Blood Reviews xxx (xxxx) xxx

Contents lists available at ScienceDirect

Blood Reviews

journal homepage: www.elsevier.com/locate/issn/0268960X

Review

Advancing CAR T-cell therapies: Preclinical insights and clinical translation for hematological malignancies

Arun K. Arunachalam, Céline Grégoire, Beatriz Coutinho de Oliveira, Jan Joseph Melenhorst *

Cell Therapy & Immuno-Engineering Program, Center for Immunotherapy and Precision Immuno-Oncology, Lerner College of Medicine, Cleveland Clinic, Cleveland, OH 44195, United States of America

ARTICLE INFO

Keywords:
CAR T-cells
Hematological malignancies
Preclinical studies
Long-term remissions
Predictors of response
Translational immunology

ABSTRACT

Chimeric antigen receptor (CAR) T-cell therapy has achieved significant success in achieving durable and potentially curative responses in patients with hematological malignancies. CARs are tailored fusion proteins that direct T cells to a specific antigen on tumor cells thereby eliciting a targeted immune response. The approval of several CD19-targeted CAR T-cell therapies has resulted in a notable surge in clinical trials involving CAR T cell therapies for hematological malignancies. Despite advancements in understanding response mechanisms, resistance patterns, and adverse events associated with CAR T-cell therapy, the translation of these insights into robust clinical efficacy has shown modest outcomes in both clinical trials and real-world scenarios. Therefore, the assessment of CAR T-cell functionality through rigorous preclinical studies plays a pivotal role in refining therapeutic strategies for clinical applications. This review provides an overview of the various in vitro and animal models used to assess the functionality of CAR T-cells. We discuss the findings from preclinical research involving approved CAR T-cell products, along with the implications derived from recent preclinical studies aiming to optimize the functionality of CAR T-cells. The review underscores the importance of robust preclinical evaluations and the need for models that accurately replicate human disease to bridge the gap between preclinical success and clinical efficacy.

1. Introduction

Chimeric Antigen Receptor (CAR) T-cell therapy has transformed the cancer field by inducing long-term and potentially curative responses in patients with hematological malignancies [1,2]. CAR T-cells are reengineered T cells specifically designed to target antigens expressed predominantly by tumor cells [2,3]. Structurally, CARs are fusion proteins that combine: a) an antigen-recognition domain that contains the antibody-derived tumor-targeting variable regions, also called as single-chain variable fragment (scFv), with b) a T cell activation domain (T cell receptor-derived constant regions, i.e., CD3ζ), that are connected by c) a spacer hinge and transmembrane spanning elements. These CARs are usually introduced to the T cells using lentiviral or retroviral vectors, redirecting T cells to specific surface antigens in a manner independent of major histocompatibility complex (MHC) restriction. This approach avoids tumor cell escape from the immune system through Human Leukocyte Antigen (HLA) downregulation or mutations in proteasomal antigen processing, just to name a few known escape mechanisms [2,4]. First-generation CARs included only the CD3ζ signaling domain, providing insufficient activation [5]. Second and third-generation CARs incorporate one or two costimulatory domains, respectively, allowing full activation and memory pool formation of CAR T-cells with sustained tumor control [2]. The most commonly used costimulatory domains are CD28 and CD137 (4-1BB), with CD137 providing superior in vivo persistence of CAR T-cells [5,6].

Despite the significant success of CAR T-cell therapies in B cell malignancies, high burden and mortality rates are still associated with these diseases [7,8]. Additionally, complete response (CR) rates can vary with approximately 85 % of patients achieve CR in Acute Lymphoblastic Leukemia (ALL), 50 % in Non-Hodgkin's Lymphoma (NHL), and 25 % in Chronic Lymphocytic Leukemia (CLL) [9,10]. Despite extensive efforts in this field, achieving consistently effective curative treatments for relapsed and refractory patients remains a challenge. Some well-known mechanisms of failure upon CAR-T cell therapy include tumor resistance to apoptosis [11], loss of target antigens [12,13], upregulation of inhibitory receptors [14], and T cell-intrinsic deficiencies [15].

* Corresponding author at: 9500 Euclid Avenue, NA2-012, Cleveland Clinic Lerner Research Institute, Cleveland Clinic, Cleveland, OH 44195, United States of America.
E-mail address: melenhj@ccf.org (J.J. Melenhorst).

https://doi.org/10.1016/j.blre.2024.101241

Available online 12 September 2024

However, depending on the aggressiveness of the disease and the design of the CAR T construct, these mechanisms may vary, and there are still knowledge gaps which need to be addressed [16,17].

