. Preclinical results of an allogeneic, universal CD19/CD7-targeting CAR-T cell therapy (GC502) for B cell Malignancies. *Blood*. (2021) 138:1722. doi: 10.1182/blood-2021-148500

40. Li S, Yuan Z, Liu L, Li Y, Martina S, Liu J, et al. P370: EARLY RESULTS OF A SAFETY AND EFFICACY STUDY OF ALLOGENEIC TRUUCAR™ GC502 IN

PATIENTS WITH RELAPSED/REFRACTORY B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (R/R B-ALL). *HemaSphere*. (2022) 6. doi: 10.1097/01.HS9.0000844368.41871.e3

41. Cha S, Charbonneau M, Brito A, Habibi A, Pham C, Nguyen C. ATA3431: allogeneic CD19/CD20 bispecific CAR EBV T cells for the treatment of B-cell Malignancies. *Transplant Cell Ther*. (2024) 30:S11. doi: 10.1016/j.jtct.2023.12.033

42. Aranda-Orgilles B, Chion-Sotinel I, Skinner J, Grudman S, Mumford B, Dixon C, et al. Preclinical evidence of an allogeneic dual CD20xCD22 CAR to target a broad spectrum of patients with B-cell Malignancies. *Cancer Immunol Res*. (2023) 11:946–61. doi: 10.1158/2326-6066.CIR-22-0910

43. Hay KA, Gauthier J, Hirayama AV, Voutsinas JM, Wu Q, Li D, et al. Factors associated with durable EFS in adult B-cell ALL patients achieving MRD-negative CR after CD19 CAR T-cell therapy. *Blood*. (2019) 133:1652–63. doi: 10.1182/blood-2018-11-883710

44. Donohoue P, Pacesa M, Lau E, Vidal B, Irby MJ, Nyer DB, et al. Highly specific cas9 and cas12a engineering of human T cells for generation of novel allogeneic cell therapies. *Transplant Cell Ther*. (2024) 30:S237. doi: 10.1016/j.jtct.2023.12.310

45. Li P, Su Q, Xu Y, He J, Wang L, Wang Y, et al. Multiplex genome editing of human T cells with innovative transformer base editor (tBE) for construction of next generation CAR-T therapies. *Blood*. (2023) 142:6825–. doi: 10.1182/blood-2023-178504

46. Pomeroy EJ, Anzalone AV, Podracky CJ, Bloch NB, Chang R, Dwivedi AA, et al. Multiplex prime editing and PASSIGE TM for non-viral generation of an allogeneic CAR-T cell product. *Blood*. (2023) 142:4803–. doi: 10.1182/blood-2023-181869

47. He P, Liu H, Zimdahl B, Wang J, Luo M, Chang Q, et al. A novel antibody-TCR (AbTCR) T-cell therapy is safe and effective against CD19-positive relapsed/refractory B-cell lymphoma. *J Cancer Res Clin Oncol*. (2023) 149:2757–69. doi: 10.1007/s00432-022-04132-9

frontiers | Frontiers in Oncology

TYPE Review
PUBLISHED 19 July 2024
DOI 10.3389/fonc.2024.1397613

OPEN ACCESS

EDITED BY
Liren Qian,
Fifth Medical Center of the PLA General Hospital, China

REVIEWED BY
Pinar Ataca Atilla,
Fred Hutchinson Cancer Research Center, United States
Srinivas Devarakonda,
The Ohio State University, United States

*CORRESPONDENCE
Nilanjan Ghosh
Nilanjan.ghosh@atriumhealth.org

†These authors have contributed equally to this work

RECEIVED 07 March 2024
ACCEPTED 01 July 2024
PUBLISHED 19 July 2024

CITATION
Medina-Olivares FJ, Gómez-De León A and Ghosh N (2024) Obstacles to global implementation of CAR T cell therapy in myeloma and lymphoma. *Front. Oncol.* 14:1397613. doi: 10.3389/fonc.2024.1397613

Obstacles to global implementation of CAR T cell therapy in myeloma and lymphoma

Fernando J. Medina-Olivares[1†], Andrés Gómez-De León[1†] and Nilanjan Ghosh[2*†]

[1]Facultad de Medicina y Hospital Universitario Dr. Jose Eleuterio Gonzalez, Universidad Autonoma de Nuevo Leon, Monterrey, Mexico, [2]Atrium Health Levine Cancer Institute, Wake Forest School of Medicine, Charlotte, NC, United States

Chimeric Antigen Receptor T-cell (CAR-T) therapies are transforming the treatment of B-cell lymphoproliferative disorders and multiple myeloma, yet global access challenges and barriers for their implementation persist. Global access disparities persist, particularly for persons living in low and middle-income countries and for underserved populations in high income countries. In this review we address patient-related factors including age, comorbidities, fitness, race and ethnicity, and geographic location for CAR-T access. Also, we review disease-related and health system barriers like disease biology, potential for short and long-term toxicity, insurance access, referrals, supply and manufacturing, regulation, costs and treatment center capacity. Lastly, alternatives for overcoming these barriers exemplified by research efforts worldwide are discussed, emphasizing the need for a multifaceted approach from all stakeholders to improve global accessibility and ensure equitable access and improved outcomes for patients worldwide.

KEYWORDS

barriers, CAR T, lymphoma, myeloma, access

Abbreviations: CAR-T, Chimeric antigen receptor T-cell therapies; ALL, B-cell acute lymphoblastic leukemia; BCMA, B-cell maturation antigen; MM, multiple myeloma; FDA, Food and Drug Administration; NHL, non-Hodgkin lymphoma; RRMM, relapsed or refractory multiple myeloma; B-ALL, B-cell precursor acute lymphoblastic leukemia; CIBMTR, Center for International Blood and Marrow Transplant Research; LA, Latin America; LBCL, large B-cell lymphoma; Axi-cel, axicabtagene ciloleucel; Tisa-cel, tisagenlecleucel; BT, bridging therapy; Cy, cyclophosphamide; hyperCVAD, cyclophosphamide, vincristine, doxorubicin, and dexamethasone; KCd, carfilzomib, cyclophosphamide, and dexamethasone; CNS, Central nervous system; EMD, extramedullary disease; CD19-CAR, CD19-targeted chimeric antigen receptor T cells; CRS, Cytokine release syndrome; ICANS, Immune Effector Cell-Associated Neurotoxicity Syndrome; CRP, C-reactive protein; SCA, single case agreements; AAV, Adeno-associated virus; CDSCO, Central Drug Standards Control Organisation

1 Introduction

Chimeric antigen receptor T-cell therapies (CAR-T) have revolutionized the management of persons with relapsed/refractory B-cell lymphoproliferative disorders (1) The first generation of CAR-T cells were not clinically effective due to low persistence (2) Subsequently, the emergence of second-generation CAR-T cells designed to target the antigen CD19 with CD28 or 4-1BB co-stimulatory domains coupled with CD3 ζ improved CAR T cell *in vivo* persistence and efficacy, leading to their establishment as a novel treatment modality for patients with B-cell non-Hodgkin Lymphomas (3) In 2013, reports of CAR T-cell therapies directed against the B-cell maturation antigen (BCMA) aimed for persons with multiple myeloma (MM) emerged. Since 2017, six CAR T products have been approved for various indications in relapsed or refractory B cell non-Hodgkin lymphoma or multiple myeloma (4).

In 2023 the Center for International Blood and Marrow Transplant Research (CIBMTR) reported data from 214 centers documenting 10,976 patients who have undergone various form of cellular therapies. Among this cohort, 6,646 patients underwent treatment for lymphoma, and 1,401 received treatment for ALL. Since their inception, the cell therapy field has undergone significant advances, but still many challenges in terms of delivery and applicability remain. Access to CAR-T cell therapy remains limited to some populations and in this review, we aim to document obstacles for their implementation and potential solutions to overcome them.

2 Patient-related factors

The scientific rationale for imposing specific chronological age restrictions in clinical trials has not been firmly established. However, it is noteworthy that 64% of trials have implemented upper age limits (5). Outside of clinical trial settings, the use of CAR-T cell therapy has been shown to be effective in older adults with NHL (6). Also, patients with myeloma may not be fit for autologous transplantation but may be considered eligible for CAR-T (7).Thus, rather than using chronological age cutoffs, a holistic assessment guided by a comprehensive geriatric assessment in older adults has been recommended to identify patients who may be at risk of complications and identify areas of opportunity for early intervention (8). On the other end of the spectrum, except for tisagenlecleucel, all approved CAR-T cells for treating persons with lymphoma and myeloma are approved for adults exclusively.

Globally speaking, country of residence perhaps remains the most important patient-centered predictor of access to CAR-T cell therapy. This treatment remains limited to only a few countries worldwide, mostly high-income countries in North America, Europe, Asia, and the Pacific, and in selected middle-income countries like China, Brazil, and India. Access runs in parallel to that of hematopoietic cell transplantation, as most procedures are performed in these world regions as well, given that it requires a similar infrastructure to CAR-T. Substantial expertise, resources, and a comprehensive network of specialists across various medical domains are necessary requisites. Most patients remain limited to what their health system has to offer with international travel only limited for the wealthy.

In China significant investment in biotechnological research and infrastructure has occurred, facilitating the rapid advancement and scaling of CAR T cell technologies. On December 11, 2017, the Chinese regulatory agency approved the first Investigational New Drug application for CAR T therapy from Nanjing Legend Biotechnology Co., Ltd. In recent years, additional technology start-ups, like Cellular Biomedicine Group, and Fosun Kite Biotechnology Co., Ltd., have emerged seeking to manufacture CAR T cells for cancer treatment. CARsgen Therapeutics Holdings Limited, specializing in innovative CAR T-cell therapies for hematologic and solid tumors, announced that the National Medical Products Administration (NMPA) of China has approved their New Drug Application (NDA) for zevorcabtagene autoleucel. This autologous CAR-T product targets BCMA and is approved for treating adult patients with relapsed or refractory multiple myeloma who have previously undergone at least three lines of therapy, including a proteasome inhibitor and an immunomodulatory agent.

A survey among groups of hematologists and transplant recipients in Latin America (LA) to assess potential CAR-T initiatives revealed active commercial studies in Brazil and Argentina. In Brazil and Mexico, projects aimed at the development of CAR-T therapy through partnerships with international academic institutions are ongoing (9,10). India's inaugural approved CAR-T, actalycabtagene autoleucel, represents a pioneering product developed in a low middle income country (LMIC) aimed at segments of society at an affordable cost (11). In countries where CAR T-cell therapies are commercially available, every facet poses obstacles to accessibility for vulnerable populations. The treatment necessitates administration at a specialized center, requiring patients to stay in proximity for at least one month. The timeframe for referral is limited, potentially exacerbating existing disparities in access and biases. Moreover, the therapy is costly, involving resource-intensive logistics, and insurance-related hurdles (12). A retrospective study in the United States conducted an evaluation of the accessibility of CAR T-cell therapy, considering both provider and patient locations and compared the travel distances of individuals undergoing CAR T-cell therapy (cohort A) from their residences to one of the 64 Foundation for the Accreditation of Cellular Therapy-accredited centers, with those of patients receiving alternative disease-based treatments (cohort B). The results indicated that patients residing in the Southern region of the United States covered significantly greater distances in travel compared to their counterparts in other regions, ranging from 17.2 to 46.7 miles versus 0.3 to 14.5 miles (13). Additionally, limited resources at referral centers further compound logistical challenges not addressed by patient assistance programs. A study conducted by Faruqi et al., which retrospectively assessed the impact of demographics and obesity on CAR T-cell therapy outcomes, found that factors such as race, ethnicity, and BMI did not have a significant influence on the effectiveness of CAR T-cell therapy or the occurrence of neurotoxicity. However, in the clinical trial environment, additional systemic barriers for racial and ethnic minority groups contribute to their underrepresentation, as clinical trial participants are mostly non-Hispanic white people, with less clinical trial openings

in regions of the United States with a higher percentage of Black residents (14). Similarly, the Pediatric World CAR Consortium has reported lack of access and fewer treatments in Black/African American patients (5.5% of the 200-patient cohort) with a greater number of prior treatment lines, more relapses, and higher rates of prior hematopoietic cell transplantation before receiving CAR T cell therapy (15).

Another patient-related barrier is immune fitness. A retrospective, multicenter, international study led by Lacoboni et al. assessed 370 patients with relapsed/refractory large B-cell lymphoma treated at 7 sites using commercially available CAR-T cell products showed that those receiving bendamustine prior to apheresis (74%) had a lower and delayed absolute peak expansion of CAR-T cells after infusion compared to the bendamustine-naïve control group. Bendamustine-containing regimens prior to CAR T-cell therapy may negatively impact T-cell numbers and composition at apheresis and subsequent CAR T-cell expansion. Patients with recent (<9 months) exposure to bendamustine and large B-cell lymphoma show worse outcomes after CAR T-cell therapy compared to those with earlier exposure to this chemotherapeutic agent (16).

To overcome immune exhaustion, the use of healthy donors for cell manufacturing is logical. Off-the-shelf readily available cell therapies that can be used without the need for patient-specific customization or harvesting of the patient's own cells is a vision for the future. In allogeneic CAR T cell recipients the risk of graft-versus-host disease is low, and the utilization of adoptive CAR T therapy using donor-derived cells has proven to be effective (17). The ability to genetically modify T cells to eliminate the endogenous T cell receptor, thereby enabling the use of T cells derived from healthy donors without the risk of graft-versus-host disease, has been studied in several platforms, including using T cell gene editing and umbilical cord-derived NK cells (18–20). Unlike autologous therapies that require individualized cell harvesting and manufacturing processes for each patient, off-the-shelf cell therapies can be mass-produced, potentially reducing costs and wait times. The allogeneic approach can lead to a faster production timeline, as the cells can be pre-manufactured and stored for use when needed.

3 Disease biology

Fast and uncontrolled growth of cancer cells can pose a significant challenge in the context of CAR T. Patients may undergo lymphocyte apheresis but experience an event of disease progression before the cells are manufactured and infused. A systematic analysis highlights a lack of consistent reporting regarding dropouts in published CD19 and BCMA CAR-T trials, specifically those linked to disease progression or manufacturing failure, which occur after enrollment but before the initiation of therapy (21). Additionally, the reasons why patients who do not receive CAR-T after enrolling are often insufficiently documented. In instances where such information is provided, a noticeable reduction in the number of patients is commonly observed from the initial enrollment stage to the actual administration of CAR-T therapy that affects the effectiveness of the treatment. For bridging therapy (BT), conventional treatments like chemotherapy or radiation therapy to stabilize the disease, is considered safe, and can achieve responses before cells are infused. A study focused on 375 adult patients with diffuse large B-cell lymphoma (LBCL) examined the modality and response of BT in relation to outcomes following the administration of axicabtagene ciloleucel (Axi-cel) or tisagenlecleucel (Tisa-cel). Most patients underwent BT using chemotherapy (57%) or radiotherapy (17%). The findings indicated that BT was well-tolerated by patients, with minimal morbidity or mortality observed with a 42% reduction in the risk of progression or death after CD19 CAR-T therapy (22).. Patients undergoing autologous chimeric antigen receptor T cell (CAR-T) therapy for multiple myeloma (MM) might necessitate BT prior to CAR-T infusion to acheive a certain level of disease control. Alkylators, like cyclophosphamide (Cy), are commonly incorporated into treatment protocols. The intensity of BT may depend on the disease kinetics and various regimens have been used (23).

Central nervous system (CNS) and other extramedullary sites of disease involvement can potentially pose challenges or limitations in the effectiveness of CAR T-cell therapy. These sites were thought to be difficult to reach limited by the blood-brain barrier or unique microenvironments, impacting cells' ability to access and eradicate cancer cells, thus the rationale for the exclusion of persons with CNS involvement in the JULIET and ZUMA-1 trials. Recent studies including a systematic review and experiences outside clinical trials have shown CAR-T to be effective in central nervous system lymphoma (24, 25). Similarly, case reports reporting positive experiences in myeloma have also been published (26).

Certainly, the overall prognosis for extramedullary multiple myeloma remains bleak, and conventional treatments have shown limited efficacy. A study from China highlighted that extramedullary disease (EMD) significantly impacted the prognosis of patients undergoing anti-BCMA CAR-T therapy for relapsed/refractory multiple myeloma (RRMM). Interestingly, patients with extra-medullary myeloma (EMM) exhibited lower rates of cytokine release syndrome (CRS) compared to those without EMM (27). Systematic analyses indicate promising initial response rates with CAR-T therapy; however, these responses tend to be transient (28).. Pan et al. conducted an analysis focusing on RRMM patients treated with CAR-T cell therapy in a clinical trial. Notably, half of the patients experiencing relapse post-CAR-T therapy had EMD. Strikingly, despite radiographically negative EMD following CAR-T treatment, most patients with initial EMD experienced subsequent relapse characterized by extramedullary disease (29).

Reduced target antigen expression is another way disease can evade CAR-T. CD19-targeted chimeric antigen receptor T cells (CD19-CAR) and blinatumomab have shown efficacy in inducing remission among relapsed or refractory B-cell acute lymphoblastic leukemia (ALL) patients. However, there's a notable association between these therapies and CD19 antigen modulation. Limited data exist concerning how prior exposure to blinatumomab might affect subsequent CD19-CAR outcomes. Blinatumomab use has

been linked to a reduction in CD19 expression. In cases where CD19 expression decreased or altered post-blinatumomab treatment, there was a heightened risk of relapse post-CD19-CAR therapy with CD19-negative disease. Several factors, such as inherent T-cell dysfunction, resistance to immunotherapy, or the adverse impact of extensive prior treatments, could contribute to the poorer outcomes observed in patients previously exposed to blinatumomab. This effect seems particularly pronounced within a patient population heavily treated with various therapies (30).

Resistance or relapse due to the absence of the target antigen (CD19 loss or downregulation) has been extensively researched. Multiple studies have indicated that instances of CD19-negative relapses occur in approximately 9% to 25% of cases of B-cell acute lymphoblastic leukemia treated with CAR T cell therapy (31). Relapses in some cases exhibit either a lack of antigen presence or lower antigen levels. In the KarMMa study, approximately 6% of patients who relapsed showed antigen loss upon immunohistochemistry assessment, while around 4% experienced serum antigen reduction measured by soluble BCMA (32). Although BCMA antigen loss occurs infrequently (4-33%) in patients treated with anti-BCMA CAR-T, one approach is to target BCMA using CARs with higher affinity or tighter binding, like biparatopic binding domains. However, this strategy may lead to more on-target toxicity, as seen in recent reports of Parkinsonian symptoms in at least six patients treated with anti-BCMA CAR T cells (33).. Various methods exist for engineering multi-specific T-cell products to counter antigen escape, such as utilizing single bicistronic vectors expressing two CARs, employing tandem vectors housing a single CAR with dual binder sequences, or co-transducing CAR T cells with separate CAR-encoding vectors (31) Multiple targets are under investigation in multiple myeloma, extending beyond BCMA to include CD19, CD38, GPRC5D, CD1, and SLAMF7 (34). Co-targeting studies of CD19/BCMA showcased a robust overall response rate of 95%, accompanied by complete response rates ranging from 16% to 57% (35) Similarly in lymphoma combinations of CD19 with CD22 are under study (36).

To overcome aggressive disease biology, several modifications to fine-tune CAR T cell design are underway. The ability to deactivate or eliminate T cells as needed, redirect universal CAR T cells using a soluble antigen recognition domain represent exciting and significant developments (37). Studies combining CAR T therapy with other treatments, such as checkpoint inhibitors, other monoclonal antibodies and small molecules are ongoing to enhance their effectiveness and durability (38).

4 Adverse events

Cytokine release syndrome (CRS) is a common and potentially severe side effect of CAR T therapy, characterized by the release of inflammatory cytokines, leading to fever, hypotension, and organ dysfunction. This phenomenon arises due to a hyperactive systemic immune reaction orchestrated by T cells, B cells, NK cells, and monocytes, resulting in the release of a substantial quantity of inflammatory mediators, including cytokines and chemokines (39). Managing CRS is crucial but challenging. Identifying pre-infusion risk factors linked to the occurrence and severity of subsequent CRS is crucial for pinpointing high-risk patients who could benefit from early intervention studies. Prophylaxis for CRS involves strategies aimed at preventing or mitigating the severity of CRS in individuals at risk, particularly those undergoing certain immunotherapies or treatments known to trigger CRS. Before starting therapies like CAR T-cell therapy, patients undergo risk assessment to identify factors that might predispose them to CRS. Using corticosteroids before or during treatment can proactively regulate the immune response, potentially lessening the severity of CRS. Tocilizumab, an IL-6 receptor antagonist, has been used to prevent or manage CRS by obstructing the IL-6 pathway, a key player in the cytokine release cascade. Rigorous patient monitoring during and post-therapy enables timely intervention upon detecting initial signs or symptoms of CRS. This comprehensive monitoring entails observing vital signs, conducting laboratory tests, and being attentive to patient-reported symptoms.

Neurological manifestations have been documented under the acronym ICANS (Immune Effector Cell-Associated Neurotoxicity Syndrome) and encompass a range of symptoms, including but not limited to headaches, cognitive disorientation, restlessness, seizures, tremors, language difficulties, comprehension challenges, aphasia, cranial nerve irregularities, and visual hallucinations (40).. Understanding and managing this toxicity remain areas of active research. Suggested factors that might predispose individuals to developing ICANS include pre-existing neurological conditions, previous occurrence of CRS, increased doses and peak expansion levels of CAR-T cells, elevated tumor burden during CAR-T infusion, reduced platelet counts at infusion, and elevated levels of inflammatory markers such as C-reactive protein (CRP) or ferritin, as well as certain cytokines like interleukin IL-1, IL-6, IL-10, and interferon-gamma. The current established approaches to treatment primarily involve corticosteroids and supportive care, with the specific role of anti-cytokine therapy yet to be precisely determined. Within the ZUMA-1 study, various safety cohorts explored alternative management or preventative strategies for CRS/ICANS and were compared against those in the pivotal cohorts. Earlier administration of corticosteroids or their prophylactic use appeared to reduce both the occurrence and severity of ICANS without visibly affecting treatment efficacy. A promising avenue of exploration involves inhibiting IL-1 signaling through the IL-1 receptor antagonist anakinra, based on preclinical research and emerging reports demonstrating its utilization in treating ICANS that doesn't respond to steroids. Access to CAR T is limited to centers which have the expertise in managing these adverse events. Establishing an effective prophylactic strategy has enabled safer administration, facilitating the delivery of these therapies with curative intent at higher doses to a broader patient population. This broader administration could encompass individuals for whom current risks are considered higher due to age or existing medical conditions, thus allowing the benefits to potentially outweigh the risks. Other relevant concerns are the long-term risks of genotoxicity.

5 Costs

Drug costs, whether within the United States or on a global scale, have witnessed significant escalation in the last two decades. Presently, the median initial price for cancer medications in the United States surpasses $155,000 USD annually (41).. The high price tag makes many drugs inaccessible to patients and poses a difficult challenge to healthcare systems worldwide. CAR T therapy is no exception and is extremely expensive. Gilead set the initial price of Yescarta (axicabtagene isoleucel) in the United States at $373,000, but it has since increased to $424,000. Remarkably, this cost is twice that of the therapy in Japan. Novartis established the price of tisagenlecleucel at $475,000, solely for the drug products, excluding expenses related to leukapheresis, lymphodepletion therapy, and the potential adverse effects associated with CAR-T immunotherapy. Llisocabtagene maraleucel, another CAR-T therapy approved for lymphoma, is priced at $410,300. Idecabtagene vicleucel, developed by Celgene (now part of Bristol Myers Squibb), is priced at $419,500. Brexucabtagene autoleucel, priced at $533,523 per patient. Lastly, Ciltacabtagene autoleucel, introduced by Janssen, is listed at $465,000. Reimbursement strategies involve navigating complex financial considerations associated with these innovative and expensive treatments. First, patients receiving CAR-T therapy often must rely on health insurance to cover a significant portion of the treatment costs. Insurance plans may vary in terms of coverage, and patients and healthcare providers need to navigate the specifics of each plan to determine the level of reimbursement. Healthcare providers, pharmaceutical companies, and payors engage in negotiations to determine the reimbursement rates for CAR-T therapies. These negotiations may involve discussions on pricing, patient access, and the overall value of the treatment. Some reimbursement strategies for CAR-T therapies include outcomes-based agreements. In these arrangements, payment may be contingent on the treatment's effectiveness, measured by predefined clinical outcomes (12, 42). This approach aligns reimbursement with treatment success and patient outcomes. Pharmaceutical companies often establish patient assistance programs to help individuals access CAR-T therapies in high income countries. These programs may offer partial financial assistance, copay support, or other forms of aid to alleviate the financial burden. In high income countries, government healthcare programs play a role in reimbursing for CAR-T therapies. A recent study from the University of Nebraska recently demonstrated that patients with private insurance often need single case agreements (SCA) and endure significant longer delays in time from intent to CAR-T to receiving the CAR-T product compared to patients with government insurance (43). Generating and using real-world evidence on the long-term effectiveness and economic impact of CAR-T therapies can support reimbursement discussions. This evidence may include data on real-world patient outcomes and the overall value of the treatment. Navigating reimbursement strategies for CAR-T therapies requires collaboration among stakeholders, including healthcare providers, pharmaceutical companies, payers, and regulatory bodies. Commercial CAR-T therapies have predominantly found distribution in key regions such as North America, Western Europe, and the Western Pacific. Conversely, in Latin America, particularly in countries like Brazil, the use of CAR-T therapies has been isolated, primarily due to the regulatory background and capacity of the health system to bear the logistical, clinical, and financial burden of such treatments.