The road from idealizing a CAR, to getting approval by regulatory agencies (i.e., the Food and Drug Administration – FDA) can be a long and hard one, and all products being developed need to achieve certain milestones regarding their function and safety. A series of preclinical assays using reliable in vitro and in vivo models are required to prove their efficacy. Usually, tumor killing activity and cytokine production are the main observations of interest, however, in order to assess if the product can produce a durable long-term remission, these CAR T cells should also be tested for their persistence after repeated tumor challenges, not only in vitro, but also in vivo.

In this review, we discuss the steps involved in translating CAR T-cell therapies from bench to bedside. While exploring the main assays and indicators that are used as predictors of efficacy, we provide insights from what the field has learned from previous clinical trials and their outcomes.

2. Background

The currently approved CAR T-cell products include CD19-directed CAR T cells for B-cell malignancies and anti-BCMA-directed CAR T cells for multiple myeloma. Two anti-CD19 CAR T-cell products utilizing the CD28 costimulatory domain are approved: axicabtagene ciloleucel (Axi-Cel) for relapsed/refractory (R/R) large B cell lymphoma (LBCL) [18–20] and follicular lymphoma (FL) [21], and brexucabtagene autoleucel (Brexu-Cel) for R/R B-cell ALL [22,23] and mantle cell lymphoma (MCL) [24]. The anti-CD19 CAR T-cell product tisagenlecleucel (Tisa-cel), which uses a 4-1BB costimulatory domain, is approved for R/R B-ALL [25], LBCL [26] and FL [27]. Another 4-1BB-based anti-CD19 CAR T-cell product, lisocabtagene maraleucel (Liso-Cel), formulated with CD4+ and CD8+ cells in a 1:1 ratio, is used in R/R LBCL [28–30], CLL [31] and MCL [32]. More recently, two 4-1BB-based CAR T-cell products have been approved in multiple myeloma: the BCMA-directed idecabtagene vicleucel (Ide-cel) [33] and the dual-BCMA construct ciltacabtagene autoleucel (Cilta-cel) [34].

These therapies have significantly improved the outcomes of patients with lymphoid malignancies, but only 20–50 % of patients experience long-term remissions [35–38] (Table 1). Therefore, current research efforts are focused not only on identifying new target antigens to broaden the applications of CAR T-cell therapies but also on designing better CAR T-cell products for lymphoid malignancies. Much can be learned from analyzing previous studies, including the results of

Table 1
Long-term results of the commercial CAR-T cell therapies.

Disease	Disease / indication	CAR T-cell product	ORR/CR	mFU (mo)	mDOR (mo)	mPFS or EFS (mo)	mOS (mo)	Long-term response / survivals (when available)	Reference
B-ALL	R/R, pediatric / AYA	Tisa-cel (CD19-BBz)	81/60 % (CRi 21 %)	38.8	NR	24	NR	3y-EFS/OS: 44/63 %	ELIANA [25,35]
		Brexu-Cel (CD19-28z)	67/29 % (CRi 38 %)	36.1	7.2 [a]	5.2 [a]	NR	/	ZUMA-4 [23]
	R/R, adults	Brexu-Cel (CD19-28z)	71/56 % (CRi 15 %)	26.8	14.6 [a]	11.6 [a]	25.4 [a]	/	ZUMA-3 [22,172]
LBCL	R/R after ≥ 2 lines of therapy	Tisa-cel (CD19-BBz)	53/39 %	40.3	NR	2.9	11.1	3y-DOR 60.4 %	JULIET [26,173]
		Axi-Cel (CD19-28z)	82/54 %	63.1	11.1	5.7	25.8	5y-EFS/OS 30.3/42.6 %	ZUMA-1 [18,36]
		Liso-cel (CD19-BBz)	73/53 %	19.9	23.1	6.8	27.3	2y-DOR/PFS/OS 49.5/40.6/50.5 %	TRANSCEND NHL 001 [28,174]
	R/R after 1st-line therapy	Axi-Cel (CD19-28z)	83/65 % (vs 50/32 % [b])	47.2	41.7 (vs 7.8)	14.7 (vs 3.7)	NR (vs 31.1)	4y-PFS/OS 41.8/54.6 % (vs 24.4/46 %)	ZUMA-7 [c] [19,37]
		Liso-cel (CD19-BBz)	80/74 % (vs 45/43 % [b])	17.5	NR (vs 9.1)	NR (vs 2.4)	NR (vs 29.9)	18mo-EFS/OS 52.6/73.1 % (vs 20.8/60.6 %)	TRANSFORM [c] [29]
		Axi-Cel (CD19-28z)	90/79 %	12	NR	11.8	NR	1y-PFS /OS 48.8/78.3 %	ALYCANTE [d] [20]
		Liso-cel (CD19-BBz)	80/54 %	12.3	12.1	9	NR	/	PILOT [e] [30]
FL	R/R after ≥ 2 lines of therapy	Tisa-cel (CD19-BBz)	86/69 %	29	NR	NR	NR	2y-DOR/PFS/OS 66.4/57.4/87.7 %	ELARA [27,175]
		Axi-Cel (CD19-28z)	92/74 %	41.7	NR	40.2	NR	3y-DOR/PFS/OS 57/54/76 %	ZUMA-5 [21,176]
MCL	R/R	Axi-Cel (CD19-28z)	91/68 %	35.6	28.2	25.8	46.6	2y-PFS 52.9 %, 30mo-OS 60.3 %	ZUMA-2 [24,177]
CLL	R/R after ≥ 2 lines of therapy [f]	Liso-cel (CD19-BBz)	43/18 %	20.8	35.5	11.9	30.3	18mo-DOR/PFS/OS 70.3/46.9/71 %	TRANSCEND CLL 004 [31]
Multiple myeloma	R/R after ≥ 4 lines of therapy [g]	Ide-cel (BCMA-BBz)	73/33 %	13.3	10.7	8.8	19.4	1y-OS 78 %	KarMMa [33]
	R/R after ≥ 2 lines of therapy [g]	Cilta-cel (dual BCMA-BBz)	97/67 %	27.7	NE	NR	NR	27mo-PFS/OS 54.9/70.4 %	CARTITUDE-1 [34,38]