6 Manufacturing chain and supply

The allocation of manufacturing slots for CAR-T therapy includes the strategic scheduling and allocation of production resources to meet the demand for these complex and personalized treatments. Manufacturers need to assess and plan their production capacity based on the anticipated demand for CAR-T therapies. This involves considering factors such as the number of patients expected to undergo treatment and the production capabilities of manufacturing facilities. The allocation of manufacturing slots is influenced by the enrollment of patients in clinical trials and later by the prescription patterns of CAR-T therapies. Manufacturers should work closely with institutions to schedule patient treatments, aligning them with available manufacturing slots and reducing the "brain-to-vein" time. The supply chain for CAR-T therapies involves various components, including the collection of patient cells, transportation, and manufacturing. Adherence to regulatory requirements is paramount in the manufacturing of CAR-T therapies. Manufacturers must ensure that their processes comply with regulatory standards, and manufacturing slots are allocated with consideration for regulatory timelines and approvals. In addition, rigorous quality control measures are essential in CAR-T manufacturing. Allocation of manufacturing slots considers the time required for thorough quality checks and assurance procedures to guarantee the safety and efficacy of the final product. Efficient use of manufacturing resources, including personnel, equipment, and facilities, is a critical factor in slot allocation. Close collaboration between manufacturers and the treatment team is crucial in the slot allocation process and may represent a significant barrier to access for individuals with lymphoma and multiple myeloma (44). Following the popularity of the therapy and limited treatment alternatives in multiply treated myeloma patients bottlenecks in the manufacturing space have been reported (45). The 'vein to vein' duration, referring to the time between apheresis and infusion of CAR T cells, stands another critical factor influencing patient outcomes (46). Prolonged vein-to-vein timelines create issues due to the rapid disease progression, impacting the eligibility of end-stage patients for CAR T treatment. Companies actively explore strategies to minimize vein-to-vein time, such as optimizing time-constrained quality control processes, aimed at gaining a competitive edge (47). An alternative strategy to reduce vein-to-vein time, lower production costs, and enhance scalability involves exploring decentralized CAR-T production models. This approach entails producing CAR-T products within academic hospitals using GMP-grade facilities or employing automated CAR-T manufacturing systems like the Miltenyi Prodigy or Lonza Cocoon incubator. In Switzerland, institutions have ventured into manufacturing CAR T cell therapies at a cost ranging from US$150,000 to US$200,000,

approximately half the price of most approved CAR-T cell therapies. However, in the United States, regardless of production site (academic or industry), CAR-T cell therapies are subjected to identical regulatory processes and pre-market approval as drugs, constraining their widespread implementation (12). Addressing this barrier involves exploring the development of allogeneic CAR-T cells. Allogeneic CAR T-cell therapy holds the potential to establish repositories of cryopreserved cells within individual hospitals, enabling quicker timelines for patients. Advances in genome editing tools, via CRISPR/Cas9, can allow us to overcome the two main limitations of allogeneic CAR T cells i.e., graft-vs.-host disease and host allorejection. Advancing next-generation allogeneic CAR T cells is a focal point of ongoing research to tackle these challenges (48). These manufacturing challenges have led to notably high fixed expenses, resulting in reluctance to extend CAR T therapy to a wider group of patients. Timely communication about patient schedules, treatment plans, and any unforeseen challenges can help streamline the manufacturing process and timely availability of the CAR T cell product.

The T2EVOLVE initiative, part of the Innovative Medicine Initiative (IMI) consortium, aims to accelerate the development of CAR and TCR engineered T cell therapies within the EU. By utilizing tools available in the current EU regulatory framework, T2EVOLVE strives to support an iterative and adaptive learning approach across various product versions that share similar design elements or are based on the same platform technology. As the understanding of the connections between product quality attributes, manufacturing processes, clinical efficacy, and safety improves through both development and post-licensure phases, new opportunities are arising to streamline regulatory submissions, enhance clinical studies, and extrapolate data across different product versions, thereby minimizing the need for repetitive studies.

7 Hospitals and pathways of care

The burden on referral centers, including limited capacity, a shortage of trained personnel, and a constrained number of hospital beds, poses challenges in the context of inpatient CAR-T therapy. In response to this, one potential solution is the implementation of outpatient CAR-T therapy. The scarcity of specialized centers, skilled personnel, and available hospital beds dedicated to CAR-T therapy can impede the timely and efficient administration of treatments. During the initial stages of development of CAR-T therapy, inpatient hospital stay due to monitoring and management of potential side effects was required in all cases. This strategy can strain hospital resources and limit the number of patients who can receive treatment. Implementing outpatient CAR-T therapy offers a potential solution to address these challenges minimizing the need for prolonged inpatient stays. This approach enhances treatment accessibility, reduces the burden on inpatient facilities, and can streamline the treatment process. Outpatient management of lymphoma and myeloma patients has become standard. Evidence from trials indicates that a notable portion of patients treated in outpatient settings eventually require hospitalization due to severe side effects, notably CRS and ICANS. Data on the safety of outpatient CAR T-cell therapy has been reported for specific products like tisa-cel and liso-cel. Initial reports demonstrate outpatient infusions using tisa-cel in approximately 24% to 27% of patients in studies for B-cell ALL and DLBCL, respectively (49). The University of Pennsylvania also reported relatively safe outpatient administration of tisa-cel, with a 31% admission rate post-infusion (50) Moreover, Bachier et al. indicated that patients receiving liso-cel could be safely monitored in outpatient settings, with 59% requiring hospitalization post-infusion within a median of 5.5 days, and only 8% hospitalized within three days of infusion (51). Hence, an evaluation of a patient's risk for these side effects, the onset time of CRS and ICANS, and the availability of trained providers and caregivers is essential to determine outpatient suitability. To mitigate risks, outpatient facilities must establish comprehensive safety protocols and possess adequate resources to manage CRS and ICANS, encompassing both physical resources (like hospital access and medications) and proper training of clinical staff.

In Latin America, implementing outpatient CAR T-cell therapy would require adaptation to regional healthcare systems. Establishing specialized centers with robust safety measures and well-trained staff could facilitate safe outpatient administration. This approach demands careful consideration of resource allocation and training programs tailored to the region's healthcare landscape to ensure effective and safe outpatient treatment. It is essential to carefully assess patient safety and the feasibility of outpatient management, considering factors such as the specific CAR-T construct used, the patient's health status, and the potential for adverse reactions. Close monitoring and coordination with healthcare providers are crucial elements of successful outpatient CAR-T therapy implementation.

Products manufactured in the point-of-care from non-commercial academic sources like University Hospitals offer flexibility in patient treatment and can come with lower costs for health systems. Notably, in Spain, local vectors which encode a CAR against CD19 and BCMA product have been developed (ARI-001 and ARI-002, respectively) with cell manufactured implemented with the Miltenyi Prodigy® manufacturing platform. The anti-CD19 product has obtained the approval of the Spanish regulatory agency and has been given a PRIME designation by the EMA (52). Spanish patients who are not candidates for commercial therapies may receive academic CAR-T in several institutions that have incorporated this alternative into their care pathway. This product and its point of care manufacturing platform is under study in several countries in Europe, Latin America, Asia, and is posed to be the first academic product to compete with products distributed by pharmaceutical companies. Recently, the results of the Spanish BCMA product have been published showing comparable safety and efficacy to commercially available products in RRMM (53). While not strictly an academic effort, the Cocoon system has been studied through the implementation of local CAR-T manufacturing closed systems in several centers in real time with monitoring by the sponsor in the Galapagos trials (54). Access to effective vectors becomes a limiting step for institutions aiming for POC manufacturing and academic CAR-T. Caring Cross, a

nonprofit organization, is actively driving progress in CAR T accessibility by spearheading a humanitarian licensing approach. This strategy aims to streamline commercialization processes while concurrently fostering sustainable access in low- and middle-income countries (LMICs) (55) This initiative is designed to encourage widespread standardization across the industry, laying a robust foundation for academic institutions and early-stage startups to manufacture lentiviral and Adeno-associated virus (AAV) vectors that align with potential clinical development. Even with an accessible vector, POC CAR-T manufacturing is a challenging procedure. It requires specialized manufacturing facilities, skilled personnel, and infrastructure for transportation and administration. Assessment of the apheresis service and cell processing laboratory is necessary to ensure compliance with release product specifications. This evaluation may entail acquiring extra equipment for product collection and final product storage, improving the capacity to transport and receive cellular products, and ensuring an adequate workforce for executing these procedures (56). Developing and maintaining these resources can be a barrier to its widespread adoption. Furthermore, the low numbers of hematopoietic cell transplants performed in LMICs reflect the lack of necessary infrastructure and resources that will likely impact the capacity for implementation of academic CAR-T therapy.

Even in places with established transplant programs, manufacturing CAR T-cells is a complex and time-consuming process. This delay can be critical for patients with aggressive diseases who need rapid intervention. Strategic investments in optimizing the CAR T-cell manufacturing process are crucial for reducing production time. Leveraging automation and advanced techniques can significantly expedite this phase. Establishing centralized CAR T-cell manufacturing facilities enhances efficiency and widens the reach to serve a larger patient population promptly, minimizing wait times and expediting treatment. Rigorous adherence to quality control standards during CAR T-cell production is imperative for ensuring patient safety. Delays may arise if the manufactured CAR T-cells fail to meet these stringent standards, necessitating re-manufacturing. One of the key objectives in the manufacturing process is to invest in a robust quality control system to minimize the likelihood of non-conforming products. This involves comprehensive testing of cells for purity, potency, and safety. The implementation of real-time monitoring systems during manufacturing detects issues early, allowing for swift corrective actions. Ensuring well-trained manufacturing staff adheres to standardized operating procedures is paramount. Proper documentation of the manufacturing process aids in identifying and rectifying issues promptly. Having backup manufacturing capacity available is essential to quickly address non-conforming products or unforeseen delays, ensuring a consistent supply of CAR T-cell therapies.

In LMICS several additional challenges have hindered the widespread adoption of CAR-T. Lack of legislation and regulation require complex frameworks and represent formidable barriers to overcome. The absence of clear guidelines for the approval, manufacturing, importation, exportation, and clinical use of CAR-T products adds further complexity. Without them, the conduct of clinical trials cannot occur, and authorization of commercial products remain a dream. The example of India could be followed, where actalycabtagene autoleucel, a CD19 product received approval from the Central Drug Standards Control Organisation (CDSCO) of India. This approval positions ImmunoACT to spearhead the development of indigenous CAR-T cell therapy within the country. NexCAR19 will be priced at $36,000-$48,000 USD, representing an approximately one-tenth of the cost compared to commercially approved therapies worldwide. This competitive pricing could potentially attract patients from neighboring counties who don't have access to CAR T, offering access to an exclusive therapy at a substantially lower

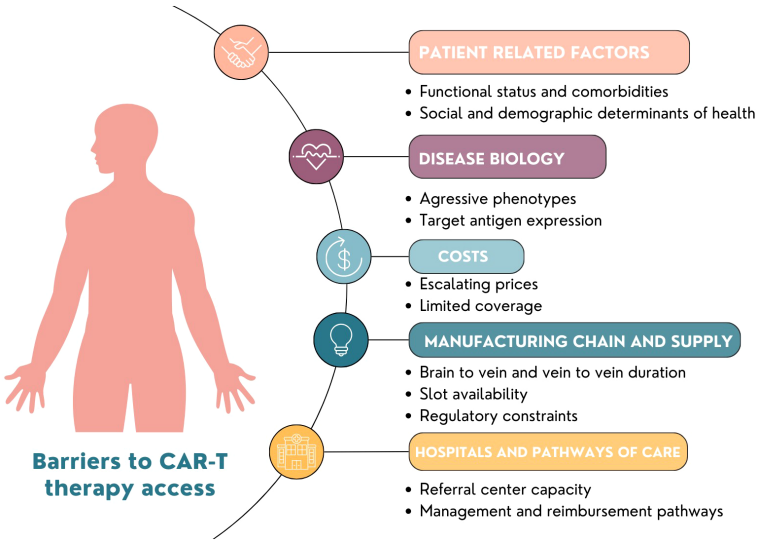


FIGURE 1
Barriers to Global Implementation of CAR-T.

cost. Nevertheless, it would remain financially inaccessible for the vast majority of individuals within India. Even after authorization, securing reimbursement for CAR-T treatments within healthcare systems becomes the next challenge, primarily due to limited financial support and reimbursement mechanisms for these advanced and expensive therapies in the context of limited resources, healthcare expenditures and alternative priorities of care.

Therefore, cost-effectiveness studies performed across different settings are needed.

Addressing these obstacles necessitates a comprehensive strategy involving collaboration among regulatory bodies, healthcare systems, and international organizations and institutions. The focus should be on developing transparent regulatory pathways, enhancing capacity for HSCT procedures, and establishing mechanisms for the approval and reimbursement of CAR-T therapies within resource-constrained financial environments. Additionally, initiatives for education and awareness are vital to ensure that healthcare professionals and policymakers in LMICs are well-informed about the potential benefits and challenges associated with CAR-T therapies.

8 Conclusion

While CAR T therapy has made significant strides in recent years, addressing these obstacles is essential to make this revolutionary treatment more accessible, safe, and effective for a broader range of cancer patients. Studies reporting current access to CAR T and using specific interventions to improve access are needed to overcome these challenges. In addition to reporting the outcomes of patients who receive CAR T, studies should report the number of patients who were eligible for CAR T but were not able to receive them and detail the barriers for receiving CAR-T. In summary, overcoming these obstacles in CAR T-cell therapy for aggressive diseases requires a combination of scientific innovation, process and supply chain op? imization, alternative pathways for care and quality control measures to ensure timely and effective treatment while prioritizing patient safety (Figure 1).

Author contributions

FM-O: Writing – original draft, Writing – review & editing. AG-D: Writing – original draft, Writing – review & editing. NG: Conceptualization, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors express their deep appreciation to the Leon Levine Foundation and the Kerry and Simone Vickar Family Foundation for their support.

Conflict of interest

NG has received consulting fees from BMS, Kite Pharma and Novartis. NG has also received research support from BMS.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Trias E, Juan M, Urbano-Ispizua A, Calvo G. The hospital exemption pathway for the approval of advanced therapy medicinal products: an underused opportunity? The case of the CAR-T ARI-0001. *Bone Marrow Transplant*. (2022) 57:156–9. doi: 10.1038/s41409-021-01463-y

2. Eshhar Z, Waks T, Grosst G, Schindler DG. Specific activation and targeting of cytotoxic lymphocytes through chimeric single chains consisting of antibody-binding domains and the y or C subunits of the immunoglobulin and T-cell receptors. *Proc Natl Acad Sci USA*. (1993) 90:720–24. doi: 10.1073/pnas.90.2.720

3. Wang J, Hu Y, Huang H. Current development of chimeric antigen receptor T-cell therapy. *Stem Cell Investig*. (2018) 5:44–4. doi: 10.21037/sci

4. Suzanne Rose First-ever CAR T-cell therapy approved in U.S. *Cancer Discovery*. (2017) 7:OF1–1. doi: 10.1158/2159-8290.CD-NB2017-126

5. Jaggers J, Klepin HD, Wildes TM, Olin RL, Artz AS, Wall SA, et al. Characterizing inclusion and exclusion criteria in clinical trials for CAR-T cellular therapy among adults with hematologic Malignancies. *Blood*. (2019) 134:5819–9. doi: 10.1182/blood-2019-131504

6. Chihara D, Liao L, Tkacz J, Franco A, Lewing B, Kilgore KM, et al. Real-world experience of CAR T-cell therapy in older patients with relapsed/refractory diffuse large B-cell lymphoma. *Blood*. (2023) 142:1047–55. doi: 10.1182/blood.2023020197

7. Vic S, Lemoine J, Armand P, Lemonnier F, Houot R. Transplant-ineligible but chimeric antigen receptor T-cells eligible: a real and relevant population. *Eur J Cancer*. (2022) 175:246–53. doi: 10.1016/j.ejca.2022.08.019

8. Hernandez Torres C, Hsu T. Comprehensive geriatric assessment in the older adult with cancer: A review. *Eur Urol Focus*. (2017) 3:330–9. doi: 10.1016/j.euf.2017.10.010

9. Cornetta K, Bonamino M, Mahlangu J, Mingozzi F, Rangarajan S, Rao J. Gene therapy access: Global challenges, opportunities, and views from Brazil, South Africa, and India. *Mol Ther*. (2022) 30:2122–9. doi: 10.1016/j.ymthe.2022.04.002

10. Guzmán F, Castañeda R. INDIA TRANSFERIRÁ A MÉXICO TECNOLOGÍA CONTRA EL CÁNCER. *Selección Gaceta Politécnica*. (2022) 14:10–2.

11. Jain H, Karulkar A, Kalra D, Ravikumar S, Ghandade N, Patil R, et al. High efficacy and excellent safety profile of actalycabtagene autoleucel, a humanized CD19

CAR-T product in r/r B-cell Malignancies: A phase II pivotal trial. *Blood*. (2023) 142:4838–8. doi: 10.1182/blood-2023-185507

12. Gajra A, Zalenski A, Sannareddy A, Jeune-Smith Y, Kapinos K, Kansagra A. Barriers to chimeric antigen receptor T-cell (CAR-T) therapies in clinical practice. *Pharmaceut Med*. (2022) 36:163–71. doi: 10.1007/s40290-022-00428-w

13. Avalere Health. *Advancements in Cell Therapies Require New Patient Support Solutions* (2021). Available online at: https://avalere.com/insights/advancements-in-cell-therapies-require-new-patient-support-solutions.

14. Ahmed N, Shahzad M, Shippey E, Bansal R, Mushtaq MU, Mahmoudjafari Z, et al. Socioeconomic and racial disparity in chimeric antigen receptor T cell therapy access. *Transplant Cell Ther*. (2022) 28:358–64. doi: 10.1016/j.jtct.2022.04.008

15. Baggot C, Kunicki M L, Pacenta H, Rossoff J, Talano J A, Chao K, et al. *Inferior Outcomes Among Black Patients with Childhood Acute Lymphoblastic Leukemia Following Tisagenlecleucel*. Stanford, California: CIBMTR (2021).

16. Iacoboni G, Martin Lopez AA, Jalowiec KA, Kwon M, Rejeski K, Navarro Garcés V, 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. *Blood*. (2022) 140:1592–4. doi: 10.1182/blood-2022-169783

17. Anwer F, Shaukat AA, Zahid U, Husnain M, Mcbride A, Persky D, et al. Donor origin CAR T cells: Graft versus Malignancy effect without GVHD, a systematic review. *Immunother Future Med Ltd*. (2017) 9:123–30. doi: 10.2217/imt-2016-0127

18. Gargett T, Brown MP. The inducible caspase-9 suicide gene system as a "safety switch" to limit on-target, off-tumor toxicities of chimeric antigen receptor T-cells. *Front Pharmacol*. (2014) 5. doi: 10.3389/fphar.2014.00235

19. Chiesa R, Georgiadis C, Syed F, Zhan H, Etuk A, Gkazi SA, et al. Base-edited CAR7 T cells for relapsed T-cell acute lymphoblastic leukemia. *New Engl J Med*. (2023) 389:899–910. doi: 10.1056/NEJMoa2300709

20. Liu E, Marin D, Banerjee P, Macapinlac HA, Thompson P, Basar R, et al. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. *New Engl J Med*. (2020) 382:545–53. doi: 10.1056/NEJMoa1910607

21. Mohyuddin GR, Atieh T, Ahmed N, Sborov D, McClune B, Abdallah AO, et al. Intention to treat versus modified intention-to-treat analysis in B-cell maturation antigen and CD19 chimeric antigen receptor trials: A systematic review and meta-analysis. *Eur J Cancer*. (2021) 156:164–74. doi: 10.1016/j.ejca.2021.07.036

22. 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) 7:2872–83. doi: 10.1182/bloodadvances.2022009019

23. Zafar A, Huang CY, Lo M, Arora S, Chung A, Wong SW, et al. Intensity of cyclophosphamide-based bridging therapy before chimeric antigen receptor T cell therapy in myeloma. *Transplant Cell Ther*. (2023) 29:504.e1–7. doi: 10.1016/j.jtct.2023.05.016

24. Velasco R, Mussetti A, Villagrán-García M, Sureda A. CAR T-cell-associated neurotoxicity in central nervous system hematologic disease: Is it still a concern? *Front Neurol*. (2023) 14. doi: 10.3389/fneur.2023.1144414

25. Asghar N, Masood A, Dhaliwal A, Khurana S, Davis J, Hashmi H, et al. Chimeric antigen receptor T-cell (CAR T-cell) therapy for primary and secondary central nervous system lymphoma: A systematic review of literature. *Clin Lymphoma Myeloma Leuk*. (2023) 23:15–21. doi: 10.1016/j.clml.2022.09.008

26. Wang T, He T, Ma L, Yang Y, Feng R, Ding Y, et al. Clinical outcomes of BCMA CAR-T cells in a multiple myeloma patient with central nervous system invasion. *Front Oncol*. (2022) 12. doi: 10.3389/fonc.2022.854448

27. Que Y, Xu M, Xu Y, Almeida VDF, Zhu L, Wang Z, et al. Anti-BCMA CAR-T cell therapy in relapsed/refractory multiple myeloma patients with extramedullary disease: A single center analysis of two clinical trials. *Front Immunol*. (2021) 12. doi: 10.3389/fimmu.2021.755866

28. Zanwar S, Ho M, Kapoor P, Binder M, Buadi F, Dispenzieri A, et al. Treatment patterns for extramedullary multiple myeloma and outcomes with CAR-T therapy and bispecific antibodies. *J Clin Oncol*. (2023) 41:8022–2. doi: 10.1200/JCO.2023.41.16_suppl.8022

29. Pan D, Mouhieddine TH, Fu W, Moshier E, Parekh S, Jagannath S, et al. Outcomes after CAR T cells in multiple myeloma patients with extramedullary and paramedullary disease. *Blood*. (2023) 142:1006–6. doi: 10.1182/blood-2023-177749

30. 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) 40:932–44. doi: 10.1200/JCO.21.01405

31. Atilla PA, Atilla E. Resistance against anti-CD19 and anti-BCMA CAR T cells: Recent advances and coping strategies. *Transl Oncol*. (2022) 22:101459. doi: 10.1016/j.tranon.2022.101459

32. Pont MJ, Hill T, Cole GO, Abbott JJ, Kelliher J, Salter AI, et al. γ-Secretase inhibition increases efficacy of BCMA-specific chimeric antigen receptor T cells in multiple myeloma. *Blood*. (2019) 134:1585–97. doi: 10.1182/blood.2019000050

33. Larson RC, Kann MC, Graham C, Mount CW, Castano AP, Lee WH, et al. Anti-TACI single and dual-targeting CAR T cells overcome BCMA antigen loss in multiple myeloma. *Nat Commun*. (2023) 14:7509. doi: 10.1038/s41467-023-43416-7

34. Zhang X, Zhang H, Lan H, Wu J, Xiao Y. CAR-T cell therapy in multiple myeloma: Current limitations and potential strategies. *Front Immunol*. (2023) 14. doi: 10.3389/fimmu.2023.1101495

35. Shi X, Yan L, Shang J, Qu S, Kang L, Zhou J, et al. Tandom autologous transplantation and combined infusion of CD19 and bcma-specific chimeric antigen receptor T cells for high risk MM: initial safety and efficacy report from a clinical pilot study. *Blood*. (2018) 132:1009–9. doi: 10.1182/blood-2018-99-117964

36. Spiegel JY, Patel S, Muffly L, Hossain NM, Oak J, Baird JH, et al. CAR T cells with dual targeting of CD19 and CD22 in adult patients with recurrent or refractory B cell Malignancies: a phase 1 trial. *Nat Med*. (2021) 27:1419–31. doi: 10.1038/s41591-021-01436-0

37. Beyar-Katz O, Gill S. Advances in chimeric antigen receptor T cells. *Curr Opin Hematol*. (2020) 27:368–77. doi: 10.1097/MOH.0000000000000614

38. Grosser R, Cherkassky L, Chintala N, Adusumilli PS. Combination immunotherapy with CAR T cells and checkpoint blockade for the treatment of solid tumors. *Cancer Cell*. (2019) 36:471–82. doi: 10.1016/j.ccell.2019.09.006

39. Xu XJ, Tang YM. Cytokine release syndrome in cancer immunotherapy with chimeric antigen receptor engineered T cells. *Cancer Lett*. (2014) 343:172–8. doi: 10.1016/j.canlet.2013.10.004

40. Gatto L, Ricciotti I, Tosoni A, Di Nunno V, Bartolini S, Ranieri L, et al. CAR-T cells neurotoxicity from consolidated practice in hematological Malignancies to fledgling experience in CNS tumors: fill the gap. *Front Oncol*. (2023) 13. doi: 10.3389/fonc.2023.1206983

41. Cliff ERS, Kelkar AH, Russler-Germain DA, Tessema FA, Raymakers AJN, Feldman WB, et al. High cost of chimeric antigen receptor T-cells: challenges and solutions. *Am Soc Clin Oncol Educ Book*. (2023) 43:1–11. doi: 10.1200/EDBK_397912

42. Jørgensen J, Hanna E, Kefalas P. Outcomes-based reimbursement for gene therapies in practice: the experience of recently launched CAR-T cell therapies in major European countries. *J Mark Access Health Policy*. (2020) 8:1715536. doi: 10.1080/20016689.2020.1715536

43. Gromowsky M, Shostrom V, Schmidt-pokorny K, Armitage JO, Vose JM, Bociek RG, et al. Patients with private insurance endure significantly longer delays in time from intent to CAR-T to apheresis (Brain-2-vein) in multiple treated diffuse large B-cell lymphoma: analysis by cellular therapy intent quotient (CTIQ). *Blood*. (2023) 142:258–8. doi: 10.1182/blood-2023-181344

44. Kourelis T, Bansal R, Berdeja J, Siegel D, Patel K, Mailankody S, et al. Ethical challenges with multiple myeloma BCMA chimeric antigen receptor T cell slot allocation: A multi-institution experience. *Transplant Cell Ther*. (2023) 29:255–8. doi: 10.1016/j.jtct.2023.01.012

45. Faiman B, Khouri J, Williams LS, Anwer F. Ethical Challenges in CAR-T slot allocation. *Transplant Cell Ther*. (2023) 29:215–6. doi: 10.1016/j.jtct.2023.03.003

46. Tully S, Feng Z, Grindrod K, McFarlane T, Chan KKW, Wong WWL. Impact of increasing wait times on overall mortality of chimeric antigen receptor T-cell therapy in large B-cell lymphoma: A discrete event simulation model. *JCO Clin Cancer Inform*. (2019) 3):1–9. doi: 10.1200/CCI.19.00086

47. Nam S, Smith J, Yang G. *Driving the next wave of innovation in CAR T-cell therapies* (2019). Available online at: https://www.mckinsey.com/industries/life-sciences/our-insights/driving-the-next-wave-of-innovation-in-car-t-cell-therapies.

48. Depil S, Duchateau P, Grupp SA, Mufti G, Poirot L. 'Off-the-shelf' allogeneic CAR T cells: development and challenges. *Nat Rev Drug Discovery*. (2020) 19:185–99. doi: 10.1038/s41573-019-0051-2

49. Myers GD, Verneris MR, Goy A, Maziarz RT. Perspectives on outpatient administration of CAR-T cell therapy in aggressive B-cell lymphoma and acute lymphoblastic leukemia. *J Immunother Cancer*. (2021) 9:e002056. doi: 10.1136/jitc-2020-002056

50. Dwivedy Nasta S, Namoglu EC, Hughes ME, Chong EA, Svoboda J, Ballard HJ, et al. Hospitalization patterns with commercial CAR T-cell therapy: A single institution experience. *Blood*. (2019) 134:3240–0. doi: 10.1182/blood-2019-130650

51. Bachier CR, Palomba ML, Abramson JS, Andreadis C, Sehgal A, Godwin J, et al. Outpatient treatment with lisocabtagene maraleucel (liso-cel) in 3 ongoing clinical studies in relapsed/refractory (R/R) large B cell non-hodgkin lymphoma (NHL), including second-line transplant noneligible (TNE) patients: transcend NHL 001, outreach, and PILOT. *Biol Blood Marrow Transplantation*. (2020) 26:S25–6. doi: 10.1016/j.bbmt.2019.12.093

52. Martinez-Cibrian N, Español-Rego M, Pascal M, Delgado J, Ortiz-Maldonado V. Practical aspects of chimeric antigen receptor T-cell administration: From commercial to point-of-care manufacturing. *Front Immunol*. (2022) 52:13. doi: 10.3389/fimmu.2022.1005457

53. Ortíz-Maldonado V, Rives S, Castellà M, Alonso-Saladrigues A, Benítez-Ribas D, Caballero-Baños M, et al. CART19-BE-01: A multicenter trial of ARI-0001 cell therapy in patients with CD19+ Relapsed/refractory Malignancies. *Mol Ther*. (2021) 29:636–44. doi: 10.1016/j.ymthe.2020.09.027

54. Galápagos. *Galapagos presents new encouraging data at ASH 2023 from ongoing CD19 CAR-T studies with GLPG5201 and GLPG5101* (2023). Available online at: https://www.glpg.com/press-releases/galapagos-presents-new-encouraging-data-at-ash-2023-from-ongoing-cd19-car-t-studies-with-glpg5201-and-glpg5101/.

55. Caring Cross. *Caring Cross Launches Initiative to Enhance the Broad Application, Affordability, and Access to CAR-T Cell Therapy* (2021). Available online at: https://caringcross.org/press/car-t-cell-therapy/.

56. Curran KJ, Nikiforow S, Bachier C, Hsu YM, Maloney DG, Maus MV, et al. Robust quality infrastructure is key to safe and effective delivery of immune effector cells: how FACT-finding can help. *Blood Adv*. (2023). doi: 10.1182/bloodadvances.2023010401

frontiers | Frontiers in Oncology

TYPE Mini Review
PUBLISHED 08 August 2024
DOI 10.3389/fonc.2024.1455464

OPEN ACCESS

EDITED BY
Samir Parekh,
Icahn School of Medicine at Mount Sinai, United States

REVIEWED BY
Noffar Bar,
Yale University, United States

*CORRESPONDENCE
Issam S. Hamadeh
ish_tox@yahoo.com
Shebli Atrash
shebli.atrash@atriumhealth.org

RECEIVED 26 June 2024
ACCEPTED 25 July 2024
PUBLISHED 08 August 2024

CITATION
Hamadeh IS, Friend R, Mailankody S and Atrash S (2024) Chimeric antigen receptor T-cells: a review on current status and future directions for relapsed/refractory multiple myeloma.
Front. Oncol. 14:1455464.
doi: 10.3389/fonc.2024.1455464

Chimeric antigen receptor T-cells: a review on current status and future directions for relapsed/refractory multiple myeloma

Issam S. Hamadeh[1]*, Reed Friend[2], Sham Mailankody[3] and Shebli Atrash[2]*

[1]Clinical Pharmacy Services, Pharmacy Department, Memorial Sloan Kettering Cancer Center, New York, NY, United States, [2]Plasma Cell Disorders Division, Department of Hematologic Oncology & Blood Disorders Levine Cancer Institute, Atrium Health, Charlotte, NC, United States, [3]Myeloma Service, Division of Hematologic Oncology, Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, NY, United States

Although multiple myeloma is an incurable disease, the past decade has witnessed significant improvement in patient outcomes. This was brought about by the development of T-cell redirection therapies such as chimeric antigen receptor (CAR) T-cells, which can leverage the natural ability of the immune system to fight myeloma cells. The approval of the B-cell maturation antigen (BCMA)-directed CAR T, idecabtagene vicleucel (ide-cel), and ciltacabtagene autoleucel (cilta-cel) has resulted in a paradigm shift in the treatment of relapsed/refractory multiple myeloma. Overall response rates ranging from 73 to 97% are currently achievable. However, the limitations of KarMMa-1 and CARTITUDE-1 studies spurred the generation of real-world data to provide some insights into the effectiveness of ide-cel and cilta-cel among patients who were excluded from clinical trials, particularly those who received prior BCMA-targeted or other T-cell redirection therapies. Despite their unprecedented clinical efficacy in heavily pretreated patients, responses to CAR T remain non-durable. Although the underlying mechanisms of resistance to these agents haven't been fully elucidated, studies have suggested that resistance patterns could be multifaceted, implicating T-cell exhaustion and tumor intrinsic mechanisms such as BCMA target loss, upregulation of gamma-secretase, and others. Herein, we provide a succinct overview of the development of CAR T-cells, manufacturing process, and associated toxicities/complications. In this review, we also recapitulate the existing literature pertaining MM CAR-T as well as emerging data from some of the ongoing clinical trials designed to mitigate the shortcomings of these agents, and improve the clinical efficacy of CAR T, especially in the relapsed/refractory setting.

KEYWORDS

BCMA, CAR T-cells, T-cell redirection therapies, myeloma, immune therapy

1 Introduction

Multiple Myeloma (MM), a blood cancer that originates from plasma cells in the bone marrow, comprises about 1.8% of all new cancer cases. In 2024, an estimated 35,780 new cases and 12,540 deaths were reported (1, 2). Despite the recent advancements in understanding tumor biology and developing novel therapies, MM remains incurable, characterized by cycles of remission and relapse. With each relapse or progression, the remission period becomes shorter until the disease becomes refractory to the currently available or standard therapies (3).

A multicenter retrospective analysis, which included 275 MM patients, revealed dismal outcomes, particularly in penta-refractory patients (i.e., refractory to two proteasome inhibitors, 2 immunomodulatory agents, and 1 anti-CD38 monoclonal antibody, n=70), where the median overall survival (OS) was about 6 months (4). Such real-world data suggest that there is an unmet need to develop novel agents for heavily pretreated MM patients, which have the potential to alter the natural history of the disease.

Defects or alterations in immune surveillance that occur during tumorigenesis have recently become a topic of extensive research. This culminated in the development of novel therapeutic approaches such as immunotherapies, which can harness the intrinsic power of the immune system to treat the disease. B-cell maturation antigen (BCMA) is a transmembrane glycoprotein belonging to the tumor necrosis factor receptor superfamily 17 (TNFRSF17). Its high expression on myeloma cells made it a valuable and attractive target for MM immunotherapy, particularly in the relapsed/refractory (RR) setting (5). Chimeric antigen receptor (CAR) T-cells targeting BCMA are one type of immunotherapies that can induce both tumor-directed cytotoxicity and immunological memory; they have demonstrated unequivocal efficacy in RRMM.

This review provides a comprehensive overview of the development of CAR T-cells, published data about the clinical efficacy of the approved products, their limitations, and underlying mechanisms of resistance, and some of the investigational platforms currently in development designed to circumvent the shortcomings of the available products.

2 Overview of CAR T-cells: CAR structure, classification and manufacturing, administration, and complications

Effective eradication of cancer cells via the immune system is a multi-step process. When cancer cells shed their antigens (including tumor-associated antigens) into the bloodstream, these antigens are taken up by the antigen-presenting cells, such as dendritic cells, which process and present them to the T-cells in the context of major histocompatibility complex (MHC) molecules (6). Of note, both CD8+ and CD+4 T-cells recognize these antigens only when bound to MHC I and II molecules. Upon activation, the T-cells secrete perforins and granzymes that trigger a cascade of reactions leading to apoptosis of the tumor cells. Tumor cells can offset this T-cell-mediated immune response via several mechanisms; one of them is to intrinsically downregulate the expression of MHC molecules (7). Such findings provided the impetus to develop new modalities or approaches that have the potential to mitigate the need for MHC molecules to trigger a T-cell-mediated immune response.

2.1 The general structure of CARs

CARs are hybrid receptors that can be genetically engineered/designed and transferred to T-cells (CAR T-cells), thereby allowing the latter to identify specific tumor-associated antigens in a manner that is independent of MHC I and II molecules. From a structural standpoint, the CAR can generally be divided into four main domains or components, each of which plays an essential role in recognizing the target antigen (8).

i. An extracellular target antigen binding domain.
ii. Hinge region (which, together with the extracellular domain, constitutes the ectodomain.
iii. A transmembrane domain.
iv. The intracellular signaling domain is also referred to as the endodomain.

As the name suggests, the extracellular target antigen binding domain of the CAR confers target (antigen) specificity, given the fact that it is usually derived from the variable heavy (V_H) and light (VL) chains of monoclonal antibodies of mouse origin. A flexible linker connects the V_H and V_L chains to each other to form the so-called single chain variable fragment (scFv) (9). This allows the CAR to recognize extracellular antigens, which, in the case of multiple myeloma, is mainly the B-cell maturation antigen (BCMA); however, multiple myeloma cells express on their surfaces a multitude of other antigens (GPCR5, CD38, FcRH5, etc.) that can be targeted by specific CARs.

The hinge region, also known as the "spacer, " serves as a bridge connecting the scFv portion to the transmembrane domain. The hinge region imparts flexibility, allowing the antigen binding domain to readily access the targeted epitope without any steric hindrance, forming a synapse with the antigen. The longer the hinge region, the more flexibility the CAR has (10).

The transmembrane domain helps anchor the CAR to the cell membrane of the T-cells through a hydrophobic α helix. Although it is the least studied component, the transmembrane domain is essential for the stability and function of the CAR-T cell (11).

The internal signaling domain is the most distal intracellular portion of the CAR; it mainly consists of CD3ζ sequences that harbor the immunoreceptor tyrosine-based activation motifs (ITAMS) which become phosphorylated when the CAR binds to its target antigen. Hence, the internal domain is responsible for signal transmission into the cell interior (12).

CARs that consist of the antigen binding domain (scFv) and CD3ζ (intracellular domain) are often referred to as "first-generation" CARs. These have fallen out of favor due to their modest clinical efficacy, exemplified by their limited activation, expansion, and persistence.

2.2 Different generations of CAR T-cells

To overcome these shortcomings, modifications have been made to the structure of the endodomain through the incorporation of costimulatory molecules, which led to the inception of several generations of CAR T-cells.

2.2.1 Second-generation CAR T-cells

In addition to CD3ζ, the intracellular domain contains co-stimulatory molecules such as CD28 and 4-1BB to boost the immune signal. The two FDA-approved CAR T-cell products for multiple myeloma, idecabtagene vicleucel (ide-cel) and ciltacabtagene autoleucel (cilta-cel), have 4-1BB as a co-stimulatory molecule. The main advantages of 4-1BB over CD28 are slower expansion and longer persistence. Because of these properties, 4-1BB containing CAR T-cells are less prone to exhaustion (13, 14).

2.2.2 Third-generation CAR T-cells

These have 2 co-stimulatory domains, 4-1BB and CD28, to amplify the intracellular signaling. Subsequently, third generation CAR T-cells have better expansion and differentiation into memory T-cells (15).

2.2.3 Fourth generation CAR T-cells

They are also referred to as "T-cells redirected for universal cytokine killing, TRUCKs" because they are constructed or designed in a manner where they secrete inflammatory cytokines such as interleukin-2 (IL-12) upon activation of antigen binding domain. This unique characteristic further enhances the proliferation and function of the CAR T-cells (16).

2.2.4 Fifth generation CAR T-cells

They are currently being designed for the sole purpose of improving the safety of the product. Fifth generation CAR T-cells contain more additional intracellular domains and drug-dependent ON or OFF-switches to circumvent some of the "off-tumor" activity of the product (17).

2.3 Production/manufacturing of CAR T-cells and their administration for treating patients with multiple myeloma

Engineering/manufacturing of CAR T-cells is complex and involves several phases (18). This journey begins with collection of the patient's peripheral blood mononuclear cells through leukapheresis. Because the patient's own cells are utilized, the final product is often referred to as "autologous CAR T-cell therapy" to distinguish it from products where the T-cells are collected from a donor. The T-cells are subsequently selected using anti-CD4 and anti-CD8 microbeads and then activated through various *in-vitro* activation methods to facilitate their *in-vitro* expansion.

Insertion of the CAR into the genome of the T-cells occurs through either viral vectors (retroviral or lentiviral vectors) or transposons followed by expansion in a bioreactor in a medium with anti-CD3/CD28 monoclonal antibodies and cytokines. When the required cell dose is reached, the CAR T-cells are isolated and transferred to a bag, where they are resuspended in an infusion-compatible medium. Because the manufacturing process is lengthy, as described above (it takes about 4-5 weeks), patients with high disease burdens may require bridging chemotherapy before administering CAR T-cells.

Prior to CAR T-cell infusion, patients should receive a lymphodepleting conditioning regimen, which creates a suitable environment for the *in vivo* expansion of these cells. Fludarabine (30 mg/m^2) in combination with cyclophosphamide (300 mg/m^2) is the most commonly used preparative regimen in multiple myeloma. The regimen is administered for three consecutive days, i.e., on days -5, -4, and -3, prior to the infusion of the CAR T-cells on day 0.

2.4 Major complications of CAR T cells therapy

Some of the acute or early complications with CAR T-cells are cytokine release syndrome (CRS) and neurotoxicity/immune effector cell-associated neurotoxicity syndrome (ICANS) (19). Although the pathophysiology of these syndromes is beyond the scope of this paper, it has been demonstrated that tumor cell destruction following CAR T-cell activation results in a drastic surge in the levels of inflammatory cytokines [interferon-γ, tumor necrosis factor-α (TNF-α), granulocyte-macrophage colony-stimulating factor, interleukin (IL)-10, IL-6 and others]. This high concentration of cytokines generates a systemic inflammatory response or cytokine storm that impairs internal organ function. The cardinal manifestations of CRS are fever, hypotension, and hypoxia (20). The median time to onset of CRS varies with each product as illustrated in Table 1. The underlying pathophysiology of neurotoxicity/ICANS remains poorly understood; it has been postulated that disruption of the blood-brain barrier, as well as activation of the endothelial cells, results in T-cell infiltration into the brain parenchyma. In addition, the neurologic complications could exhibit in a biphasic pattern i.e. acute and/or delayed (20). Early signs and symptoms of acute neurotoxicity include confusion, disturbances in writing and language, agitation and obtundation; severe acute events are characterized by seizures and cerebral edema. Delayed neurotoxicity is associated with movement, cognitive and personality changes, and its median time to onset is about 27 days (range: 14-108 days) (25).

Because timely recognition of CRS and neurotoxicity/ICANS is central to reducing morbidity and mortality, several guidelines were crafted to help with grading and managing these serious complications CAR T-cell therapy (26, 27).

Some of the long-term complications of CAR T-cells include B-cell aplasia and hypogammaglobulinemia, both of which predispose patients to infections, thereby warranting antibiotic prophylaxis and intravenous immunoglobulin support.

TABLE 1 Reported adverse effects with ide-cel and cilta-cel.

Study	CRS	CRS, grades 3/4	Median time to onset	Median duration	ICANS	ICANS grade 3/4	Median time to onset	Median duration
KarMMa-1 (21)	Overall: 84%	Overall: 5%	1 day (range: 1-12)	5 days (range: 1-63)	Overall: 18%	Overall: 3%	2 days (range: 1-10)	3 days (range: 1-26)
	D1: 50%	D1: 0%			D1: 0%	D1: 0%		
	D2: 76%	D2: 6%			D2: 17%	D2: 1%		
	D3: 95%	D3: 6%			D3: 20%	D3: 6%		
CARTITUDE-1 (22)	95%	4%	7 days (IQR: 5-8)	4 days (IQR: 3-6)	21%	9%	8 days (IQR: 6-8)	4 days (IQR: 3-7)
KarMMa-3 (23)	88%	4%	1 day (range:1-14)	4 days (range: 1-51)	15%	3%	3 days (1–317)	2 days (range: 1-37)
CARTITUDE-4 (24)	76%	1%	8 days (range: 1-23)	3 days (range: 1-17)	21%	8%	10 days (6–15)	2 days (range: 1-6)

3 Clinical efficacy of the approved and investigational CAR T-cells for relapsed/refractory multiple myeloma

Ide-cel and cilta-cel are the only two FDA-approved CAR T-cells for treating relapsed/refractory multiple myeloma (18–20). Both target the BCMA antigen, which is heavily expressed on the surface of these cells. From a structural standpoint, ide-cel has a single BCMA binding domain, whereas cilta-cel has two BCMA binding domains.

Based on the pivotal KarMMa-1 and CARTITUDE-1 trials, these products were initially reserved for heavily pretreated MM patients who received at least 4 lines of therapy, including an immunomodulatory agent, proteasome inhibitor and an anti-CD38 monoclonal antibody. The key findings from these trials are summarized in Table 2. However, the recent data from the KarMMa-3 and CARTITUDE-4 prompted the FDA in April of 2024 to expand the indications for both ide-cel and cilta-cel and approve them for earlier lines of treatment in patients with RRMM (23, 24). According to the new approval, ide-cel is approved for treating patients who received at least two prior lines of therapy, including a proteasome inhibitor, an immunomodulatory agent, and a CD-38 monoclonal antibody. Cilta-cel can also be considered to treat patients who have received at least one line of therapy, including a proteasome inhibitor and an immunomodulatory agent, and are refractory to lenalidomide.

3.1 Real-world data for approved CAR T-cells

The stringent inclusion criteria that were set forth in the KarMMa-1 and CARTITUDE-1 studies (inadequate organ function, prior exposure to BCMA-targeted therapies, cytopenias, performance status, etc.) precluded a group of patients from participating in these pivotal studies who resembled those in the real world. Hence, there has been great interest in evaluating/replicating the efficacy of ide-cel and cilta-cel in real-world settings. The study by Hansen et al. included 159 patients who received commercial ide-cel at 11 institutions (median dose: $407.0x10^6$ cells, range:154.1-456.4); the number of prior lines of therapy was 7 (4–18), 21% (n=33) had prior exposure to BCMA-targeted therapies and 46% (n=73) met 2 of the exclusion criteria in the KarMMa-1 study (28). The clinical efficacy was in line with what was previously noted or reported, where the overall response (OR) and at least complete response (CR) rates were 84% and 42%, respectively. The median progression free survival (PFS) was 8.5 months (95% CI: 6.5-NR), and OS was 12.5 months (95% CI: 11.3-NR). In the subgroup of patients who were previously exposed to BCMA-targeted therapies, the ORR (73%) and at least CR rate (33%) did not differ significantly from those who had no prior BCMA-targeted therapies (p=0.2). The PFS differed significantly based on the type of prior BCMA-targeted therapy; The PFS was significantly shorter among the patients who were previously treated with belantamab mafodotin (n=25; 5.3 months, 95% CI: 3.0-NR, p=0.0043) or BCMA bispecific T-cell engager antibody (n=4; 2.7 months, 95% CI: 1.9-NR, p=0.00069). Alternatively, the difference in PFS did not reach statistical significance among those with prior CAR-T cells (n=4, p=0.72). CRS rates occurred in 82% of patients (grade 2: 20%) and ICANS in 18% (grade 2: 11%).

Dima et al. assessed the efficacy of ide-cel as a standard of care (SOC) in 69 RRMM patients who did not meet the eligibility criteria of the KarMMa-1 study at 3 US academic institutions (part of the US Myeloma Innovations Research Collaborative, USMIRC) (29). Compared to KarMMa-1, SOC ide-cel demonstrated improved efficacy with and ORR and at least CR rate of 93% and 48%, respectively. The median PFS was comparable between the two studies, 8.5 months (95% CI, 6.2–10.9). Furthermore, there were 18 patients who were previously treated with BCMA-directed therapies (belantamab mafodotin: 16 and BCMA-directed CAR T-cells: 2). Interestingly, patient outcomes were not impacted by prior treatment with BCMA-directed therapies where the ORR in this subgroup was 90%, at least CR was 47% and median PFS was 6.2 months.

The study by Sidana et al. also provided some insights into the real-world efficacy of ide-cel among 603 patients using the CIBMTR

10.3389/fonc.2024.1455464

TABLE 2 Summary of the findings from clinical studies evaluating ide-cel and cilta-cel.

	KarMMa-1 (N=128)	CARTITUDE-1 (N=97)	KarMMa-3 (N=254)	CARTITUDE-4
Patient age, years (range)	61 (33–78)	61 (56-68)	63 (30-81)	61.5 (27-78)
Extramedullary disease, %	39	13	24	21.2
High risk cytogenetic features, %	35	24	42	59.4
High tumor burden, %	51	22	28	20.4
R-ISS stage III, %	16	14	12	5.8
Bridging, %	88	75	84	100
Prior lines of therapy	6 (range: 3-16)	6 (range 4-8)	3 (range: 2-4)	1 (32.7%)
				2 (39.9%)
				3 (27.4%)
CAR- T cells	Idecabtagene vicleucel	Ciltacabtagene autoleucel	Idecabtagene vicleucel	Ciltacabtagene autoleucel
Target dose	D1: $150x10^6$	$0.75x10^6$/kg (range: $0.5x10^6$-$1x10^6$)	$150x10^6$-$450x10^6$	$0.75x10^6$/kg
	D2: $300x10^6$			
	D3: $450x10^6$			
Overall response rate, %	Overall: 73	97 (95% CI: 91.2-99.4)	71 (95% CI: 66-77)	84.60%
	D1: 50			
	D2: 69			
	D3: 81			
Complete response	Overall: 33	67	39 (95% CI: 33-45)	73.1
	D1: 25			
	D2: 29			
	D3: 39			
MRD at 10^{-5} sensitivity, %	26 (95% CI: 19-34)	27	20 (95% CI: 15.2-25)	60.6
PFS	Overall: 8.8 months (95% CI: 5.6-11.6)	34.9 months (95% CI: 25.2-NE) CR	13.3 months (95% CI: 11.8-16.1)	12 months: 75.9% (95% CI: 69.4-81.1%)
	D1: 2.8 months (95% CI: 1.0- NE)			
	D2: 5.8 months (95% CI: 4.2-8.9)			
	D3: 12.1 months (95% CI: 8.8-12.3)			
OS	19.4 months (95% CI: 18.2- NE)	36 months: Overall: 62.9%		12 months: 84.1%
		≥ CR: 59.8%		
		12-month sustained MRD negativity: NE		
		12-month sustained MRD negativity-CR: NE		
Reference:	(21)	(22)	(23)	(24)

database (30). The number of prior lines of therapy was 7 (4–21), and 5% (n=28) had prior exposure to BCMA-directed CAR T cell therapy. The ide-cel dose ranged from 300-460x10^6 cells. The ORR was noted in 71% (n=421) of the patients; of whom 27% (n=162) achieved a CR. The 6- month PFS and OS rates were 62% (95% CI: 58-66%) and 82% (95% CI: 79-85%), respectively.