ORR: overall response rate; CR: complete remission; DOR: duration of response; mo: months; B-ALL: B-cell acute lymphoblastic leukemia; R/R: relapsed/refractory; AYA: adolescent and young adults; CRi: complete remission with incomplete hematological recovery; (m)EFS: (median) event-free survival; (m)OS: (median) overall survival; (m)RFS: (median) relapse-free survival; NR: not reached; LBCL: large B-cell lymphoma; (m)PFS: (median) progression free survival; SOC: standard of care; FL: follicular lymphoma; MCL: mantle cell lymphoma; CLL: chronic lymphocytic leukemia; BCMA: B-cell maturation antigen.

[a] Censored for subsequent treatment.

[b] SOC arm: immunochemotherapy + autologous hematopoietic stem cell transplantation.

[c] <12 mo after 1st-line therapy, eligible for autologous hematopoietic stem cell transplantation.

[d] <12 mo after 1st-line therapy, non-eligible for autologous hematopoietic stem cell transplantation.

[e] After 1st-line therapy, eligible for autologous hematopoietic stem cell transplantation.

[f] including a Bruton tyrosine kinase (BTK) inhibitor and a B-cell lymphoma 2 (BCL-2) inhibitor.

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

correlative studies that have identified important determinants of response and resistance to CAR T-cell therapies. Furthermore, in the absence of standardized and validated assays for predicting the efficacy of CAR T-cell therapies, reviewing the preclinical evaluations from both successful and unsuccessful clinical trials might provide valuable insights into the key parameters to consider when developing new CAR T-cell products.

3. Preclinical models and assays to evaluate CAR potency

Several biological factors have been reported and associated with clinical response, especially in the context of anti-CD19 CAR T-cell treated patients [39,40]. Some determinants of response are related to the in vivo expansion/peak in CAR T levels in apheresed samples [1,15,18,39], persistence of circulating CAR T in the patient after a prolonged follow-up [15,41,42], and early memory phenotype [15]. On the other hand, products that express genes linked to cellular exhaustion resulted in reduced clinical responses in patients with B-cell lymphoma [15,43]. At the perspective of non-responding patients, an increase in CD8+ cells with an exhausted/senescent phenotype and a distinct transcriptional signature associated with terminal differentiation and exhaustion was characteristic in this population, as well as the presence of regulatory T cells (Treg) in the CAR T product [15,44]. These findings underscore the importance of thoroughly assessing the functional characteristics of CAR T cells before infusion, in addition to their phenotypic analysis. The main read-outs of in vitro and in vivo assays are summarized in Fig. 1.

3.1. In vitro potency assays

The information obtained from in vitro assays are a crucial part of quality control for final products, and they have the potential to serve as valuable biomarkers that are translated into efficacy in vivo [45]. These assays are essential not only to assess their cytotoxic function during preclinical development, but also to ensure that the manufacturing process and final product meet standards for quality, consistency, and stability for each batch release [46]. Additionally, potency assays provide a basis for assessing comparability after scale-up during process development, site transfer, and/or the introduction of new starting materials (e.g., a new patient sample). These steps should be established during early clinical development and are required before moving forward to a clinical trial.

The main assays performed to assess CAR T-cell potency are cytotoxicity assays and cytokine production. Cytotoxicity assays can be