Real-world data for cilta-cel demonstrated an ORR of 80% and a CR rate of 40% among 139 patients with RR disease treated at 12 academic centers in the U.S. 36% (n=50) had penta-refractory and the number of prior lines of therapy was 6 (2–18) (31). The reduced efficacy of cilta-cel in this study could be explained by the higher percentage of patients with extramedullary disease (35% compared to 13% in CARTITUDE-1) and high-risk cytogenetic features (41% vs. 24%).

3.2 Efficacy of approved CAR T-cells following antibody-drug conjugates and other bispecific T-cell engager antibodies

Recently, other T-cell redirection therapies or bispecific T-cell engager antibodies (teclistamab, elranatamab, and talquetamab) have been approved for RRMM. Despite the availability of several options to treat patients in this setting, the proper sequencing of these agents (CAR T-cells and bispecific T-cell engager antibodies) has become a dilemma. This has also posed the question of whether CAR-T cells retain their efficacy following BCMA-directed therapies or bispecific T-cell engager antibodies.

Given this gap in our knowledge, Cohen et al. sought to investigate the efficacy of cilta-cel in a small cohort of 20 patients with RRMM who were enrolled in the CARTITUDE-2 study and previously treated with noncellular BCMA-directed therapies (either an antibody-drug conjugate or a bispecific antibody) (32). The authors noted an ORR of 60% (95% CI: 36.1-80.9%), with 30% achieving at least a CR. The PFS was 9.1months (95% CI: 1.5-NE), and OS was not reached at the time of data cut-off. Further stratification based on the type of prior BCMA therapy revealed an ORR of 61.5% (95% CI: 31.6-861%) in the antibody drug conjugate (ADC) exposed group and 57.1% (95% CI: 18.4-90.1%) in the bispecific antibody exposed group. The two main factors that predicted response to cilta-cel were the treatment duration of prior anti-BCMA therapy (29.5 days in responders vs. 63.5 days in non-responders) and the elapsed time between the two treatment modalities (235 days in responders vs. 117.5 days in non-responders). The median PFS appeared to be longer in the ADC exposed (9.5 months, 95% CI:1-NE) relative to the bispecific antibody exposed group (5.3 months, 95% CI: 0.6-NE). Despite the small number of patients, 13 patients with prior BCMA-ADC and 7 patients with prior BCMA-bispecific antibody, these findings suggested that cilta-cel might be considered a viable option for select heavily pretreated MM patients who received prior BCMA-directed therapies, namely anti-BCMA ADC. In a case series of 5 heavily pretreated MM patients who received a median of 7 lines of therapy (range: 5-8) including prior BCMA-directed CAR T-cells (3 investigational CAR T-cells and 2 ide-cel), Attar N et al. reported an ORR rate of 80% (n=4) and a CR of 60% (n=3). The PFS rate at 6 months was 75%. Of note, the time elapsed between the two CAR T-cells ranged from 8 to 38 months (33).

The retrospective study by Ferreri et al, evaluated the clinical efficacy of commercial ide-cel in 50 patients who were previously treated with BCMA-targeted therapies (38 received belantamab mafodotin, 7 bispecific antibody, and the rest investigational CAR T-cells) (34). The median number of prior lines of therapy was 9 (range: 4-18), and 86% (n=43) received bridging therapy before ide-cel infusion, where the median dose was 403.3 x10^6 (range: 154.1-454). The ORR was 74%, with 29% achieving at least a CR. The median PFS was 3.2 months, and OS has not been reached given the short follow-up period with an estimated 6-month OS rate of 72%. Response stratification based on the type of BCMA-targeted therapy showed that the patients who had prior CAR T-cells had the highest response rate (100%), whereas those who received belantamab mafodotin had the lowest response rate (68%); the ORR among patients treated with bispecific antibodies was 86%. Similarly, patients previously treated with CAR T-cells had a longer PFS (NR) compared to those with prior exposure to belantamab mafoditin (PFS: 3.2 months) or bispecific antibodies (PFS: 2.8 months) (34).

Although these studies were small, they provided evidence of CAR T-cells' efficacy post other T-cell redirection therapies; nevertheless, the duration of response appeared to be modest compared to what was described in KarMMa-1 and CARTITUDE-1. Larger studies may be warranted to corroborate these findings to help not only select patients who are likely to benefit from these agents, but also guide the optimal sequencing of these therapies.

3.3 Investigational CAR T-cells for multiple myeloma: a delve beyond BCMA

While further investigation is underway, Tables 3, 4 highlight some of the recent advancements which are exploring additional targets to overcome some of the current limitations of BCMA targeted- CAR T-cells such as antigen escape, relapse, and toxicity. Some promising candidates include GPRC5D, SLAMF7, CD38, CD138, and CD19 Table 5 and Table 6.

GPRC5D is a transmembrane protein with limited expression in normal tissues but selectively highly expressed in MM cells. In 2020, GPRC5D was investigated as a novel target for CAR T-cell therapy in MM (35). The study highlights the toxicity and efficacy of GPRC5D-targeted CAR T-cells in eliminating MM cells in 17 patients with MM. Importantly, targeting GPRC5D did not result in notable off-tumor toxicity, underscoring the specificity and safety of this approach. Dysgeusia is a known side effect of GPRC5D bispecific T-cell engager antibodies. With GPRC5D-targeted CAR T-cells, dysgeusia was seen in only 2 out of 17 patients. CRS and nail changes were seen in 88% and 65% of patients. Overall responses were notable in about 70% of patients, even those who had received prior BCMA-based therapy. This study marks a promising step forward in developing next-generation CAR T-cell therapies for MM (35).

SLAMF7, also known as CS1, is highly expressed on myeloma cells and natural killer (NK) cells. However, SLAMF7 is also

expressed on activated T-cells, which raises concerns about CAR-T cell fratricide (54, 55).

Similarly, fratricide is a concern in CD38 CAR T-cells (56). CD38 is another well-established target in MM, with drugs like daratumumab and isatuximab (CD38 monoclonal antibodies). However, CD38 is expressed in normal T/NK cells. CD38-CAR T- cells have shown preclinical success and are being evaluated in early-phase clinical trials (57). CD38 CAR T-cells remains in the early phase of development.

CD138 is highly expressed on plasma cells and is another potential target for CAR T-cell therapy. One major obstacle is CD138 expression on other cell types, including subsets of epithelial and endothelial cells (58). CD138-CAR T-cells have shown promise in preclinical studies and are currently under investigation in clinical trials (NCT03672318).

Dual-targeting CAR T-cells that simultaneously target BCMA and another antigen (e.g., CD19, GPRC5D, CD38, or SLAMF7) are under development. These bispecific CAR T-cells aim to prevent antigen escape and increase durable responses by targeting multiple pathways simultaneously. One innovative strategy to enhance the efficacy of CAR T-cell therapy is the combination of CD19 and BCMA, which has shown significant promise among the various dual-targeting strategies.

CART-ddBCMA is another unique BCMA CAR-T product with a D-binding Domain, comprising 73 amino acids, and offering a highly stable bond with reduced immunogenicity. The CART-ddBCMA showed an ORR of 100% in a phase 1 trial (59). Responses were deep where 22 of the 37 patients achieved sCR/CR, 7 VGPR, and 2 PR. Of 22 patients who were evaluable for MRD, 19 were MRD negative at 10^{-5} or lower (59).

TABLE 4 Current or ongoing clinical trials with cilta-cel.

	CARTITUDE-5	CARTITUDE-6
Experimental arm	NDMM, transplant deferred, treated with VRd induction followed by cilta-cel	NDMM, transplant eligible, following DVRd followed by cilta-cel
Control arm	VRd followed by Rd maintenance	DVRd followed by ASCT
Trial Phase	3	3
Primary end point	PFS	PFS, sustained MRD neg CR
NCT	04923893	05257083

NDMM, newly diagnosed Multiple Myeloma; VRd, Bortezomib, Lenalidomide, dexamethasone; Rd, Lenalidomide, dexamethasone; DVRd, Daratumumab, Bortezomib, Lenalidomide, dexamethasone; PFS, progression free survival; MRD, minimal residual disease; CR, complete response.

CD19 is a protein commonly found on B cells, and has been successfully targeted in B-cell malignancies such as acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL). Although CD19 is not typically found on myeloma cells, it is present in a subset of early B-lineage precursors and plasmablasts that can eventually develop into myeloma cells (9, 60). This dual-targeting approach addresses the limitations of single-target CAR T-cell therapies by targeting both malignant plasma cells and their progenitors. Both preclinical studies and early-phase clinical trials have investigated CAR T-cells' effectiveness in targeting CD19 and BCMA. Early-phase clinical trials have shown promising results. For example, a study by Zhao et al. reported the outcomes of a phase 1/2 trial that treated 21 patients with dual-targeting CD19 and BCMA CAR T-cells. The combination

TABLE 3 Current or ongoing clinical trials with ide-cel.

	Elranatamab in RRMM	KarMM-7	KarMMa-9
Experimental arm	Elranatamab given after (SOC) Ide-cel	Arm A: Ide-cel + CC-220 (+/- low dose dex) Arm B: Ide-cel + BMS-986405	Ide-cel + Len maintenance
Control arm	NA (single arm study)	NA (single arm study)	Len maintenance
Trial Phase	2	1/2	3
Prior lines of therapy to enroll	≥4 including PI, IMID, anti-CD38 mAb	≥3 for both Arms except Arm A, cohort 2 can receive at least 1 but no >3 LOT	4-6 cycles of induction therapy (incl PI, IMID) and single ASCT 80-120 days prior to consent
Primary end point	CR or sCR post consolidation therapy; PFS	DLT, CRR	PFS
NCT	06138275	04855136	06045806

SOC, standard of care; NA, not applicable; PI, proteosome inhibitor; IMID, immunomodulatory; mAb, monoclonal antibody; CR, complete response; sCR, stringent complete response; LOT, lines of therapy; DLT, dose limiting toxicity; CRR, complete response rate; Len, Lenalidomide; LOT, lines of therapy; PFS, progression free survival.

TABLE 5 New targets for CART.

New targets for CART		
Target	Extra properties	Trial
SLAMF7/CS1	incorporating on/off suicide gene	NCT03958656 and NCT03710421
	Off the shelf, UCARTCS1	NCT04142619
CD38	–	NCT03464916
	dual-specificity anti-CD38/BCMA CAR T-cell	NCT03767751
CD19	–	NCT02135406
	dual-specificity anti-CD19/BCMA CAR T-cell	NCT03455972
TACI	Targeting BCMA and TACI	NCT03287804
κ light chain	Costimulating domain CD28	NCT00881920
Lewis Y	Costimulating domain CD28	NCT01716364
NY-ESO-1	34% of the HLA-A2 positive expressed NY-ESO-1 and/or LAGE-1	NCT03638206
NKG2D ligands	Costimulating domain DAP10	NCT02203825

TABLE 6 New CAR-Ts designed to offset resistance mechanisms.

New CAR-Ts designed to offset resistance mechanisms							
Resistance mechanism	Mechanism of action	CAR-T properties	No. Of patients	Median follow up	Response	Toxicity	Reference
Antigen escape	Dual-targeted CAR T-cell therapy	BCMA/CD38 bispecific CAR T-cells	23 R/R MM	9 months	ORR 87%, sCR 52% PR 33%	CRS (87%), ICANS (0%), infections (22%)	(36)
			16 RRMM	11.5 months	ORR 88%, CR 81%, PR 6%	CRS (75%), HLH (6%), infections (38%)	(37)
		BCMA/CS1 bispecific CAR T-cells	16 RRMM	290 days	ORR 100%, sCR 31% PR 13%	CRS (38%)	(38)
		BCMA/GPRC5D bispecific CAR T-cells	Na	Na	Na	Na	NCT05431608
		CD38 and SLAMF7	Na	Na	Na	Na	(39)
	Combined infusion	anti BCMA and anti-CD38 CAR T- cells	22 RRMM	24 months	ORR 91%, CR 55%	CRS (100%), ICANS (14%), infections (17%)	(40)
		anti BCMA and anti-CD19 CAR T- cells	62 RRMM	21 months	ORR 92%, CR 60%, PR 21%	CRS (95%), ICANS (11%), B cell aplasia (30%), infections (45%)	(41, 42)
		anti BCMA and anti-CD19 CAR T- cells and maintenance	10 RRMM 20 high risk	Range: 248-966 days	ORR 23%, CR 6% PR 6%	CRS (90%), ICANS (3%)	(43)
		Anti-BCMA and anti-CD19 CAR T- cells followed by lenalidomide after auto-HSCT	10	42 months	ORR 100%, sCR 90%, CR 10%	CRS (100%), infections (100%)	(44)
		Combined infusion of anti-BCMA and anti-CD19 FasT CAR T-cells	13 high-risk NDMM	5.3 months	ORR 95% sCR 69%	CRS (23%)	(45)
		Combined CAR T-cells with gamma secretase	18 RRMM	10 months. sBCMA levels ≤ 3.0 ng/mL (low) at day 60 PFS of 31 months vs. (high) PFS of 5.4 months	ORR 89%, sCR 44%	CRS (94%) ICANS (39%)	(46)
	Different target	anti-GPRC CAR T-cell (OriCAR-017)	10 RRMM	238 days	ORR 100% sCR 60% 40% VGPR	CRS(100%), no ICANS	(47)
		anti-GPRC CAR T-cells (MCARH109)	17 RRMM	10 months	ORR 70%	CRS (88%), ICANS (6%), Cerebellar disorder (12%)	(35)

(Continued)

TABLE 6 Continued

New CAR-Ts designed to offset resistance mechanisms							
Resistance mechanism	Mechanism of action	CAR-T properties	No. Of patients	Median follow up	Response	Toxicity	Reference
CAR T-cell exhaustion	Allogeneic BCMA-targeting CAR T-cells	ALLO-715	43 RRMM	10 months	ORR (56%), with dose: 320 × 106 CAR+ T (n = 24), ORR (71%), VGPR (45.8%), CR (25%)	CRS (55%), infections (44%)	(48, 49)
		P-BCMA-ALLO1	24 RRMM	–	ORR Post-BCMA (60%)	CRS (14%), ICANS (4%)	(50, 51)
	Enrich for memory-like T cells	bb21217	72 RRMM	9 months	ORR (69%)	CRS (75%), ICANS (15%)	(52)
	Improve potency and phenotypic attributes	BMS-986354 (NEX-T process)	60 RRMM	DOR 10.8 Months	ORR (95%)	CRS (82%), ICANS (9%)	(53)

Na, Not available.

achieved an overall response rate of 95% in infused patients, with 43% of patients experiencing complete remissions (41).

The future of CAR T-cell therapy for MM is promising, with ongoing research focused on identifying and validating new targets beyond BCMA. Antigens such as GPRC5D, SLAMF7, CD38, and CD138 represent exciting avenues for exploration, offering hope for more effective and durable treatments. As these novel CAR T-cell therapies advance through clinical trials, they hold the potential to transform the landscape of MM treatment, providing new options for patients who have exhausted current therapies.

4 Potential mechanisms of resistance to approved CAR T-cells

The mechanisms of resistance to CAR T-cells have not been fully elucidated. In this section, we describe some of the proposed mechanisms. Because these patterns of resistance are multifactorial, we divided them into the following:

4.1 Tumor intrinsic mechanisms of resistance

4.1.1 Bi allelic loss of *BCMA*

It is a rare event and accounts for about 6% of the cases at the time of relapse (61). Bi allelic loss of BCMA was first described by Samur et al. in a patient who received ide-cel at a dose of $150x10^6$ CAR+ T cells following 4 prior lines of therapy (including a proteasome inhibitor, an immunomodulatory agent, and an anti-CD38 monoclonal antibody) (62). The patient achieved a partial response; nonetheless, the disease relapsed at 9 months post CAR T-cells, which necessitated a second infusion of ide-cel at a higher dose ($450x10^6$). Unfortunately, the patient derived no clinical benefit from the second dose. The lack of response evoked further investigation to help uncover the underlying mechanisms of resistance. Transcriptomic analysis revealed deletion of 16p in the majority of the multiple myeloma cells; it is worth noting that the BCMA gene (*TNFRSF17*) is located on 16p13.13. This finding was further substantiated by whole exome sequencing of purified CD138+ cells which also identified the presence of a loss of function mutation (p.Q38*) in BCMA in 70%. The authors attributed this lack of response to a lack of BCMA expression secondary to the biallelic loss of BCMA (monoallelic loss of 16 p and second copy loss-of-function mutation), which provides the molecular basis for the lack of BCMA expression in MM cells at the time of relapse. Da Via MC also reported a case of homozygous (biallelic) BCMA gene loss or deletion in a 71-year-old male who received ide-cel at a dose of $450x10^6$. At the time of disease progression, BCMA expression was undetectable compared to baseline (P<6.2×10−94) (63).

4.1.2 Gamma secretase production

BCMA can be cleaved from the surface of myeloma cells by the protease, gamma-secretase, thereby increasing the concentration of soluble/circulating BCMA in plasma (64). The reduced density of surface BCMA may in fact compromise the activity of BCMA targeting therapies such as ide-cel and cilta-cel. Indeed, the study by Chen et al. demonstrated that circulating BCMA levels exceeding 156 ng/mL in samples obtained from 379 patients with RRMM were associated with reduced binding of anti-BCMA antibodies to myeloma cells (65). Hence, inhibition of γ-secretase activity could increase the efficacy of anti-BCMA CAR T-cell therapy via upregulation of BCMA density on plasma cells (66). The combination of a gamma-secretase (crenigacestat) with BCMA targeting CAR T-cells was evaluated in the phase I study of Cowan et al. (46) Of the 18 patients included in the study, 7 had prior exposure to BCMA-targeted therapy. Administration of 3 doses of crenigacestat of 25 mg every other day prior to lymphodepleting chemotherapy resulted in an increase in the

BCMA binding sites in all patients, including those with prior BCMA-targeted therapies. The ORR in this subgroup of patients (prior to BCMA-targeted therapy) was 71%, with 43% achieving at least a very good partial response. However, the PFS was shorter (2.6 months) compared to 28.8 months among those with no prior BCMA-targeted targeted therapies. Given the small number of patients, larger studies are needed to validate the efficacy of this approach.

4.2 T-cell exhaustion mechanisms

The expansion kinetics of CAR T-cells are crucial for effectively eliminating tumor cells. The success of CAR T-cell therapy is closely linked to the fitness and memory-like characteristics of the cells (67). Less-differentiated T-cells exhibit robust proliferative potential and resistance to exhaustion, with decreased expression of inhibitory receptors such as checkpoint inhibitors, leading to enhanced CAR T-cell expansion. Achieving optimal CAR T-cell expansion by day +7 was an independent and dynamic indicator of treatment response (68). In the early phase 1 clinical trial of cilta-cel, the CAR T-cells were undetectable in the peripheral blood of most patients at four months; the CAR T-cells persisted up to 10 months in merely 16% of the patients (69). CAR T-cell persistence at 3 and 12 months was noted in 86% and 20% of the patients treated with ide-cel respectively (70).

Myeloma cells can evade elimination by CAR T-cells, even if they still have the BCMA target antigen. This is due to CART-cell exhaustion or alteration of the internal apoptotic machinery in plasma cells (71). CAR T-cell exhaustion can happen because plasma cells interact with inhibitory ligands on T-cells (like TIGIT, TIM3, and/or LAG3), or over express PDL1 or lack the expression of costimulatory ligands like CD58 on plasma cells (67). Additionally, using CAR T-cell therapies in earlier lines of therapy was associated with impressive overall response rates and progression-free survival (KarMMa-3 and CARTITUDE4).

Several patient-related factors could impact CAR T-cell efficacy. For instance, using alkylating agents prior to lymphocyte collection may hinder CAR T-cell fitness and decrease the CD4+/CD8+ ratio, while using selinexor might improve lymphocyte fitness (72–74). Even after lymphocyte collection, bridging chemotherapy might affect the absolute lymphocyte count (ALC) before lymphodepletion chemotherapy and modulate CAR T-cell efficacy. The response after CAR T-cell therapy was significantly higher in patients with a high pre-lymphodepletion ALC, which was defined as 0.75 ≥ 10^9/L (76% versus 41%; P = .002). Patients with a low pre-lymphodepletion ALC had a significantly inferior OS (15.4 months) and PFS (8.4 months) compared with those with a high pre-lymphodepletion ALC (OS: not reached, p<0.001; PFS: 27.3 months, <0.001). Notably, higher pre-lymphodepletion ALC was not correlated with higher CRS rates (73). Alternatively, one study demonstrated that a higher baseline ALC was associated with higher CRS/ICANS rates; however, this did not translate into improved survival rates (75). Regardless of the baseline ALC, Saldarriaga et al. showed that the maximum ALC within 15 days after the CAR-T infusion at a dose of 1.0 x 10^3/uL was an independent predictor of PFS (76). These conflicting findings require validation in future studies to better understand the contribution of ALC to patient outcomes and complications with CAR T-cells.

Additionally, underlying comorbidities may also play a role in determining CAR T-cell eligibility. A retrospective analysis indicated that the presence of renal impairment was associated with prolonged cytopenias (77). With advancing age, there is an accumulation of antigen-experienced and dysfunctional T-cell subsets, such as TEMRA, TEX, and TTD cells, as well as selective retention of antigen-inexperienced T cells with memory-like features and NK cell-like markers (67, 78). Data from the CIBMTR registry suggested that there were no significant differences in ORR, median OS, and treatment-related mortality between patients aged 70 or older and younger patients who received ide-cel (79). Regarding toxicity, the older population exhibited higher rates of low grade (1 and 2) neurotoxicity; rates of severe neurotoxicity (grade 3 and above) was comparable between the two age groups. The study also looked at frail patients with score of ≥2 using the simplified frailty index. Frail patients showed similar PFS and OS, but had higher rates of prolonged cytopenia, clinically significant infections, and neurotoxicity of any grade without an increase in high-grade adverse events (79).

Patient-related factors could be modifiable and significantly impact CAR T-cell fitness. According to a prospective pilot study, a six-month physical activity intervention led to notable reductions in levels of T-cell exhaustion markers such as PD-1, TIGIT, TIM3, and/or LAG3 at the end of the intervention compared to the baseline (80).

4.3 FDA warnings about CAR T-cells

The FDA utilized the Adverse Event Reporting System (FAERS) to uncover a significant risk of T-cell malignancies linked to all currently approved BCMA-directed and CD19-directed genetically modified autologous CAR T- cell immunotherapies. As a result, in January 2024, the FDA implemented safety labeling revisions across this therapeutic class.

The analysis involved 12,394 adverse event records related to CAR T-cell therapy in the FAERS database. Among these entries, 536 (about 4.3%) documented secondary primary malignancies along with other adverse reactions. Axicabtagene ciloleucel was associated with 51.7% of these cases, while tisagenlecleucel was linked to 33%. However, -ide-cel and cilta-celcomprised 4% and 3% of the reported cases, respectively (81).

The most frequently observed secondary cancers after CAR T-cell therapy were leukemias, comprising 2.7% of all reported incidents. Skin cancers emerged as the second most common, accounting for 0.4% of the overall adverse event reports. Additionally, seventeen instances of T-cell non-Hodgkin lymphomas were recorded, predominantly featuring anaplastic large T-cell lymphomas. Furthermore, two cases of large granular T-cell leukemia were identified, bringing the total T-cell malignancy reports within the FAERS database to 19.

5 Discussion

The treatment landscape of RRMM is ever evolving with the identification of new targets and the development of novel treatment modalities. The addition of ide-cel and cilta-cel CAR T-cells to the therapeutic armamentarium of multiple myeloma translated into dramatically improving patient outcomes. The LocoMMotion study demonstrated that standard therapies have minimal activity in triple-class exposed patients (i.e. received an immunomodulatory agent, a proteasome inhibitor, and an anti-CD38 monoclonal antibody) where response rates were approximately 30% (95% CI: 24.2–36.0) (82). Additionally, median PFS and OS were 4.6 (95% CI: 3.9–5.6) and 12.4 months (95% CI: 10.3–NE), respectively. CAR T-cells, as well as other T-cell redirection therapies, were developed to address this urgent or unmet demand for more potent and effective therapies for heavily pretreated multiple myeloma patients. Although CAR T-cell therapy has significantly increased response rates by 2 to 3-fold, the disease will inevitably relapse as treatment-resistant clones start to emerge. As discussed earlier, our deeper understanding of the molecular mechanisms that confer resistance to CAR T-cells has set the framework for some of the clinical trials listed herein. If these investigational CAR T-cells are granted approval, this will pose great challenges to the treating physician as treatment options for RRMM continue to grow. Hence, further research is still needed to not only better sequence these agents but also identify the optimal strategy which can alter the natural history of the disease and thereby lead to a cure.

Author contributions

SA: Writing – original draft, Writing – review & editing. IH: Writing – original draft, Writing – review & editing. RF: Writing – original draft, Writing – review & editing. SM: Writing – original draft, Writing – review & editing, Conceptualization, Data curation, Formal Analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors express their deep appreciation to the Leon Levine Foundation and the Kerry and Simone Vickar Family Foundation for their support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Siegel RL, Giaquinto AN, Jemal A. Cancer statistics, 2024. *CA Cancer J Clin*. (2024) 74:12–49. doi: 10.3322/caac.21820

2. Siegel RL, Giaquinto AN, Jemal A. Erratum to "Cancer statistics, 2024. *CA Cancer J Clin*. (2024) 74:203. doi: 10.3322/caac.21830

3. Anderson KC, Kyle RA, Rajkumar SV, Stewart AK, Weber D, Richardson P, et al. Clinically relevant end points and new drug approvals for myeloma. *Leukemia*. (2008) 22:231–9. doi: 10.1038/sj.leu.2405016

4. Gandhi UH, Cornell RF, Lakshman A, Gahvari ZJ, McGehee E, Jagosky MH, et al. Outcomes of patients with multiple myeloma refractory to CD38-targeted monoclonal antibody therapy. *Leukemia*. (2019) 33:2266–75. doi: 10.1038/s41375-019-0435-7

5. Cho SF, Anderson KC, Tai YT. Targeting B cell maturation antigen (BCMA) in multiple myeloma: potential uses of BCMA-based immunotherapy. *Front Immunol*. (2018) 9:1821. doi: 10.3389/fimmu.2018.01821

6. Alnefaie A, Albogami S, Asiri Y, Ahmad T, Alotaibi SS, Al-Sanea MM, et al. Chimeric antigen receptor T-cells: an overview of concepts, applications, limitations, and proposed solutions. *Front Bioeng Biotechnol*. (2022) 10:797440. doi: 10.3389/fbioe.2022.797440

7. Garrido F, Aptsiauri N, Doorduijn EM, Garcia Lora AM, van Hall T. The urgent need to recover MHC class I in cancers for effective immunotherapy. *Curr Opin Immunol*. (2016) 39:44–51. doi: 10.1016/j.coi.2015.12.007

8. Dagar G, Gupta A, Masoodi T, Nisar S, Merhi M, Hashem S, et al. Harnessing the potential of CAR-T cell therapy: progress, challenges, and future directions in hematological and solid tumor treatments. *J Transl Med*. (2023) 21:449. doi: 10.1186/s12967-023-04292-3

9. Sterner RC, Sterner RM. CAR-T cell therapy: current limitations and potential strategies. *Blood Cancer J*. (2021) 11:69. doi: 10.1038/s41408-021-00459-7

10. Guest RD, Hawkins RE, Kirillova N, Cheadle EJ, Arnold J, O'Neill A, et al. The role of extracellular spacer regions in the optimal design of chimeric immune receptors: evaluation of four different scFvs and antigens. *J Immunother*. (2005) 28:203–11. doi: 10.1097/01.cji.0000161397.96582.59

11. Bridgeman JS, Hawkins RE, Bagley S, Blaylock M, Holland M, Gilham DE. The optimal antigen response of chimeric antigen receptors harboring the CD3zeta transmembrane domain is dependent upon incorporation of the receptor into the endogenous TCR/CD3 complex. *J Immunol*. (2010) 184:6938–49. doi: 10.4049/jimmunol.0901766

12. Rafiq S, Hackett CS, Brentjens RJ. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. *Nat Rev Clin Oncol*. (2020) 17:147–67. doi: 10.1038/s41571-019-0297-y

13. Imai C, Mihara K, Andreansky M, Nicholson IC, Pui CH, Geiger TL, et al. Chimeric receptors with 4-1BB signaling capacity provoke potent cytotoxicity against acute lymphoblastic leukemia. *Leukemia*. (2004) 18:676–84. doi: 10.1038/sj.leu.2403302

14. Harding FA, McArthur JG, Gross JA, Raulet DH, Allison JP. CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. *Nature*. (1992) 356:607–9. doi: 10.1038/356607a0

15. Ramos CA, Rouce R, Robertson CS, Reyna A, Narala N, Vyas G, et al. *In vivo* fate and activity of second- versus third-generation CD19-specific CAR-T cells in B cell non-Hodgkin's lymphomas. *Mol Ther*. (2018) 26:2727–37. doi: 10.1016/j.ymthe.2018.09.009

16. Chmielewski M, Abken H. TRUCKs: the fourth generation of CARs. *Expert Opin Biol Ther*. (2015) 15:1145–54. doi: 10.1517/14712598.2015.1046430

17. Kagoya Y, Tanaka S, Guo T, Anczurowski M, Wang CH, Saso K, et al. A novel chimeric antigen receptor containing a JAK-STAT signaling domain mediates superior antitumor effects. *Nat Med*. (2018) 24:352–9. doi: 10.1038/nm.4478

18. Vormittag P, Gunn R, Ghorashian S, Veraitch FS. A guide to manufacturing CAR T cell therapies. *Curr Opin Biotechnol*. (2018) 53:164–81. doi: 10.1016/j.copbio.2018.01.025

19. Neelapu SS, Tummala S, Kebriaei P, Wierda W, Gutierrez C, Locke FL, et al. Chimeric antigen receptor T-cell therapy - assessment and management of toxicities. *Nat Rev Clin Oncol*. (2018) 15:47–62. doi: 10.1038/nrclinonc.2017.148

20. Adkins S. CAR T-cell therapy: adverse events and management. *J Adv Pract Oncol*. (2019) 10:21–8.

21. Munshi NC, Anderson LD, Shah N, Madduri D, Berdeja J, Lonial S, et al. Idecabtagene vicleucel in relapsed and refractory multiple myeloma. *N Engl J Med*. (2021) 384:705–16. doi: 10.1056/NEJMoa2024850

22. 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) 41:1265–74. doi: 10.1200/JCO.22.00842

23. 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) 388:1002–14. doi: 10.1056/NEJMoa2213614

24. 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) 389:335–47. doi: 10.1056/NEJMoa2303379

25. Cohen AD, Parekh S, Santomasso BD, Gállego Pérez-Larraya J, van de Donk NWCJ, Arnulf B, et al. Incidence and management of CAR-T neurotoxicity in patients with multiple myeloma treated with ciltacabtagene autoleucel in CARTITUDE studies. *Blood Cancer J*. (2022) 12:32. doi: 10.1038/s41408-022-00629-1

26. Lee DW, Santomasso BD, Locke FL, Ghobadi A, Turtle CJ, Brudno JN, et al. ASTCT consensus grading for cytokine release syndrome and neurologic toxicity associated with immune effector cells. *Biol Blood Marrow Transpl*. (2019) 25:625–38. doi: 10.1016/j.bbmt.2018.12.758

27. Santomasso BD, Nastoupil LJ, Adkins S, Lacchetti C, Schneider BJ, Anadkat M, et al. Management of immune-related adverse events in patients treated with chimeric antigen receptor T-cell therapy: ASCO guideline. *J Clin Oncol*. (2021) 39:3978–92. doi: 10.1200/JCO.21.01992

28. Hansen DK, Sidana S, Peres LC, Colin Leitzinger C, Shune L, Shrewsbury A, et al. Idecabtagene vicleucel for relapsed/refractory multiple myeloma: real-world experience from the myeloma CAR T consortium. *J Clin Oncol*. (2023) 41:2087–97. doi: 10.1200/JCO.22.01365

29. Dima D, Rashid A, Davis JA, Shune L, Abdallah AO, Li H, et al. Efficacy and safety of idecabtagene vicleucel in patients with relapsed-refractory multiple myeloma not meeting the KarMMa-1 trial eligibility criteria: A real-world multicentre study. *Br J Haematol*. (2024) 204:1293–9. doi: 10.1111/bjh.19302

30. Sidana S, Ahmed N, Akhtar OS, Heim M, Brazauskas R, Hansen DK, et al. Real world outcomes with idecabtagene vicleucel (Ide-cel) CAR-T cell therapy for relapsed/refractory multiple myeloma. *Blood*. (2023) 142:1027–7. doi: 10.1182/blood-2023-181762

31. Hansen DK, Patel KK, Peres LC, Kocoglu MH, Shune L, Simmons G, et al. Safety and efficacy of standard of care (SOC) ciltacabtagene autoleucel (Cilta-cel) for relapsed/refractory multiple myeloma (RRMM). *JCO*. (2023) 41:8012–2. doi: 10.1200/JCO.2023.41.16_suppl.8012

32. Cohen AD, Mateos MV, Cohen YC, Rodriguez-Otero P, Paiva B, van de Donk NWCJ, et al. Efficacy and safety of cilta-cel in patients with progressive MM after exposure to other BCMA-targeting agents. *Blood*. (2022) 140(Supplement 1):4646–8. doi: 10.1182/blood-2022-158563

33. Attar N, Cirstea D, Branagan AR, Yee AJ, Frigault MJ, Raje N. Use of cilta-cel CAR T cells following previous use of a BCMA-directed CAR T-cell product in heavily treated patients with relapsed/refractory multiple myeloma: A single institution case series. *Blood*. (2023) 142:6924–4. doi: 10.1182/blood-2023-182212

34. 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) 13:117. doi: 10.1038/s41408-023-00886-8

35. Mailankody S, Devlin SM, Landa J, Nath K, Diamonte C, Carstens EJ, et al. GPRC5D-targeted CAR T cells for myeloma. *N Engl J Med*. (2022) 387:1196–206. doi: 10.1056/NEJMoa2209900

36. Mei H, Li C, Jiang H, Zhao X, Huang Z, Jin D, et al. A bispecific CAR-T cell therapy targeting BCMA and CD38 in relapsed or refractory multiple myeloma. *J Hematol Oncol*. (2021) 14:161. doi: 10.1186/s13045-021-01170-7

37. Tang Y, Yin H, Zhao X, Jin D, Liang Y, Xiong T, et al. High efficacy and safety of CD38 and BCMA bispecific CAR-T in relapsed or refractory multiple myeloma. *J Exp Clin Cancer Res*. (2022) 41:2. doi: 10.1186/s13046-021-02214-z

38. Li C, Wang X, Wu Z, Luo W, Zhang Y, Kang Y, et al. Bispecific CS1-BCMA CAR-T cells are clinically active in relapsed or refractory multiple myeloma: an updated clinical study. *Blood*. (2022) 140:4573–4. doi: 10.1182/blood-2022-170686

39. Roders N, Nakid-Cordero C, Raineri F, Fayon M, Abecassis A, Choisy C, et al. Dual chimeric antigen receptor T cells targeting CD38 and SLAMF7 with independent signaling demonstrate preclinical efficacy and safety in multiple myeloma. *Cancer Immunol Res*. (2024) 12:478–90. doi: 10.1158/2326-6066.CIR-23-0839

40. Zhang H, Liu M, Xiao X, Lv H, Jiang Y, Li X, et al. A combination of humanized anti-BCMA and murine anti-CD38 CAR-T cell therapy in patients with relapsed or refractory multiple myeloma. *Leuk Lymphoma*. (2022) 63:1418–27. doi: 10.1080/10428194.2022.2030476

41. Yan Z, Cao J, Cheng H, Qiao J, Zhang H, Wang Y, et al. A combination of humanised anti-CD19 and anti-BCMA CAR T cells in patients with relapsed or refractory multiple myeloma: a single-arm, phase 2 trial. *Lancet Haematol*. (2019) 6:e521–9. doi: 10.1016/S2352-3026(19)30115-2

42. Wang Y, Cao J, Gu W, Shi M, Lan J, Yan Z, et al. Long-term follow-up of combination of B-cell maturation antigen and CD19 chimeric antigen receptor T cells in multiple myeloma. *J Clin Oncol*. (2022) 40:2246–56. doi: 10.1200/JCO.21.01676

43. 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 Discovery*. (2023) 4:118–33. doi: 10.1158/2643-3230.BCD-22-0074

44. Shi X, Yan L, Shang J, Kang L, Yan Z, Jin S, et al. Anti-CD19 and anti-BCMA CAR T cell therapy followed by lenalidomide maintenance after autologous stem-cell transplantation for high-risk newly diagnosed multiple myeloma. *Am J Hematol*. (2022) 97:537–47. doi: 10.1002/ajh.26486

45. Du J, Fu W, Lu J, Qiang W, He H, Liu J, et al. Phase I open-label single-arm study of BCMA/CD19 dual-targeting fasTCAR-T cells (GC012F) as first-line therapy for transplant-eligible newly diagnosed high-risk multiple myeloma. *Blood*. (2022) 140:889–90. doi: 10.1182/blood-2022-162295

46. Cowan AJ, Pont MJ, Sather BD, Turtle CJ, Till BG, Libby EN, et al. γ-Secretase inhibitor in combination with BCMA chimeric antigen receptor T-cell immunotherapy for individuals with relapsed or refractory multiple myeloma: a phase 1, first-in-human trial. *Lancet Oncol*. (2023) 24:811–22. doi: 10.1016/S1470-2045(23)00246-2

47. Zhang M, Wei G, Zhou L, Zhou J, Chen S, Zhang W, et al. GPRC5D CAR T cells (OriCAR-017) in patients with relapsed or refractory multiple myeloma (POLARIS): a first-in-human, single-centre, single-arm, phase 1 trial. *Lancet Haematol*. (2023) 10:e107–16. doi: 10.1016/S2352-3026(22)00372-6

48. Mailankody S, Matous JV, Chhabra S, Liedtke M, Sidana S, Oluwole OO, et al. Publisher Correction: Allogeneic BCMA-targeting CAR T cells in relapsed/refractory multiple myeloma: phase 1 UNIVERSAL trial interim results. *Nat Med*. (2023) 29:3271. doi: 10.1038/s41591-023-02306-7

49. 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) 29:422–9. doi: 10.1038/s41591-022-02182-7

50. Dholaria B, Shune L, Kin A, McArthur K, Eskew JD, Martin CE, et al. Abstract CT071: Clinical activity of P-BCMA-ALLO1, a B-cell maturation antigen (BCMA) targeted allogeneic chimeric antigen receptor T-cell (CAR-T) therapy, in relapsed refractory multiple myeloma (RRMM) patients (pts) following progression on prior BCMA targeting therapy. *Cancer Res*. (2024) 84:CT071–1. doi: 10.1158/1538-7445.AM2024-CT071

51. Dholaria B, Kocoglu MH, Kin A, Asch AS, Ramakrishnan A, Bachier C, et al. Early safety results of P-BCMA-ALLO1, a fully allogeneic chimeric antigen receptor T-cell (CAR-T), in patients with relapsed / refractory multiple myeloma (RRMM). *Blood*. (2023) 142:3479–9. doi: 10.1182/blood-2023-182430

52. Raje NS, Shah N, Jagannath S, Kaufman JL, Siegel DS, Munshi NC, et al. Updated clinical and correlative results from the phase I CRB-402 study of the BCMA-targeted CAR T cell therapy bb21217 in patients with relapsed and refractory multiple myeloma. *Blood*. (2021) 138:548–8. doi: 10.1182/blood-2021-146518

53. Costa LJ, Kumar SK, Atrash S, Liedtke M, Kaur G, Derman BA, et al. Results from the first phase 1 clinical study of the B-cell maturation antigen (BCMA) nex T chimeric antigen receptor (CAR) T cell therapy CC-98633/BMS-986354 in patients (pts) with relapsed/refractory multiple myeloma (RRMM). *Blood*. (2022) 140:1360–2. doi: 10.1182/blood-2022-160038

54. Zhang X, Zhang H, Lan H, Wu J, Xiao Y. CAR-T cell therapy in multiple myeloma: Current limitations and potential strategies. *Front Immunol*. (2023) 14:1101495. doi: 10.3389/fimmu.2023.1101495

55. O'Neal J, Ritchey JK, Cooper ML, Niswonger J, Sofia González L, Street E, et al. CS1 CAR-T targeting the distal domain of CS1 (SLAMF7) shows efficacy in high tumor burden myeloma model despite fratricide of CD8+CS1 expressing CAR-T cells. *Leukemia*. (2022) 36:1625–34. doi: 10.1038/s41375-022-01559-4

56. Liao C, Wang Y, Huang Y, Duan Y, Liang Y, Chen J, et al. CD38-specific CAR integrated into CD38 locus driven by different promoters causes distinct antitumor activities of T and NK cells. *Adv Sci (Weinh)*. (2023) 10:e2207394. doi: 10.1002/advs.202207394

57. Mihara K, Bhattacharyya J, Kitanaka A, Yanagihara K, Kubo T, Takei Y, et al. T-cell immunotherapy with a chimeric receptor against CD38 is effective in eliminating myeloma cells. *Leukemia*. (2012) 26:365–7. doi: 10.1038/leu.2011.205

58. Palaiologou M, Delladetsima I, Tiniakos D. CD138 (syndecan-1) expression in health and disease. *Histol Histopathol*. (2014) 29:177–89.

59. Frigault M, Rosenblatt J, Dhakal B, Raje N, Cook D, Gaballa MR, et al. Phase 1 study of CART-ddbcma for the treatment of subjects with relapsed and /or refractory multiple myeloma. *Blood*. (2022) 140:7439–40. doi: 10.1182/blood-2022-163827

60. Robillard N, Wuillème S, Moreau P, Béné MC. Immunophenotype of normal and myelomatous plasma-cell subsets. *Front Immunol*. (2014) 5:137. doi: 10.3389/fimmu.2014.00137

61. Lee H, Ahn S, Maity R, Leblay N, Ziccheddu B, Truger M, et al. Mechanisms of antigen escape from BCMA- or GPRC5D-targeted immunotherapies in multiple myeloma. *Nat Med*. (2023) 29:2295–306. doi: 10.1038/s41591-023-02491-5

62. Samur MK, Fulciniti M, Aktas Samur A, Bazarbachi AH, Tai YT, Prabhala R, et al. Biallelic loss of BCMA as a resistance mechanism to CAR T cell therapy in a patient with multiple myeloma. *Nat Commun*. (2021) 12:868. doi: 10.1038/s41467-021-21177-5

63. Da Vià MC, Dietrich O, Truger M, Arampatzi P, Duell J, Heidemeier A, et al. Homozygous BCMA gene deletion in response to anti-BCMA CAR T cells in a patient with multiple myeloma. *Nat Med*. (2021) 27:616–9. doi: 10.1038/s41591-021-01245-5

64. Laurent SA, Hoffmann FS, Kuhn PH, Cheng Q, Chu Y, Schmidt-Supprian M, et al. γ-Secretase directly sheds the survival receptor BCMA from plasma cells. *Nat Commun*. (2015) 6:7333. doi: 10.1038/ncomms8333

65. Chen H, Li M, Xu N, Ng N, Sanchez E, Soof CM, et al. Serum B-cell maturation antigen (BCMA) reduces binding of anti-BCMA antibody to multiple myeloma cells. *Leuk Res*. (2019) 81:62–6. doi: 10.1016/j.leukres.2019.04.008

66. Pont MJ, Hill T, Cole GO, Abbott JJ, Kelliher J, Salter AI, et al. γ-Secretase inhibition increases efficacy of BCMA-specific chimeric antigen receptor T cells in multiple myeloma. *Blood*. (2019) 134:1585–97. doi: 10.1182/blood.2019000050

67. Mehta PH, Fiorenza S, Koldej RM, Jaworowski A, Ritchie DS, Quinn KM. T cell fitness and autologous CAR T cell therapy in haematologic Malignancy. *Front Immunol*. (2021) 12:780442. doi: 10.3389/fimmu.2021.780442

68. Baur K, Buser A, Jeker LT, Khanna N, Läubli H, Heim D, et al. CD4+ CAR T-cell expansion is associated with response and therapy related toxicities in patients with B-cell lymphomas. *Bone Marrow Transpl*. (2023) 58:1048–50. doi: 10.1038/s41409-023-02016-1

69. Zhao WH, Liu J, Wang BY, Chen YX, Cao XM, Yang Y, et al. A phase 1, open-label study of LCAR-B38M, a chimeric antigen receptor T cell therapy directed against B cell maturation antigen, in patients with relapsed or refractory multiple myeloma. *J Hematol Oncol*. (2018) 11:141. doi: 10.1186/s13045-018-0681-6

70. Raje N, Berdeja J, Lin Y, Siegel D, Jagannath S, Madduri D, et al. Anti-BCMA CAR T-cell therapy bb2121 in relapsed or refractory multiple myeloma. *N Engl J Med*. (2019) 380:1726–37. doi: 10.1056/NEJMoa1817226

71. Rejeski K, Jain MD, Smith EL. Mechanisms of resistance and treatment of relapse after CAR T-cell therapy for large B-cell lymphoma and multiple myeloma. *Transplant Cell Ther*. (2023) 29:418–28. doi: 10.1016/j.jtct.2023.04.007

72. Cooke RE, Quinn KM, Quach H, Harrison S, Prince HM, Koldej R, et al. Conventional treatment for multiple myeloma drives premature aging phenotypes and metabolic dysfunction in T cells. *Front Immunol*. (2020) 11:2153. doi: 10.3389/fimmu.2020.02153

73. Liu Y, Chen W, Yu M, Li H, Cheng H, Cao J, et al. Absolute lymphocyte count prior to lymphodepletion impacts outcomes in multiple myeloma patients treated with chimeric antigen receptor T cells. *Transplant Cell Ther*. (2022) 28:118.e1–5. doi: 10.1016/j.jtct.2021.11.016

74. Stadel R, Liu R, Landesman Y, Wald D, Hosahalli Vasanna S, De Lima MJG. Sequential administration of selinexor then CD19 CAR-T cells exhibits enhanced efficacy in a mouse model of human non-Hodgkin's lymphoma. *Blood*. (2022) 140:7413–4. doi: 10.1182/blood-2022-164443

75. Pan DD, Mouhieddine TH, Fu W, Moshier E, Parekh S, Jagannath S, et al. Inflammatory biomarkers and outcomes in multiple myeloma patients after CAR T-cell therapy. *Blood*. (2023) 142:92–2. doi: 10.1182/blood-2023-188310

76. Mejia Saldarriaga M, Pan D, Unkenholz C, Mouhieddine TH, Velez-Hernandez JE, Engles K, et al. Absolute lymphocyte count after BCMA CAR-T therapy is a predictor of response and outcomes in relapsed multiple myeloma. *Blood Adv*. (2024) 8 (15):3859–69. doi: 10.1182/bloodadvances.2023012470

77. Sidana S, Peres LC, Hashmi H, Hosoya H, Ferreri C, Khouri J, et al. Idecabtagene vicleucel chimeric antigen receptor T-cell therapy for relapsed/refractory multiple myeloma with renal impairment. *Haematologica*. (2024) 109:777–86. doi: 10.3324/haematol.2023.283940

78. Quinn KM, Fox A, Harland KL, Russ BE, Li J, Nguyen THO, et al. Age-related decline in primary CD8+ T cell responses is associated with the development of senescence in virtual memory CD8+ T cells. *Cell Rep*. (2018) 23:3512–24. doi: 10.1016/j.celrep.2018.05.057

79. Akhtar OS, Hashmi H, Oloyede T, Brazauskas R, Bye M, Sidana S, et al. Real world outcomes of older adults and frail patients with relapse/refractory multiple myeloma receiving Idecabtagene vicleucel. *Transplant Cell Ther*. (2024) 30:S184–5. doi: 10.1016/j.jtct.2023.12.239

80. Joseph JM, Hillengass M, Cannioto R, Tario JD, Wallace PK, Attwood K, et al. T cell exhaustion markers in multiple myeloma patients are lower after physical activity intervention. *Clin Lymphoma Myeloma Leuk*. (2024) 26:S2152-2650(24)00153-8. doi: 10.1016/j.clml.2024.04.006

81. Elsallab M, Ellithi M, Lunning MA, D'Angelo C, Ma J, Perales MA, et al. Second primary Malignancies after commercial CAR T-cell therapy: analysis of the FDA Adverse Events Reporting System. *Blood*. (2024) 143:2099–105. doi: 10.1182/blood.2024024166

82. Mateos MV, Weisel K, De Stefano V, Goldschmidt H, Delforge M, Mohty M, et al. LocoMMotion: a prospective, non-interventional, multinational study of real-life current standards of care in patients with relapsed and/or refractory multiple myeloma. *Leukemia*. (2022) 36:1371–6. doi: 10.1038/s41375-022-01531-2

frontiers | Frontiers in Oncology

TYPE Mini Review
PUBLISHED 15 August 2024
DOI 10.3389/fonc.2024.1397186

OPEN ACCESS

EDITED BY
Jean El Cheikh,
American University of Beirut Medical Center, Lebanon

REVIEWED BY
Matthew Mei,
City of Hope National Medical Center, United States
Manali Kamdar,
University of Colorado, United States

*CORRESPONDENCE
Tamara K. Moyo
tamara.moyo@atriumhealth.org

RECEIVED 07 March 2024
ACCEPTED 22 July 2024
PUBLISHED 15 August 2024

CITATION
Moyo TK and Vaidya R (2024) Re-examining the role of hematopoietic stem cell transplantation in relapsed large B-cell lymphoma in the era of chimeric antigen receptor (CAR) T-cell therapy.
Front. Oncol. 14:1397186.
doi: 10.3389/fonc.2024.1397186

Re-examining the role of hematopoietic stem cell transplantation in relapsed large B-cell lymphoma in the era of chimeric antigen receptor (CAR) T-cell therapy

Tamara K. Moyo[1]* and Rakhee Vaidya[2]

[1]Department of Hematologic Oncology and Blood Disorders, Atrium Health Levine Cancer Institute, Charlotte, NC, United States, [2]Department of Hematology and Oncology, Atrium Health Wake Forest Baptist Comprehensive Cancer Center, Winston Salem, NC, United States

Historically, salvage chemoimmunotherapy with consolidative autologous hematopoietic stem cell transplantation (ASCT) was the only potentially curative therapeutic option for patients with relapsed/refractory large B-cell lymphoma (LBCL). Treatment options were few and outcomes poor for patients whose lymphoma failed to respond to salvage chemotherapy/ASCT and for patients not eligible for ASCT. The approval of chimeric antigen receptor (CAR) T-cell therapy for relapsed/refractory LBCL revolutionized the treatment landscape with unprecedented response rates and durability of responses. As a result, earlier intervention with CAR T-cell therapy has been explored, and the enthusiasm for CAR T-cell therapy has overshadowed ASCT. In this article, we will review the data available for ASCT and CAR T-cell therapy in relapsed LBCL and will examine the role for ASCT in relapsed/refractory LBCL in the era of CAR T-cell therapy.

KEYWORDS

relapsed large B-cell lymphoma (LBCL), CAR T-cell therapy, autologous stem cell transplant (ASCT), tisagenlecleucel, axicabtagene ciloleucel, lisocabtagene maraleucel

Introduction

Large B-cell lymphoma (LBCL) including *de novo* diffuse large B-cell lymphoma (DLBCL), primary mediastinal B-cell lymphoma (PMBCL), transformed indolent lymphoma, and high grade B-cell lymphoma (HGBL) have a typically aggressive course but are treatable and potentially curable types of B-cell non-Hodgkin lymphoma (B-NHL). The likelihood of achieving cure is variable and influenced by lymphoma characteristics such as histology and cytogenetics, by patient specific factors such as age, comorbidities and

access to care, and by the first line treatment strategy employed. All current first line treatment regimens for LBCL include multi-agent anthracycline-based chemotherapy, a steroid and an anti-CD20 antibody. The addition of the anti-CD20 antibody rituximab to first line therapy for LBCL in the early 2000s had a major impact on response rates and overall survival (1–3). More recently, in the phase 3 POLARIX study replacing vincristine in the standard R-CHOP regimen (rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone) with polatuzumab vedotin, an anti-CD79b antibody-drug conjugate, led to higher response rates in patients with advanced DLBCL (78% complete response rate in patients treated with pola-R-CHP vs. 74% with R-CHOP (4). Dose adjusted R-EPOCH (rituximab, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin), a high intensity regimen with extended infusions and escalating doses of chemotherapy, did not improve outcomes in DLBCL patients in the Alliance/CALGB 50303 study (5) but is still employed for some subtypes of LBCL based on single arm prospective studies or retrospective data (6, 7).

Although many LBCL patients may be cured with first line therapy, many will relapse. 30-40% of DLBCL patients may be refractory to or relapse after first line treatment with the highest risk of relapse within the first 2 years (8). In a multi-center retrospective analysis of high grade B-cell lymphoma with *MYC* and *BCL2* rearrangements (so called double hit lymphoma), 2y PFS and OS after first line chemoimmunotherapy were 40 and 49%, respectively (7). For those LBCL patients who either do not respond to first line therapy or who relapse after an initial response, curative treatment options are limited, and the likelihood of cure is slim. In the SCHOLAR-1 pooled analysis of patients with refractory DLBCL, defined as progressive or stable disease as best response to any line of therapy or relapse within 1 year after ASCT, who were included in 4 clinical trial and observational cohort datasets, response rates to salvage chemotherapy were uniformly low with a pooled response rate 26% and a pooled complete response rate just 7%, underscoring the need for alternative therapies for patients in this group (9). In an analysis of patients with relapsed aggressive LBCL treated with high intensity regimens like R-EPOCH in the first line setting, the overall response rate to second line chemoimmunotherapy was 44% but the median PFS just 3 months and OS 8 months (10). Patients who relapsed in a later time frame after first line therapy did have improved outcomes (8, 11). In general, a short duration of first remission or failure to achieve remission have categorized patients into a high risk group.

The treatment landscape is evolving for LBCL patients who either do not respond to or who relapse after first line therapy. Historically, patients with relapsed LBCL have been treated with salvage chemoimmunotherapy. Responses to second line chemoimmunotherapy may be consolidated with high dose chemotherapy followed by an ASCT in fit patients. However, only a subset of patients with chemosensitive relapsed LBCL will achieve a durable response to salvage therapy. Thus, there was still an unmet need for improved salvage therapies, particularly for patients who relapsed early after an initial response to first line therapy or who had chemo-refractory disease. Chimeric antigen receptor (CAR) T-cell therapy was developed in this space and revolutionized the treatment landscape for relapsed LBCL, offering another potentially curative treatment option to patients including those with chemo-refractory disease. This article will review the evolution of the treatment paradigm for patients with relapsed/refractory LBCL and explore the role for ASCT in the modern era of cellular therapy.

Salvage chemotherapy and ASCT in relapsed/refractory LBCL

In the pre-CAR T-cell era, the only potentially curative option for patients whose LBCL relapsed after anthracycline-containing induction chemotherapy was salvage chemotherapy followed by consolidative ASCT. Consolidative ASCT improves duration of remission, PFS, and OS in patients who respond to salvage chemotherapy. In the pre-rituximab era, the Parma group demonstrated a clear benefit of ASCT in patients who had chemo-sensitive relapsed B-NHL. In the Parma study, patients with relapsed B-NHL who had a response after 2 cycles of salvage DHAP (dexamethasone, cisplatin and cytarabine) were randomized either to receive an additional 4 cycles of DHAP + radiotherapy or to ASCT + radiotherapy. For those who responded to DHAP, outcomes were significantly better in the group who also received high dose chemotherapy and ASCT. At 5 years, both event free survival (EFS) and overall survival (OS) were significantly higher in the ASCT group compared to no ASCT (5y EFS: 46% vs 12%, 5y OS: 53% vs 32%) (12). As a result of this study, high dose chemotherapy and ASCT became standard of care for patients with relapsed LBCL that responded to second-line chemotherapy.

Multiple studies have shown that outcomes are similarly improved after ASCT in the rituximab era. The addition of rituximab to second-line therapy improved the likelihood of achieving a response which made ASCT more attainable (13). In the CORAL study, patients treated with rituximab-containing second-line therapy followed by high dose chemotherapy and ASCT had a 3 year PFS of 53% (14). In a retrospective European Blood and Marrow Transplantation registry study, 5 year disease free survival (DFS) after ASCT in the rituximab era was 48% (15). The more recent ORCHARRD study randomized patients with relapsed DLBCL to second line treatment with R-DHAP followed by ASCT or ofatumumab-DHAP (O-DHAP) followed by ASCT. There were no statistically significant differences in PFS, EFS or OS between the patients treated with R-DHAP or O-DHAP. Fewer than 40% of patients on either arm achieved a complete or partial response to second-line therapy and received ASCT per protocol. For those patients who did receive consolidative ASCT, outcomes were similar to those in PARMA and CORAL (2 year PFS and OS in the ORCHARRD study for patients treated with R-DHAP + ASCT were 52% and 68%, respectively). Highlighting the need for alternative non-chemotherapy salvage regimens, the 2y PFS and OS of patients treated with R-DHAP irrespective of transplant status were 26% and 38%, respectively) (16).

Duration of response to first-line therapy has consistently demonstrated an impact on outcomes after transplant. Patients with primary refractory LBCL or early relapse (e.g. relapse <12 months from diagnosis) are more likely to be chemo-refractory and

have a relatively poor prognosis. In a multiregression analysis of prognostic factors predicting a response to DHAP salvage therapy in the Parma study, patients who relapsed > 12 months after diagnosis were nearly 3 times as likely to achieve a response (17). Regardless of whether they had relapsed early or late after first line therapy, the relative risk of progression was similarly increased in patients who responded to DHAP but who did not receive ASCT as compared to those who received ASCT. However, more than half of patients with early relapse who received ASCT after DHAP had progressed in the first year, whereas patients with late relapse had median PFS closer to 5 years (17). In the CORAL study patients who received rituximab-containing therapy and relapsed within 12 months of diagnosis had 3y PFS 39% with ASCT vs 14% without ASCT (14), providing further evidence that ASCT does improve outcomes regardless of time to relapse. However, the 3y PFS in the patients with early relapse still fell short of the 53% 3y PFS in all patients treated with ASCT on the study regardless of time to relapse (14). In both Parma and CORAL, ASCT improved PFS, but there was a significant gap in outcomes of patients with early relapse receiving ASCT compared to the entire group who received ASCT. The ORCHARRD study also demonstrated improved outcomes in patient with late relapse (>12 months from first-line therapy) as compared to patients who relapsed early after or who had suboptimal response to first-line therapy (16).

Response to therapy prior to ASCT and depth of response are important predictors of outcomes after ASCT in relapsed LBCL. Although ASCT may prolong the disease free interval, patients with active disease at the time of ASCT typically fail to achieve a cure. In an international multicenter study of patients with refractory LBCL, zero of 34 patients with refractory disease transplanted without ever having achieved remission were alive at 3 years (18). Patients who had an initial response to first line therapy but no response to second-line therapy had 3y DFS 14% after ASCT, whereas patients with chemo-sensitive lymphoma who achieved a response after both first and second line therapy had 3y DFS 30% after ASCT (18). Improved PFS was seen in patients who achieved complete response to rituximab containing salvage regimens prior to ASCT in the CORAL study as compared to patients who had only a partial response to therapy (14).

Multiple studies have demonstrated the prognostic importance of 18-fluorodeoxyglucose positron emission tomography (FDG-PET) after salvage chemotherapy but before ASCT in relapsed LBCL. In one study, only 3 of 30 patients with a negative FDG-PET prior to ASCT had relapsed with a median PFS of 1083 days, whereas only 4 patients with a positive FDG-PET prior to ASCT were still in remission with median PFS 402 days (19). In another study, median PFS of patients with a negative FDG-PET prior to ASCT was not reached vs 15.4 months in those with a positive FDG-PET (20). In an analysis of 129 patients with relapsed/refractory DLBCL, pre-ASCT FDG-PET response was the only pre-transplant risk factor that predicted both PFS and OS after ASCT. The 3y PFS and OS for patients with chemo-sensitive relapsed LBCL who achieved a complete metabolic response (Deauville score 1-3) on pre-transplant FDG-PET was 77% and 86% respectively versus 49% and 54% respectively in patients with partial response (Deauville 4) on FDG-PET prior to ASCT (21). Patients in the ORCHARRD study who had a complete response based on FDG-PET imaging after 3 cycles of salvage R-DHAP/O-DHAP also had significantly improved PFS/OS after ASCT than patients who had a positive FDG-PET scan (16).

Although consolidative ASCT has demonstrated improved outcomes in patients who respond to salvage chemoimmunotherapy, it is important to consider that many patients do not respond to salvage therapy or are not candidates for ASCT. In the Parma study, more than 40% of patients did not achieve a response to salvage DHAP and were not eligible for ASCT (12). Although many studies have shown that it is feasible to transplant elderly patients with relapsed lymphoma (22–24), the data are challenging to interpret in large part due to the retrospective nature of the studies with inherent selection bias and variable definitions of "elderly". Perhaps more important than age, patient comorbidities may impact the risk of non-relapse mortality with ASCT (25). Patients typically must undergo rigorous testing to ensure fitness for ASCT. For patients who have no response or only partial response to salvage therapy or who may be unfit for transplantation due to comorbidities, a more accessible approach with similarly improved chance to achieve disease control/cure was lacking until the advent of CAR T-cell therapy.

CAR T-cell therapy in relapsed LBCL

CAR T-cell therapy was developed to address the unmet need for effective therapies with durable response in patients with relapsed/refractory LBCL. A detailed description of CAR T-cell features and manufacturing is beyond the scope of this review article. Simply put, autologous CAR T-cell therapy for lymphoma involves the collection of a patient's own T-cells through leukapheresis and genetic modification of the T-cells to express a chimeric receptor with coactivation domains that home the activated T cells to the lymphoma upon reinfusion into the patient. All currently approved CAR T-cell products for LBCL target the CD19 protein on the surface of the lymphoma cells, although other targets are actively under investigation. Lymphodepletion chemotherapy (LDC), typically incorporating fludarabine and cyclophosphamide, is given prior to CAR T-cell therapy to create a more hospitable environment for CAR T-cells to flourish and proliferate.

There are now three approved autologous CAR T-cell products for relapsed/refractory LBCL available in the United States. Axicabtagene ciloleucel (axi-cel) was the first approved CD-19 directed CAR T-cell therapy for relapsed/refractory LBCL after two prior lines of therapy. In the ZUMA-1 phase 2 clinical trial, axi-cel was administered to 111 patients with refractory DLBCL, PMBCL, or transformed follicular lymphoma (tFL). Response rates were unprecedented, with an 82% objective response rate (ORR) and 54% complete response (CR) rate (26). After a median follow-up of 63 months, median duration of response to axi-cel was 11.1 months but 31% of patients had ongoing response at 5 years (27). In the TRANSCEND NHL 001 study, 269 patients with relapsed or refractory LBCL were treated with lisocabtagene

maraleucel (liso-cel). At a median follow-up of 19.9 months, the ORR and CR rate to liso-cel were 73% and 53% respectively. The median duration of response to liso-cel was 23.1 months (28). In the JULIET study, after a median follow-up of 40 months, ORR and CR rate in 93 patients treated with tisagenlecleucel (tisa-cel) were 53% and 39% respectively (29). As a result of these studies, axi-cel, liso-cel, and tisa-cel received initial regulatory approvals for relapsed/refractory LBCL after two prior lines of therapy. Real world analyses have largely confirmed the responses to CAR T-cell therapy seen in the registrational clinical trials.

Whereas side effects of consolidative high dose chemotherapy followed by ASCT are similar to side effects typical of salvage chemoimmunotherapy, CAR T-cell therapy has a unique side effect profile. Acute side effects of CAR T-cell therapy include cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS), but patients receiving CAR T-cell therapy may also experience prolonged cytopenias, hypogammaglobulinemia, and increased risk of infections. The risk of side effects varies widely amongst the three CAR T-cell products. Westin, et al. presented a comparison of safety and efficacy of the three commercially available anti-CD19 CAR T-cell products in the ZUMA-1, TRANSCEND and JULIET trials, with rates of any grade CRS ranging from 42% - 92% and of any grade ICANS 21% - 67%. The majority of CRS and ICANS events were grade 1/2, regardless of the product (30).

Although adverse events due to CAR T-cell therapy are common, the majority are low grade and manageable with conventional means including tocilizumab for CRS or steroids for ICANS. Thus, elderly or unfit patients are not excluded from CAR T-cell therapy. Patients > 70 years treated with CAR T-cell therapy had similar kinetics of T cell expansion, similar rates of grade ≥ 3 CRS or ICANS, and similar outcomes as younger patients undergoing the same therapy. Objective response rate was 63% in patients > 70 years and CR rate 46% (31). PFS and OS at 1 year were 32% and 69% respectively (31). Although poor performance status and comorbidities did predict for inferior survival after CAR T-cell therapy, presence of comorbidities was not associated with incidence of CRS, ICANS or admission to ICU (32).

In addition to the distinct side effects of CRS and ICANS, there are shared side effects between ASCT and CAR T-cell therapy. The risk of cytopenias after CAR T-cell therapy is variable and influenced by the lymphodepletion chemotherapy, the CAR T-cell product, inflammatory responses to CAR T-cell therapy (e.g. severity of CRS or immune-effector cell-associated hemophagocytic syndrome or IEC-HS), presence of infections, disease burden, among others. In a subset of patients, cytopenias may be severe and/or prolonged. Up to 16% of patients remained neutropenic and up to 38% thrombocytopenic more than 3 months after CAR T-cell infusion on the ZUMA-1, TRANSCEND and JULIET trials (26, 28, 29). For comparison, in one large retrospective study including 1182 patients who received ASCT, none had neutropenia persisting beyond 30 days, whereas 9.6% had thrombocytopenia beyond 90 days after ASCT (33). Bacterial and viral infections are also common after both ASCT and CAR T-cell therapy, with the risk increased in patients with a history of infections prior to CAR T-cell therapy and in patients treated with corticosteroids for CRS or ICANS (34). Finally, the incidence of second primary malignancy after CAR T-cell therapy is an area of particular interest after the FDA released a report of secondary T-cell neoplasms (35). A large multi-center retrospective study of 582 patients who received CAR T-cell therapy for relapsed LBCL, identified 45 cases (8.2%) with second primary malignancy, the most common of which was myelodysplastic syndrome, diagnosed at a median of 19.3 months after CAR T-cell infusion (36). The incidence of second primary malignancy after ASCT is estimated to be 10-15% at 15 years after ASCT (37, 38).

The impact of tumor burden on CAR T-cell efficacy has also been explored in retrospective analyses. Although the registrational studies required patients undergoing CAR T-cell therapy to have measurable disease, retrospective real-world analyses have shown that CAR T-cell therapy may perform better with lower tumor burden. Patients with low metabolic tumor volume treated with axi-cel for relapsed/refractory LBCL had improved OS and PFS as compared to patients with high metabolic tumor volume (39). Wudhikarn et al. reported good outcomes for patients who had no residual lymphoma at the time of CAR T-cell therapy. At 1 year post CAR T-cell therapy, only 39.4% of patients had relapsed and OS was 81.3% (40). In fact optimal bridging therapy may reduce the risk of disease progression or death by as much as 40% after CAR T-cell therapy (41). Radiation has been explored as bridging therapy and was found to effectively cytoreduce bulky tumors, lower LDH, and reduce metabolic tumor volume, all of which have been associated with poor responses to CAR T-cell therapy (42, 43). Importantly, bridging radiation had no adverse impact on safety outcomes.

CAR T-cell therapy versus ASCT in relapsed LBCL

Given the unprecedented responses to CAR T-cell therapy in patients with relapsed/refractory LBCL after at least 2 lines of therapy, attention turned to optimizing the risk/benefit ratio of CAR T-cell therapy through a variety of mechanisms. One consideration was whether the efficacy could be improved and risks reduced by employing CAR T-cell therapy earlier in the treatment paradigm for LBCL. Prospective clinical trials were launched that randomized LBCL patients in first relapse to receive standard of care therapy with second line chemoimmunotherapy followed by consolidative ASCT in patients with chemo-sensitive relapse or a commercially available CAR T-cell product. Salvage chemotherapy plus ASCT was compared to axi-cel in the ZUMA-7 clinical trial, to liso-cel in the TRANSFORM study, and to tisa-cel in the BELINDA clinical trial (Table 1).

In the phase 3 ZUMA-7 study, 180 patients with primary refractory/early relapsed LBCL were randomized to receive axi-cel, and 179 patients to receive salvage chemoimmunotherapy plus ASCT as per standard of care (SOC). Bridging chemotherapy was not allowed in the axi-cel arm. Notably drop-out in the SOC arm was high, mostly due to lack of response to salvage chemotherapy, with only 64 (36%) randomized patients receiving salvage chemotherapy and ASCT per protocol as compared to 96% of patients randomized to the axi-cel arm who received the infusion

(44). Median progression free survival in the axi-cel arm was 14.7 months and 3.7 months in the SOC arm. Axi-cel was favored as second line treatment overall (HR 0.40, 95% CI 0.31-0.51). There were no subgroups in which SOC was favored (44). Despite that more than 50% of patients treated on the SOC arm went on to receive cellular immunotherapy off protocol, there was improved overall survival in the axi-cel arm (median not reached vs 35.1 months in the SOC arm), although the difference was not statistically significant. Interestingly, a naïve T-cell phenotype ($CCR7^+CD45RA^+$) thought to represent stem memory T-cells was more prevalent in patients treated with axi-cel in ZUMA-7 as compared to ZUMA-1 and were associated with improved progression free survival and duration of response but not toxicity, providing a plausible scientific explanation for the improved PFS in the ZUMA-7 trial and suggesting that T-cells may be more effective if collected and engineered to become axi-cel earlier in the treatment algorithm for relapsed LBCL (47).

In the phase 3 TRANSFORM study, patients with primary refractory or early relapsed LBCL were randomized to receive liso-cel or SOC with salvage chemo-immunotherapy and ASCT if chemo-sensitive. Bridging therapy was common (63%) in the liso-cel arm. Nearly 46% of patients randomized to SOC did receive

TABLE 1 Pivotal trials comparing CAR T-cell therapy and Standard of Care in first relapse.

	ZUMA-7[a]		TRANSFORM[b]		BELINDA[c]	
	Axi-cel N=180	SOC N=179	Liso-cel N=92	SOC N=92	Tisa-cel N=162	SOC N=160
Patient characteristics						
Median age, years (range)	58 (21 - 80)	60 (26-81)	60 (20 - 74)	58 (26 - 75)	59.5 (19 - 79)	58 (19 - 77)
Age ≥ 65 years, no. (%)	51 (28)	58 (32)	36 (39)	25 (27)	54 (33.3)	46 (28.8)
Male sex, no. (%)	110 (61)	127 (71)	44 (48)	61 (66)	103 (63.6)	98 (61.2)
White, no. (%)	145 (81)	152 (85)	Not reported	Not reported	128 (79)	128 (80)
AA-IPI ≥2[a, b] or IPI ≥2 [c], no. (%)	82 (46)	79 (44)	36 (39)	37 (40)	106 (65.4)	92 (57.5)
ECOG PS 1, no. (%)	85 (47)	79 (44)	44 (48)	35 (38)	70 (43.2)	65 (40.6)
Clinical characteristics						
Stage III or IV, no. (%)	139 (77)	146 (82)	68 (74)	63 (68)	107 (66.0)	98 (61.3)
High grade B-cell lymphoma, no. (%)	31 (17)	26 (15)	22 (24)	21 (23)	39 (24.1)	27 (16.9)
Double expressor lymphoma, no. (%)	57 (32)	62 (35)	Not reported	Not reported	Not reported	Not reported
Relapse at ≤12 months after first line therapy, no. (%)	47 (26)	48 (27)	25 (27)	22 (24)	55 (34.0)	53 (33.1)
Refractory to first-line therapy, no. (%)	133 (74)	131 (73)	67 (73)	70 (76)	107 (66.0)	107 (66.9)
Received bridging therapy, no. (%)	65 (36)	—	58(63)	—	135 (83.3)	—
Type of bridging therapy allowed	glucocorticoids	—	platinum based therapy (1 cycle)	—	platinum based therapy	—
Received CAR-T or ASCT, per protocol, no. (%)	170 (94)	64 (36)	89 (97)	43 (47)	155 (97.5)	52 (32.5)
Received CAR-T crossover, no. (%)	—	N/A	—	58 (63)	—	81 (50.6)
Efficacy						
Median EFS, months (95% CI)	8.3 (4.5 - 15.8)	2.0 (1.6 - 2.8)	NR (9.5 - NR)	2.4 (2.2 - 4.9)	3.0 (3.0 - 3.5)	3.0 (2.9 - 4.2)
Estimated 2-year[a] or 18-month[b] EFS, % (95% CI)	41 (33 - 48)	16 (11 - 22)	52.6 (42.3 - 62.9)	20.8 (12.2 - 29.5)	Not reported	Not reported
CR, no. (%)	110 (61)	61 (34)	68 (74)	40 (43)	46 (28.4)	44 (27.5)
Median OS, months (95% CI)	NR (28.3 - NE)	35.1 (18.5 - NE)	NR (29.5 - NR)	29.9 (17.9 - NR)	16.9 (11.1 - NE)	15.3 (12.3 - NE)
Median PFS, months (95% CI)	14.7 (5.4 - NE)	3.7 (2.9 – 5.3)	NR (12.6 - NR)	6.2 (4.3 - 8.6)	Not reported	Not reported
Estimated 2-year[a] or 18-month[b] PFS, % (95% CI)	46 (38 - 53)	27 (20 - 35)	58.2 (47.7 - 68.7)	28.8 (17.7 - 40.0)	Not reported	Not reported

(Continued)

TABLE 1 Continued

	ZUMA-7[a]		TRANSFORM[b]		BELINDA[c]	
	Axi-cel N=180	SOC N=179	Liso-cel N=92	SOC N=92	Tisa-cel N=162	SOC N=160
Safety						
Grade ≥3 Adverse Event, no. (%)	155 (91)	140 (83)	85 (92)	81 (89)	136 (84)	144 (90)
Grade ≥3 Neutropenia, no. (%)	118 (69)	69 (41)	75 (82)	47 (52)	65 (40.1)	63 (39.4)
Grade ≥3 Thrombocytopenia, no. (%)	25 (15)	95 (57)	46 (50)	62 (68)	52 (32.1)	76 (47.5)
Any Grade ≥3 prolonged cytopenia, no. (%)	49 (29)	12 (19)	40 (43)	3 (3)	Not reported	Not reported
Grade ≥3 Febrile Neutropenia, no. (%)	4 (2)	46 (27)	11 (12)	21 (23)	21 (13.0)	40 (25.0)
Grade ≥3 CRS, no. (%)	11 (6)	—	1 (1)	—	8 (5.2)	—
Grade ≥3 Neurologic Event, no. (%)	36 (21)	1 (1)	4 (4)	Not reported	3 (1.9)	Not reported

AA-IPI age adjusted international prognostic index; ASCT autologous stem cell transplant; Axi-cel axicabtagene ciloleucel; CAR-T chimeric antigen receptor T-cell therapy; CI confidence interval; CR complete response; CRS cytokine release syndrome; ECOG PS Eastern Cooperative Oncology Group Performance Status; EFS event free survival; IPI international prognostic index; Liso-cel lisocabtagene maraleucel; NE not estimable; NR not reached; OS overall survival; PFS progression free survival; SOC standard of care (e.g. salvage chemotherapy +/- ASCT); Tisa-cel tisagenlecleucel.
[a]ZUMA-7 (44).
[b]TRANSFORM (45).
[c]BELINDA (46).

ASCT. At a median follow-up of 17.5 months, median EFS was significantly prolonged in the liso-cel group (not reached versus 2.4 months in the SOC arm). Differences in median overall survival favored liso-cel but did not reach statistical significance (mOS not reached with liso-cel versus 6.2 months in the SOC arm; HR = 0.724; p 0.0987)) (45).

In the phase 3 BELINDA study, patients with refractory or early relapsed LBCL were randomized to receive tisa-cel (n= 162) or SOC chemoimmunotherapy and ASCT (n=160). There were important differences in the treatment groups, with a higher percentage of patients with HGBL and high IPI scores in the tisa-cel group. A majority of patients randomized to receive tisa-cel received bridging therapy (83%). Notably, patients treated on the SOC arm had response assessed after 6 weeks of therapy and if response was inadequate, they could receive another SOC therapy prior to ASCT. 54% of patients in the SOC arm did in fact receive more than one SOC salvage therapy, and yet only 32.5% went on to receive ASCT. The ORR and CR rates were not statistically different in the tisa-cel or SOC therapy arms. Likewise median survival was not statistically different in the two arms (median EFS 3.0 months in both groups and median OS 16.9 months in the tisa-cel arm vs 15.3 months in the SOC arm) (46). The results of the BELINDA trial did not support use of tisa-cel in the second line setting, potentially because of the inclusion of patients with higher risk in the tisa-cel arm or because manufacturing time was significantly longer than with the other two anti-CD19 CAR T-cell products, allowing more time for relapse to occur before tisa-cel infusion.

Despite the disappointing results in the BELINDA trial, the prospective ZUMA-7 and TRANSFORM studies underscore the potential for axi-cel and liso-cel in a high risk group of patients with LBCL enriched for chemo-refractory lymphoma and with expectedly poor outcomes with ASCT. As a result of these studies, axi-cel and liso-cel have received approval for treatment of LBCL patients in the second line who are either refractory to first line therapy or who relapse early (within 12 months of first-line therapy). Additionally, liso-cel is approved for second line therapy in patients who are not transplant-eligible based on age or comorbidities, regardless of timing of relapse with respect to their first line therapy.

Recent registry database studies may re-kindle the fire for ASCT in patients with chemo-sensitive relapsed LBCL. One study included patients with relapsed LBCL in partial response at the time of ASCT or CAR T included in the Center for International Blood & Marrow Transplant Research (CIBMTR) registry database. There were lower relapse rates and improved 2y OS in the ASCT group as compared to the axi-cel group (relapse rate 40% with ASCT vs 53% with axi-cel; 2y OS 69% for the patients who received ASCT vs 47% for patients who received axi-cel) (48). Similarly for patients with relapsed LBCL in the CIBMTR database who received additional chemoimmunotherapy and achieved a complete remission prior to CAR T-cell therapy or ASCT, outcomes were better for patients whose response was consolidated with ASCT as compared to CAR T-cell therapy. Relapse rate and OS at 2 years were 48% and 65.6% respectively in patients treated with CAR T-cell therapy as compared to 27.8% and 78.9% respectively in the ASCT group (49). Higher 2y relapse rate and inferior 2y PFS were also seen in the subgroup of patients with early relapse who achieved CR prior to CAR T-cell therapy as compared to ASCT (relapse rate 45.9% in CAR T-cell cohort vs 22.8% in ASCT; 2y PFS 48.3% after CAR T-cell therapy vs 70.9% after ASCT) (49). Although intriguing, the majority of patients treated with CAR T-cell therapy in this study received tisa-cel, which in both the JULIET and BELINDA studies had less favorable outcomes after CAR T-cell therapy as compared to outcomes of axi-cel or liso-cel in their respective phase 3 studies. The applicability of these findings at centers where the preferred CAR T-cell product is other than tisa-cel is uncertain. Prospective studies will be necessary to fully elucidate the optimal strategy for sequencing transplant and

cellular therapy in patients with relapsed LBCL, especially those who are not at the extreme ends of relapse risk.

Discussion

For decades, the only potentially curative option for patients with relapsed DLBCL was ASCT which was associated with 5-year OS of approximately 50% (12). Unfortunately, two thirds of patients with relapsed DLBCL were not candidates for ASCT due to advanced age, comorbidities, or chemo refractory disease. Long term outcomes in these patients was exceedingly poor. The availability of CAR T-cell therapy drastically improved outcomes in these patients, with more than 30% of patients achieving a sustained and durable remission after a single infusion of CD19-directed CAR T-cells. These impressive results in the third line (and beyond) setting led to randomized studies comparing CAR T-cell therapy to ASCT in second line. Results for two of the 3 randomized studies showed significantly improved outcomes with CAR-T in the second line as compared to salvage chemoimmunotherapy followed by ASCT. Notably, only one third of the patients who were randomized to the ASCT arm received ASCT; this is because a high proportion of patients had chemo refractory disease after salvage chemotherapy and were eventually treated with CAR T-cells off protocol.

These data clearly highlight the superiority of CAR T-cell therapy over ASCT in patients who have primary refractory disease or early relapse after front line chemoimmunotherapy. A significant number of these patients have high risk disease characteristics such as double hit lymphoma or high grade B-cell lymphoma intermediate between DLBCL and Burkitt. Our consensus for this group of patients is to proceed with CAR T-cell therapy since additional cytotoxic chemotherapy is unlikely to achieve long term disease control.

Another group of patients where CAR T-cell therapy is clearly superior to ASCT is older patients who are not candidates for ASCT due to frailty or other medical comorbidities. Real-world data from CAR T-cell therapy shows that patients who are older and have co-morbidities have similar outcomes after CAR T-cell therapy as those who were treated on the initial pivotal CAR-T clinical trials which had stringent inclusion and exclusion criteria (31, 50). The toxicities of CAR T-cell therapy including CRS and neurotoxicity can often be successfully treated with early recognition, prompt escalation of care, and medications such as tocilizumab and steroids. On the contrary, ASCT carries significantly higher toxicity due to the intensity of conditioning therapy which causes a significant period of cytopenias, mucosal toxicity, and risk of infection. Thus, our consensus is to proceed with CAR T-cell therapy for patients who are not candidates for ASCT due to older age, frailty, or co-morbidities, regardless of the timing of relapse after front line chemoimmunotherapy.

While the role of ASCT in treatment of relapsed DLBCL has significantly declined after availability of CAR T, there is still a sub group of patients where ASCT may be superior to CAR T-cell therapy. This subset includes patients who have disease relapse in a later timeframe after front line therapy and who are fit for ASCT. In this group of patients, it is reasonable to discuss pros and cons of ASCT and consider two to three cycles of platinum-containing salvage chemotherapy. Approximately half of the patients receiving salvage chemotherapy will have chemo refractory disease and will not be able to proceed with ASCT, eventually requiring CAR T-cell therapy. However, based on retrospective data from CIBMTR, a proportion of patients who achieve a complete or partial response to salvage chemotherapy may have better outcomes with ASCT as compared to CAR T-cell therapy (49). Similarly for patients who have already received second line chemoimmunotherapy prior to their referral for cellular therapy, the CIBMTR data would support proceeding to ASCT if they have achieved an optimal response. If relapse occurs after ASCT in these patients, the efficacy of CAR T-cell therapy in later lines of therapy is well-established with 5 year PFS of approximately 40%.

There are additional factors which could impact the decision making process around sequencing of therapies for patients with relapsed/refractory LBCL. While the efficacy of CAR T-cell therapy is well-established in patients who fail salvage chemotherapy and ASCT, it is unknown if a patient who failed second line CAR T-cell

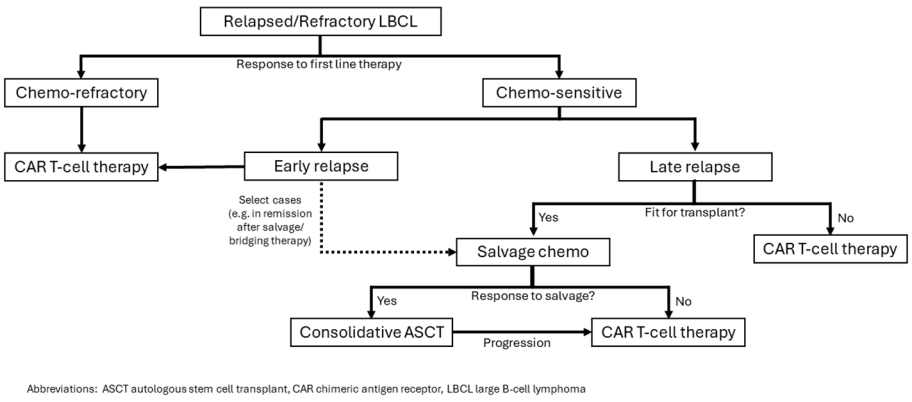


FIGURE 1
Our approach to selection of CAR T-cell therapy or ASCT for patients with relapsed/refractory LBCL.

therapy might benefit from consolidation with ASCT after later lines of therapy or if autologous stem cell collection would even be feasible after CAR T-cell therapy. The advent of newer therapies may force us to re-examine the role of CAR T-cell therapy and sequencing of therapies for relapsed/refractory LBCL in the future. As an example, two CD20 x CD3 bispecific antibodies epcoritamab and glofitamab have been approved for use in relapsed LBCL, eliciting durable responses with lower rates of CRS or neurotoxicity as compared to CAR T-cell therapy, although no prospective studies have directly compared bispecific antibodies to CAR T-cell therapy. The incorporation of bispecific antibodies into earlier lines of therapy is an area of active investigation. It is unknown if bispecific antibody therapy could lead to T cell exhaustion or alteration of the T cell milieu in a way that could impact the manufacturing or efficacy of CAR T-cell therapy.

In summary, CAR T-cell therapy has superseded ASCT in the treatment of relapsed DLBCL for a vast majority of patients. A small subset of young, fit patients who have a late relapse and chemotherapy sensitive disease may still have better outcomes with ASCT. Our approach (Figure 1) is to consider CAR T-cell therapy in patients who fail to achieve CR during first line of therapy or patients who relapse and are unfit for ASCT. For fit patients with late relapse, salvage chemoimmunotherapy followed by consolidative ASCT is favored. For patients fit for transplant who have an initial response to first line chemotherapy but relapse early, a careful weighing of the pros and cons of each approach may be warranted. Proceeding directly to CAR T-cell therapy with axi-cel or liso-cel would be supported by the ZUMA-7 and TRANSFORM studies. However, if the patient achieved a complete response to bridging chemoimmunotherapy, consolidation with ASCT and reserving CAR T-cell therapy for later relapse could be considered and supported by the CIBMTR data analyses.

Author contributions

TM: Writing – original draft, Writing – review & editing. RV: Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that no financial support was received for the research, authorship, and/or publication of this article.

Acknowledgments

The authors express their deep appreciation to the Leon Levine Foundation and the Kerry and Simone Vickar Family Foundation for their support.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Coiffier B, Lepage E, Briere J, Herbrecht R, Tilly H, Bouabdallah R, et al. CHOP chemotherapy plus rituximab compared with CHOP alone in elderly patients with diffuse large-B-cell lymphoma. *N Engl J Med.* (2002) 346:235–42. doi: 10.1056/NEJMoa011795

2. Habermann TM, Weller EA, Morrison VA, Gascoyne RD, Cassileth PA, Cohn JB, et al. Rituximab-CHOP versus CHOP alone or with maintenance rituximab in older patients with diffuse large B-cell lymphoma. *J Clin Oncol.* (2006) 24:3121–7. doi: 10.1200/JCO.2005.05.1003

3. Pfreundschuh M, Trumper L, Osterborg A, Pettengell R, Trneny M, Imrie K, et al. CHOP-like chemotherapy plus rituximab versus CHOP-like chemotherapy alone in young patients with good-prognosis diffuse large-B-cell lymphoma: a randomised controlled trial by the MabThera International Trial (MInT) Group. *Lancet Oncol.* (2006) 7:379–91. doi: 10.1016/S1470-2045(06)70664-7

4. Tilly H, Morschhauser F, Sehn LH, Friedberg JW, Trneny M, Sharman JP, et al. Polatuzumab vedotin in previously untreated diffuse large B-cell lymphoma. *N Engl J Med.* (2022) 386:351–63. doi: 10.1056/NEJMoa2115304

5. Bartlett NL, Wilson WH, Jung SH, Hsi ED, Maurer MJ, Pederson LD, et al. Dose-adjusted EPOCH-R compared with R-CHOP as frontline therapy for diffuse large B-cell lymphoma: clinical outcomes of the phase III intergroup trial alliance/CALGB 50303. *J Clin Oncol.* (2019) 37:1790–9. doi: 10.1200/JCO.18.01994

6. Dunleavy K, Pittaluga S, Maeda LS, Advani R, Chen CC, Hessler J, et al. Dose-adjusted EPOCH-rituximab therapy in primary mediastinal B-cell lymphoma. *N Engl J Med.* (2013) 368:1408–16. doi: 10.1056/NEJMoa1214561

7. Petrich AM, Gandhi M, Jovanovic B, Castillo JJ, Rajguru S, Yang DT, et al. Impact of induction regimen and stem cell transplantation on outcomes in double-hit lymphoma: a multicenter retrospective analysis. *Blood.* (2014) 124:2354–61. doi: 10.1182/blood-2014-05-578963

8. Maurer MJ, Ghesquieres H, Jais JP, Witzig TE, Haioun C, Thompson CA, et al. Event-free survival at 24 months is a robust end point for disease-related outcome in diffuse large B-cell lymphoma treated with immunochemotherapy. *J Clin Oncol.* (2014) 32:1066–73. doi: 10.1200/JCO.2013.51.5866

9. Crump M, Neelapu SS, Farooq U, Van Den Neste E, Kuruvilla J, Westin J, et al. Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. *Blood.* (2017) 130:1800–8. doi: 10.1182/blood-2017-03-769620

10. Ayers EC, Li S, Medeiros LJ, Bond DA, Maddocks KJ, Torka P, et al. Outcomes in patients with aggressive B-cell non-Hodgkin lymphoma after intensive frontline treatment failure. *Cancer.* (2020) 126:293–303. doi: 10.1002/cncr.32526

11. Hilton LK, Ngu HS, Collinge B, Dreval K, Ben-Neriah S, Rushton CK, et al. Relapse timing is associated with distinct evolutionary dynamics in diffuse large B-cell lymphoma. *J Clin Oncol.* (2023) 41:4164–77. doi: 10.1200/JCO.23.00570

12. Philip T, Guglielmi C, Hagenbeek A, Somers R, van der Lelie H, Bron D, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. *N Engl J Med.* (1995) 333:1540–5. doi: 10.1056/NEJM199512073332305

13. Vellenga E, van Putten WL, van 't Veer MB, Zijlstra JM, Fibbe WE, van Oers MH, et al. Rituximab improves the treatment results of DHAP-VIM-DHAP and ASCT in relapsed/progressive aggressive CD20+ NHL: a prospective randomized HOVON trial. *Blood.* (2008) 111:537–43. doi: 10.1182/blood-2007-08-108415

14. Gisselbrecht C, Glass B, Mounier N, Singh Gill D, Linch DC, Trneny M, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. *J Clin Oncol.* (2010) 28:4184–90. doi: 10.1200/JCO.2010.28.1618

15. Mounier N, Canals C, Gisselbrecht C, Cornelissen J, Foa R, Conde E, et al. High-dose therapy and autologous stem cell transplantation in first relapse for diffuse large B

cell lymphoma in the rituximab era: an analysis based on data from the European Blood and Marrow Transplantation Registry. *Biol Blood Marrow Transplant*. (2012) 18:788–93. doi: 10.1016/j.bbmt.2011.10.010

16. van Imhoff GW, McMillan A, Matasar MJ, Radford J, Ardeshna KM, Kuliczkowski K, et al. Ofatumumab versus rituximab salvage chemoimmunotherapy in relapsed or refractory diffuse large B-cell lymphoma: the ORCHARRD study. *J Clin Oncol*. (2017) 35:544–51. doi: 10.1200/JCO.2016.69.0198

17. Guglielmi C, Gomez F, Philip T, Hagenbeek A, Martelli M, Sebban C, et al. Time to relapse has prognostic value in patients with aggressive lymphoma enrolled onto the Parma trial. *J Clin Oncol*. (1998) 16:3264–9. doi: 10.1200/JCO.1998.16.10.3264

18. Philip T, Armitage JO, Spitzer G, Chauvin F, Jagannath S, Cahn JY, et al. High-dose therapy and autologous bone marrow transplantation after failure of conventional chemotherapy in adults with intermediate-grade or high-grade non-Hodgkin's lymphoma. *N Engl J Med*. (1987) 316:1493–8. doi: 10.1056/NEJM198706113162401

19. Spaepen K, Stroobants S, Dupont P, Vandenberghe P, Maertens J, Bormans G, et al. Prognostic value of pretransplantation positron emission tomography using fluorine 18-fluorodeoxyglucose in patients with aggressive lymphoma treated with high-dose chemotherapy and stem cell transplantation. *Blood*. (2003) 102:53–9. doi: 10.1182/blood-2002-12-3842

20. Derenzini E, Musuraca G, Fanti S, Stefoni V, Tani M, Alinari L, et al. Pretransplantation positron emission tomography scan is the main predictor of autologous stem cell transplantation outcome in aggressive B-cell non-Hodgkin lymphoma. *Cancer*. (2008) 113:2496–503. doi: 10.1002/cncr.23861

21. Sauter CS, Matasar MJ, Meikle J, Schoder H, Ulaner GA, Migliacci JC, et al. Prognostic value of FDG-PET prior to autologous stem cell transplantation for relapsed and refractory diffuse large B-cell lymphoma. *Blood*. (2015) 125:2579–81. doi: 10.1182/blood-2014-10-606939

22. Elstrom RL, Martin P, Hurtado Rua S, Shore TB, Furman RR, Ruan J, et al. Autologous stem cell transplant is feasible in very elderly patients with lymphoma and limited comorbidity. *Am J Hematol*. (2012) 87:433–5. doi: 10.1002/ajh.23108

23. Sun L, Li S, El-Jawahri A, Armand P, Dey BR, Fisher DC, et al. Autologous stem cell transplantation in elderly lymphoma patients in their 70s: outcomes and analysis. *Oncologist*. (2018) 23:624–30. doi: 10.1634/theoncologist.2017-0499

24. Dogu MH, Cagirgan S, Ocakci S, Kaya AH, Ilkkilic K, Sanli NM, et al. Autologous stem cell transplantation and stem cell mobilization kinetics in elderly patients with B cell non-Hodgkin lymphoma. *Transfus Apher Sci*. (2017) 56:814–8. doi: 10.1016/j.transci.2017.11.012

25. Wildes TM, Augustin KM, Sempek D, Zhang QJ, Vij R, Dipersio JF, et al. Comorbidities, not age, impact outcomes in autologous stem cell transplant for relapsed non-Hodgkin lymphoma. *Biol Blood Marrow Transplant*. (2008) 14:840–6. doi: 10.1016/j.bbmt.2008.05.002

26. 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) 377:2531–44. doi: 10.1056/NEJMoa1707447

27. Neelapu SS, Jacobson CA, Ghobadi A, Miklos DB, Lekakis LJ, Oluwole OO, et al. Five-year follow-up of ZUMA-1 supports the curative potential of axicabtagene ciloleucel in refractory large B-cell lymphoma. *Blood*. (2023) 141:2307–15. doi: 10.1182/blood.2022018893

28. 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) 396:839–52. doi: 10.1016/S0140-6736(20)31366-0

29. 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) 380:45–56. doi: 10.1056/NEJMoa1804980

30. Westin JR, Kersten MJ, Salles G, Abramson JS, Schuster SJ, Locke FL, et al. Efficacy and safety of CD19-directed CAR-T cell therapies in patients with relapsed/refractory aggressive B-cell lymphomas: Observations from the JULIET, ZUMA-1, and TRANSCEND trials. *Am J Hematol*. (2021) 96:1295–312. doi: 10.1002/ajh.26301

31. Ram R, Grisariu S, Shargian-Alon L, Amit O, Bar-On Y, Stepensky P, 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) 107:1111–8. doi: 10.3324/haematol.2021.278288

32. Kittai AS, Huang Y, Gordon M, Denlinger N, Mian A, Fitzgerald L, 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) 27:46–52. doi: 10.1016/j.bbmt.2020.09.028

33. Lutfi F, Skelton WP IV, Rosenau E, Farhadfar N, Murthy HS, May WS, et al. Poor Graft Function Following Autologous Stem Cell Transplant (ASCT): A Retrospective Analysis over Two Decades at a Transplant Center. *Blood*. (2017) 130:4529. doi: 10.1182/blood.V130.Suppl_1.4529.4529

34. Wudhikarn K, Palomba ML, Pennisi M, Garcia-Recio M, Flynn JR, Devlin SM, et al. Infection during the first year in patients treated with CD19 CAR T cells for diffuse large B cell lymphoma. *Blood Cancer J*. (2020) 10:79. doi: 10.1038/s41408-020-00346-7

35. Furlow B. FDA investigates risk of secondary lymphomas after CAR-T immunotherapy. *Lancet Oncol*. (2024) 25:21. doi: 10.1016/S1470-2045(23)00631-9

36. Melody M, Epperla N, Shouse G, Romancik J, Allen P, Moyo TK, et al. Subsequent Malignant neoplasms in patients previously treated with anti-CD19 CAR T-cell therapy. *Blood Adv*. (2024) 8:2327–31. doi: 10.1182/bloodadvances.2024012573

37. Danylesko I, Shimoni A. Second Malignancies after hematopoietic stem cell transplantation. *Curr Treat Options Oncol*. (2018) 19:9. doi: 10.1007/s11864-018-0528-y

38. Forrest DL, Nevill TJ, Naiman SC, Le A, Brockington DA, Barnett MJ, et al. Second Malignancy following high-dose therapy and autologous stem cell transplantation: incidence and risk factor analysis. *Bone Marrow Transplant*. (2003) 32:915–23. doi: 10.1038/sj.bmt.1704243

39. Dean EA, Mhaskar RS, Lu H, Mousa MS, Krivenko GS, Lazaryan A, et al. High metabolic tumor volume is associated with decreased efficacy of axicabtagene ciloleucel in large B-cell lymphoma. *Blood Adv*. (2020) 4:3268–76. doi: 10.1182/bloodadvances.2020001900

40. Wudhikarn K, Tomas AA, Flynn JR, Devlin SM, Brower J, Bachanova V, et al. Low toxicity and excellent outcomes in patients with DLBCL without residual lymphoma at the time of CD19 CAR T-cell therapy. *Blood Adv*. (2023) 7:3192–8. doi: 10.1182/bloodadvances.2022008294

41. 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) 7:2872–83. doi: 10.1182/bloodadvances.2022009019

42. Hubbeling H, Silverman EA, Michaud L, Tomas AA, Shouval R, Flynn J, et al. Bridging radiation rapidly and effectively cytoreduces high-risk relapsed/refractory aggressive B cell lymphomas prior to chimeric antigen receptor T cell therapy. *Transplant Cell Ther*. (2023) 29:259 e1– e10. doi: 10.1016/j.jtct.2022.12.021

43. Sim AJ, Jain MD, Figura NB, Chavez JC, Shah BD, Khimani F, et al. Radiation therapy as a bridging strategy for CAR T cell therapy with axicabtagene ciloleucel in diffuse large B-cell lymphoma. *Int J Radiat Oncol Biol Phys*. (2019) 105:1012–21. doi: 10.1016/j.ijrobp.2019.05.065

44. 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) 386:640–54. doi: 10.1056/NEJMoa2116133

45. 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) 141:1675–84. doi: 10.1182/blood.2022018730

46. 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) 386:629–39. doi: 10.1056/NEJMoa2116596

47. Filosto S, Vardhanabhuti S, Canales MA, Poire X, Lekakis LJ, de Vos S, 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 Discovery*. (2024) 5:21–33. doi: 10.1158/2643-3230.BCD-23-0112

48. 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) 139:1330–9. doi: 10.1182/blood.2021013289

49. Shadman M, Wooahn K, Kaur M, Kharfan-Dabaja MA, Herrera AF, Sauter CS, et al. Autologous transplant (auto-HCT) is associated with improved clinical outcomes compared to CAR-T therapy in patients (pts) with large B-cell lymphoma (LBCL) achieving a complete remission. *Blood*. (2023) 142:781. doi: 10.1182/blood-2023-173536

50. Jacobson CA, Munoz J, Sun F, Kanters S, Limbrick-Oldfield EH, Spooner C, 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– e15. doi: 10.1016/j.jtct.2023.10.017

frontiers | Frontiers in Oncology

TYPE Brief Research Report
PUBLISHED 09 October 2024
DOI 10.3389/fonc.2024.1432310

OPEN ACCESS

EDITED BY
Nilanjan Ghosh,
Levine Cancer Institute, United States

REVIEWED BY
Natalie Grover,
University of North Carolina at Chapel Hill, United States
Pooria Safarzadeh Kozani,
Tarbiat Modares University, Iran
Pouya Safarzadeh Kozani,
Tarbiat Modares University, Iran

*CORRESPONDENCE
Jörg Mahlich
Joerg.mahlich@gmail.com

RECEIVED 13 May 2024
ACCEPTED 03 September 2024
PUBLISHED 09 October 2024

CITATION
Heger J-M, Borchmann P, Riou S, Werner B, Papadimitrious MS and Mahlich J (2024) Survival outcomes of patients with diffuse large B-cell lymphoma undergoing autologous stem cell transplantation in Germany: real-world evidence from an administrative database between 2010 and 2019.
Front. Oncol. 14:1432310.
doi: 10.3389/fonc.2024.1432310

Survival outcomes of patients with diffuse large B-cell lymphoma undergoing autologous stem cell transplantation in Germany: real-world evidence from an administrative database between 2010 and 2019

Jan-Michel Heger[1], Peter Borchmann[1], Sybille Riou[2], Barbara Werner[3], Michael S. Papadimitrious[2] and Jörg Mahlich[2,4]*

[1]Department I of Internal Medicine, Center for Integrated Oncology Aachen Bonn Cologne Duesseldorf, University of Cologne, Medical Faculty and University Hospital Cologne, Cologne, Germany, [2]Health Economics and Outcomes Research, Miltenyi Biomedicine, Bergisch Gladbach, Germany, [3]Health Economics and Health Services Research, Team Gesundheit Gesellschaft für Gesunheitsmanagement mbH, Essen, Germany, [4]Duesseldorf Institute for Competition Economics (DICE), Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany

Background: Limited real-world evidence is available for patients with diffuse large B-cell lymphoma (DLBCL) who received an autologous stem cell transplantation (ASCT) in Germany.

Objectives: This study aims to describe the real-world survival outcomes of patients with DLBCL who received ASCT in Germany after diagnosis.

Design: This study is a retrospective database analysis covering the period between 2010 and 2019.

Methods: Unadjusted overall survival (OS) was plotted using the Kaplan–Meier estimator for the overall population and stratified by relapse status. A Cox regression was run to identify factors that influence OS.

Results: A total of 112 patients received an ASCT, with the average time from first-line treatment to ASCT being 11.7 months. The median OS estimated by

Kaplan–Meier was 83.4 months for the entire cohort. The only variable that significantly reduced the OS was the presence of subsequent treatment after ASCT in a time-dependent model.

Conclusion: OS after ASCT for DLBCL patients in Germany is higher than previously reported and may still be considered a valid option for carefully selected patients with relapsed/refractory DLBCL.

KEYWORDS

diffuse large B-cell lymphoma, autologous stem cell transplantation, survival, real-world evidence, claims data, Germany

Introduction

Autologous stem cell transplantation (ASCT) following high-dose chemotherapy can still be a treatment option for a well-defined group of patients with diffuse large B-cell lymphoma who relapse or are refractory after front-line therapy, even in the era of chimeric antigen receptor (CAR)-T-cell therapies. Both lisocabtagene maraleucel and axicabtagene ciloleucel, which were approved by the EMA in 2022 and 2023 for this indication (1), have demonstrated superiority over the standard of care (2, 3).

Up to 50% of patients will experience a relapse after ASCT, with the majority occurring within the first year (4). Despite the high number of relapses after ASCT, a relapse 1 year post-ASCT is associated with better survival outcomes. Limited real-world evidence relating to ASCT is available for Germany, with the exception of a single-center study in aggressive B-cell lymphomas, including diffuse large B-cell lymphoma (DLBCL). This study was conducted at the University Hospital Muenster between 2002 and 2019 (5). It reported a mean overall survival (OS) of 55 months for DLBCL patients who received ASCT. While the Kaplan–Meier (KM) curves indicated an OS benefit for patients who relapsed late (defined as ≥ 12 months after ASCT), this difference was not statistically significant. As single-center studies might suffer from limited generalizability, our study utilized a large health insurance claims database covering 6.7 million people to examine survival in German DLBCL patients after ASCT.

Materials and methods

This is a secondary analysis of a health economics study that is described elsewhere (6, 7). Briefly, the database used for this analysis is a subset of the German Statutory Health Insurance (SHI) population, covering 6.7 million people between 2010 and 2019. It was used for health service research covering multiple indications (8, 9). Deaths are reported in this database, which is not the case for claims databases in many other countries. We identified patients diagnosed with DLBCL who received an ASCT (index date) after front-line treatment. Unadjusted OS from ASCT was plotted using the KM estimator for both the overall population and stratified by (1) the presence of subsequent treatment line (yes/no) and (2) time from the start of first-line therapy to ASCT (< 12 months/≥ 12 months). Patients with a subsequent treatment line were labeled as nondurable responders, while those without a subsequent treatment line were identified as potential responders. The KM estimator was also stratified by early (subsequent treatment line is initiated < 12 months after ASCT) and late relapse (≥ 12 months after ASCT) for the nondurable responder subgroup. A Cox regression with the following covariates—age, gender, Charlson Comorbidity Index (CCI), time from the start of first-line therapy to ASCT, and presence of subsequent treatment after ASCT—was performed to identify factors influencing OS. Model fit was assessed using several diagnostic parameters, such as Akaike's information criterion (AIC), the Wald test, or the supremum test, to examine whether the proportional hazard assumption holds. If the assumption was violated, a model with time-dependent variables was applied (10). This is an extension of the Cox model and provides a feasible alternative to a landmark analysis, where subjects would have been excluded from the analysis (11). This approach avoids both the problem of selecting a landmark time and misclassification errors (12).

Results

Descriptive statistics

Among the 124 patients who received a hematopoietic stem cell transplantation (HSCT) after an initial diagnosis of DLBCL, 112

underwent ASCT and 12 received allogeneic HSCT (which is not included in this study). For patients who had one previous treatment, the average (median) follow-up time after the index was 32.0 (26.0) months. The majority (60.7%) of the cohort were men, and the mean age of patients at the index was 55.2 years old (range: 18–74). The mean pre-index CCI score was 7.8 (range: 2–16). A total of 39.3% (N = 44) received at least one subsequent treatment after ASCT, while 60.7% (n = 68) did not. The majority (65.9%) of patients who received subsequent treatments were men. The mean age of this subgroup was 56.0 years old, and the mean CCI score was 8.0. For the cohort without subsequent treatment, 57.4% were men, the mean age was 54.6 years old, and the mean CCI score was 7.7. The average time from first treatment to ASCT was 11.7 months for the entire cohort, and 11.0 and 12.1 months for the subgroups with and without subsequent treatment, respectively. The average time to subsequent treatment after ASCT was 13.5 months. In 72.7% of the cases (N = 32), subsequent treatment was initiated within 1 year after ASCT (early relapse), while 27.3% (N = 12) had subsequent treatment initiated more than 1 year after ASCT.

Survival analysis

The median OS was 83.4 months, with a 5-year survival rate of 62.2%, as estimated by the KM curve (Figure 1A). The median OS for the group with subsequent treatment was 27.8 months, and it was not estimated for patients without additional treatments ($p < 0.0001$). The KM curve for patients without subsequent treatment reached a plateau of just under 90% (Figure 1B). Stratification by the time of relapse after front-line treatment shows a slightly better (but insignificant) hazard ratio (HR) compared to those with early relapse (< 12 months). However, the median for late relapses was not reached, and survival times cannot be compared (Figure 1C). Turning to the subgroup of patients who had a relapse after ASCT, the median OS, as estimated by the KM curve, were 14.5 and 60.6 months for early and late relapse, respectively; however, these were not significantly different at the 5% level ($p = 0.0621$) (Figure 1D).

Factors influencing OS were studied using a Cox regression. Several models were examined for the entire cohort and stratified by relapse status, using the following variables: age, gender, CCI, time to receive ASCT in months, and time to subsequent treatment in months (if applicable). The best fit was observed for a time-dependent model with "subsequent treatment" as a time-dependent covariate. The model was run for the entire cohort, and Table 1 reports the results. The only significant variable influencing OS was "subsequent treatment" ($p < 0.001$), with a hazard ratio of 24.24, i.e., the risk of dying at any time point is 24.24 times higher among those who have already received subsequent therapy compared to those who have not, holding all else equal.

Discussion

To our knowledge, this is the first large-scale database study on the survival of DLBCL patients who underwent ASCT in Germany. Our findings indicate that patients in Germany are treated according to recommended treatment guidelines, as ACST is applied as second-line treatment. The 5-year survival rate in this study was 62.2%, and OS after ASCT was 83.4 months, which is broadly in line with the literature. For the USA, OS after ASCT was 64.8 months (5.4 years) for patients treated at the Mayo Clinic (4) or 72 months, as reported by Eldjerou et al., drawing on data from the CIBMTR registry (13). A study from Japan that included elderly patients (median age: 64) reported a 3-year survival rate of 49.6%, compared to 69.4% in our study (14). A more recent Japanese study covered a slightly younger patient cohort and utilized data from the Japan Society for Hematopoietic and Cellular Therapy Registry. The authors reported 5-year survival rates between 57% and 65%, depending on the high-dose chemotherapy regimen [ranimustine, etoposide, cytarabine, melphalan (MEAM), ranimustine, carboplatin, etoposide, cyclophosphamide (MCEC), or cyclophosphamide, etoposide, melphalan, dexamethasone (LEED)] (15). A recent study from Belgium presented slightly higher 5-year survival rates of 66%–69% (16).

On the other hand, the only study conducted in Germany observed lower values for both the 5-year survival rate (50%) and mean OS (55 months) (5). One potential explanation for the differences might be the higher mean age in the population studied by Wullenkord et al. (5) (63 years old) compared to our study (55 years old). In addition, their study was conducted in a single tertiary teaching hospital, whereas our study accessed a healthcare claims database for all of Germany. Furthermore, high-risk patients are typically referred to tertiary teaching hospitals, and they may not be considered representative of German clinical practice.

We observed a 10% mortality rate in the 10 months following ASCT, possibly as a result of neutropenia (5). This 10% mortality estimate is higher than expected but still within the range of what had been reported in previous research (17). Mortality was zero approximately 1 year after receiving ASCT for those without subsequent treatment. Furthermore, we observed that 39.3% of patients received subsequent treatment after ASCT in our analysis, compared to 28% in Wullenkord et al. (5).

Turning to prognostic factors, the stratified survival analysis suggests that a late relapse after ASCT is associated with an OS advantage of 46.1 months compared to early relapse, which supports previous findings (3). However, due to the small size of this subpopulation, this difference is significant only at the 10% level and not at the conventional 5% level. In Wullenkord et al. (5), the KM curves indicate an OS benefit of more than 5 years for patients who relapsed late after ASCT. However, they also determined that the OS benefit was not statistically significant. Recall that, due to data availability, we used the time from DLBCL treatment to ASCT, which is different from

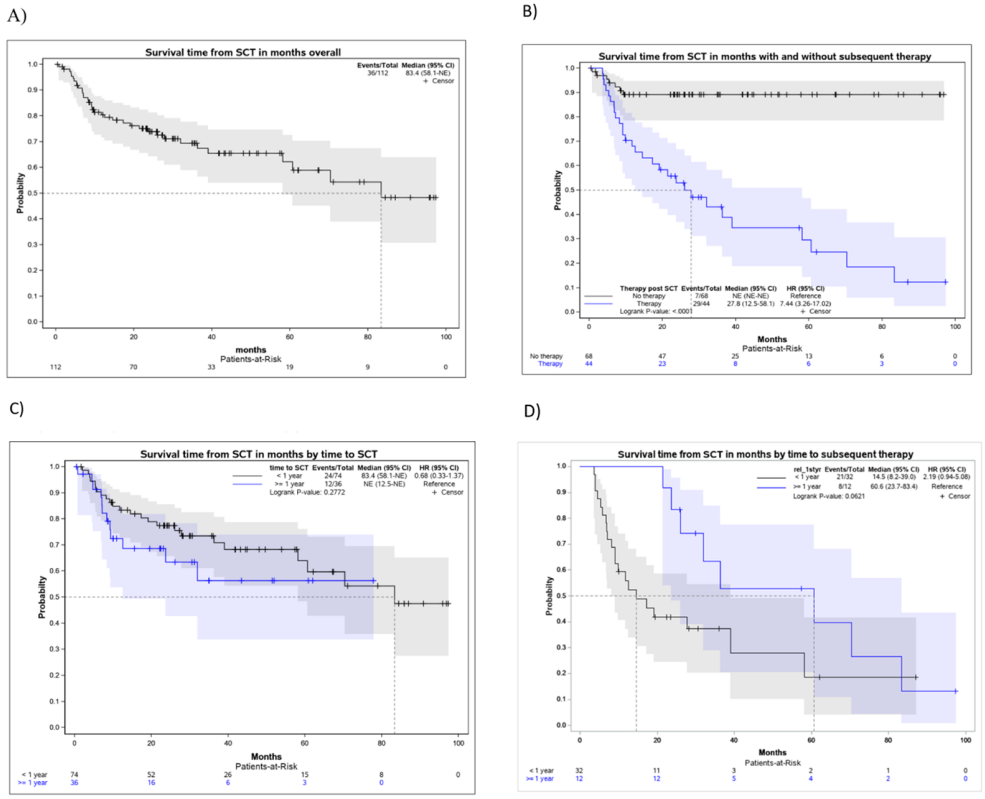


FIGURE 1
Kaplan–Meier curves for the survival times in months after ASCT. **(A)** Kaplan–Meier curve for the survival time in months from the first ASCT, overall cohort. **(B)** Kaplan–Meier curve for the survival time in months from first ASCT, stratified by subsequent therapy after ASCT. **(C)** Kaplan–Meier curves for the survival times in months from first ASCT onward, stratified by early/late relapse after first-line therapy. **(D)** Kaplan–Meier curves for the survival times in months from the first ASCT onward, stratified by early/late relapse after ASCT.

the time to relapse to ASCT used as eligibility criteria and for high-risk classification in the CAR-T trials. OS did not significantly differ between patients who relapsed early or late after first-line therapy. As the median OS for patients with a late relapse (after first-line therapy) has not yet been reached after more than 6 years, it is possible that these patients may demonstrate a long-term survival benefit. The results of the Cox regression confirm a strong association between relapse status after ASCT and OS, demonstrating that relapsed patients have a poor prognosis. No other significant relationships were found.

TABLE 1 Cox regression with a time-dependent covariate (n = 112).

Variables	p-value	Hazard ratio (HR)	95% HR confidence interval
Subsequent treatment after ASCT (time-dependent)	< 0.0001	24.236	10.092–58.204
Gender (men vs. women)	0.7465	1.127	0.545–2.333
Age (years)	0.2436	1.026	0.983–1.072
CCI	0.695	0.973	0.847–1.117
Time from initial diagnosis to ASCT (months)	0.1045	1.001	1.000–1.002

AIC: 300.2 (without covariates) and 235.8 (with covariates). Wald test: 51.983 (Chi-square), < 0.0001 (p-value).
ASCT, autologous stem cell transplantation; CCI, Charlson Comorbidity Index; AIC, Akaike's information criterion.

This retrospective study has several limitations. Claims data, in general, are not designed for research purposes and may suffer from coding errors (18). They also include only a very limited set of medical parameters, and the cause of death is not recorded. For instance, Eastern Cooperative Oncology Group (ECOG) performance status and the amount of infused cells have been reported to influence survival (4, 19) but were not included in our Cox regression due to data limitations. Other potential success factors of ASCT, such as chemosensitivity (20), were not captured in our data. Finally, and probably most importantly, the study covers the time before the advent of new treatment options such as tafasitamab (21), glofitamab (22), or loncastuximab tesirine (23) and other promising therapies (24). Even more relevant treatments are CAR-T-cell therapies (lisocabtagene maraleucel and axicabtagene ciloleucel), which are effective treatments for relapsed or refractory patients who relapse within 12 months of the end of first-line therapy (25). While there may be an urgent medical need for DLBCL patients who cannot receive ASCT, our results, echoing Shadman et al. (1), suggest that CAR-T-cell therapy and ASCT can coexist in that ASCT can still be a valid option for patients presenting with a late relapse, showing a 5-year survival rate of around 55%.

Data availability statement

Database is commercially available but not for free. Requests to access these datasets should be directed to barbara.werner@teamgesundheit.de.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

JH: Validation, Writing – original draft, Writing – review & editing. PB: Validation, Writing – original draft, Writing – review & editing. SR: Writing – original draft, Writing – review & editing. BW: Data curation, Formal analysis, Methodology, Writing – original draft, Writing – review & editing. MP: Writing – original draft, Writing – review & editing. JM: Conceptualization, Methodology, Resources, Writing – original draft, Writing – review & editing.

Funding

The author(s) declare that financial support was received for the research, and publication of this article. This study and the rapid service fee were funded by Miltenyi Biomedicine GmbH. Team Gesundheit Gesellschaft für Gesundheitsmanagement, a contract research company that received funding from Miltenyi Biomedicine to conduct the study in line with the study protocol.

Conflict of interest

JM, SR, and MP are employees of Miltenyi Biomedicine GmbH. BW is an employee of Team Gesundheit, a contract research company that received funding from Miltenyi Biomedicine to conduct the study in line with the study protocol.

The authors declare that this study received funding from Miltenyi Biomedicine. The funder had the following involvement in the study: three authors received salaries from Miltenyi Biomedicine.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Shadman M, Pasquini MC, 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) 139:1330–9. doi: 10.1182/blood.2021013289

2. Kamdar MK, Solomon S, Arnason J, Johnston PB, Glass B, Bachanova V, et al. Lisocabtagene maraleucel (liso-cel) vs standard of care (SOC) with salvage chemotherapy (CT) followed by autologous stem cell transplantation (ASCT) as second-line (2L) treatment in patients (pt) with R/R large B-cell lymphoma (LBCL): 3-year follow-up (FU) from the randomized, phase 3 TRANSFORM study. *JCO*. (2024) 42:7013–3. doi: 10.1200/JCO.2024.42.16_suppl.7013

3. 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) 389:148–57. doi: 10.1056/NEJMoa2301665

4. Tun A, Maliske S, Wang Y, Inwards DJ, Habermann TM, Micallef I, et al. Progression-free survival at 24 months as A landmark after autologous stem cell transplant in relapsed or refractory diffuse large B-cell lymphoma. *Transplant Cell Ther*. (2022) 28:610–7. doi: 10.1016/j.jtct.2022.06.015

5. Wullenkord R, Berning P, Niemann AL, Wethmar K, Bergmann S, Lutz M, et al. The role of autologous stem cell transplantation (ASCT) in aggressive B-cell lymphomas: real-world data from a retrospective single-center analysis. *Ann Hematol*. (2021) 100:2733–44. doi: 10.1007/s00277-021-04650-5

6. Borchmann P, Heger J-M, Mahlich J, Papadimitrious MS, Riou S, Werner B. Healthcare resource utilization and associated costs of German patients with diffuse large B-cell lymphoma – A retrospective health claims data analysis. *Oncol Ther*. (2023) 11:83–96. doi: 10.1007/s40487-022-00211-6

7. Borchmann P, Heger J-M, Mahlich J, Papadimitrious MS, Riou S, Werner B. Survival outcomes of patients diagnosed with diffuse large B-cell lymphoma - Real world evidence from Germany. *J Cancer Res Clin Oncol*. (2023) 149:7091–101. doi: 10.1007/s00432-023-04660-y

8. Bögemann M, Zagorska A, Akumo D, Hadad LE, Pignot M. Using data from a sickness fund claims database to assess the treatment patterns and healthcare resource utilization among patients with metastatic renal cell carcinoma in Germany. *Urol Int*. (2020) 104:982–93. doi: 10.1159/000509973

9. Mahlich J, Olbrich K, Wilk A, Wimmer A, Wolff-Menzler C. Time to treatment discontinuation in German patients with schizophrenia: long-acting injectables versus oral antipsychotics. *Clin Drug Investig*. (2021) 41:99–113. doi: 10.1007/s40261-020-00990-8

10. Zhang Z, Reinikainen J, Adeleke KA, Pieterse ME, Groothuis-Oudshoorn CGM. Time-varying covariates and coefficients in Cox regression models. *Ann Transl Med*. (2018) 6:121. doi: 10.21037/atm.2018.02.12

11. Morgan C. Landmark analysis: A primer. *J Nucl Cardiol*. (2019) 26:391–3. doi: 10.1007/s12350-019-01624-z

12. Dafni U. Landmark analysis at the 25-year landmark point. *Circ Cardiovasc Qual Outcomes*. (2011) 4:363–71. doi: 10.1161/CIRCOUTCOMES.110.957951

13. Eldjerou L, Zhang J, Keir C. Disease characteristics and outcomes in adult patients with diffuse large B-Cell lymphoma following autologous stem cell transplant. *Transplant Cell Ther*. (2018) 24:S125. doi: 10.1016/j.bbmt.2017.12.066

14. Chihara D, Izutsu K, Kondo E, Sakai R, Mizuta S, Yokoyama K, et al. High-dose chemotherapy with autologous stem cell transplantation for elderly patients with relapsed/refractory diffuse large B cell lymphoma: a nationwide retrospective study. *Biol Blood Marrow Transplant*. (2014) 20:684–9. doi: 10.1016/j.bbmt.2014.01.025

15. Koresawa-Shimizu R, Suzuki R, Uehara Y, Hiramoto N, Sawa M, Fukuda T, et al. Comparison of MEAM, MCEC and LEED high-dose chemotherapy followed by autologous stem cell transplantation in relapsed/refractory diffuse large B-cell lymphoma: data from the Japan Society for Hematopoietic and Cellular Therapy Registry. *Bone Marrow Transplant*. (2024) 59:125–7. doi: 10.1038/s41409-023-02118-w

16. Daneels W, Rosskamp M, Macq G, Saadoon EI, De Geyndt A, Offner F, et al. Real-world estimation of first- and second-line treatments for diffuse large B-cell lymphoma using health insurance data: A Belgian population-based study. *Front Oncol*. (2022) 12:824704. doi: 10.3389/fonc.2022.824704

17. Carlsten M, Jädersten M, Hellström A, Littmann K, Melén CM, Junlén HR, et al. The Karolinska experience of autologous stem-cell transplantation for lymphoma: a population-based study of all 433 patients 1994–2016. *Exp Hematol Oncol*. (2019) 8:7. doi: 10.1186/s40164-019-0131-3

18. Scheid C, Kudernatsch R, Eckart M, Libutzki B, Feig C, Mahlich J. Incidence of graft-versus-host-disease in Germany: evidence from health care claims data. *J Public Health*. (2023) 31:1609–20. doi: 10.1007/s10389-022-01736-w

19. Hu M, Watkins M, Cao Q, Raya S, Russler-Germain DA, Bachanova V, et al. Predictors of relapse and survival following autologous stem cell transplant in patients with diffuse large B-cell lymphoma. *Blood*. (2021) 138:1832. doi: 10.1182/blood-2021-146734

20. Mey U, Jha V, Strehl JW, Gorschlueter M, Rabe C, Hoebert E, et al. High dose chemotherapy with autologous stem cell transplantation in diffuse large B-cell lymphoma. *Ger Med Sci*. (2007) 5:2.

21. Salles G, Duell J, González Barca E, Tournilhac O, Jurczak W, Liberati AM, et al. Tafasitamab plus lenalidomide in relapsed or refractory diffuse large B-cell lymphoma (L-MIND): a multicentre, prospective, single-arm, phase 2 study. *Lancet Oncol*. (2020) 21:978–88. doi: 10.1016/S1470-2045(20)30225-4

22. Dickinson M, Carlo-Stella C, Morschhauser F, Bachy E, Corradini P, Iacoboni G, et al. Glofitamab for relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med*. (2022) 387:2220–31. doi: 10.1056/NEJMoa2206913

23. Caimi P, Ai W, Alderuccio J, Ardeshna KM, Hamadani M, Hess B, et al. Loncastuximab tesirine in relapsed or refractory diffuse large B-cell lymphoma (LOTIS-2): a multicentre, open-label, single-arm, phase 2 trial. *Lancet Oncol*. (2021) 22:790–800. doi: 10.1016/S1470-2045(21)00139-X

24. Gonzalez Barca E. Developing new strategies for relapsed/refractory diffuse large B-cell lymphoma. *J Clin Med*. (2023) 12:7376. doi: 10.3390/jcm12237376

25. Shargian L, Raanani P, Yeshurun M, Gafter-Gvili A, Gurion R. Chimeric antigen receptor T-cell therapy is superior to standard of care as second-line therapy for large B-cell lymphoma: A systematic review and meta-analysis. *Br J Hematol*. (2022) 198:838–46. doi: 10.1111/bjh.v198.5

Frontiers in Oncology

Advances knowledge of carcinogenesis and tumor progression for better treatment and management

The third most-cited oncology journal, which highlights research in carcinogenesis and tumor progression, bridging the gap between basic research and applications to imrpove diagnosis, therapeutics and management strategies.

Discover the latest Research Topics

See more →

Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact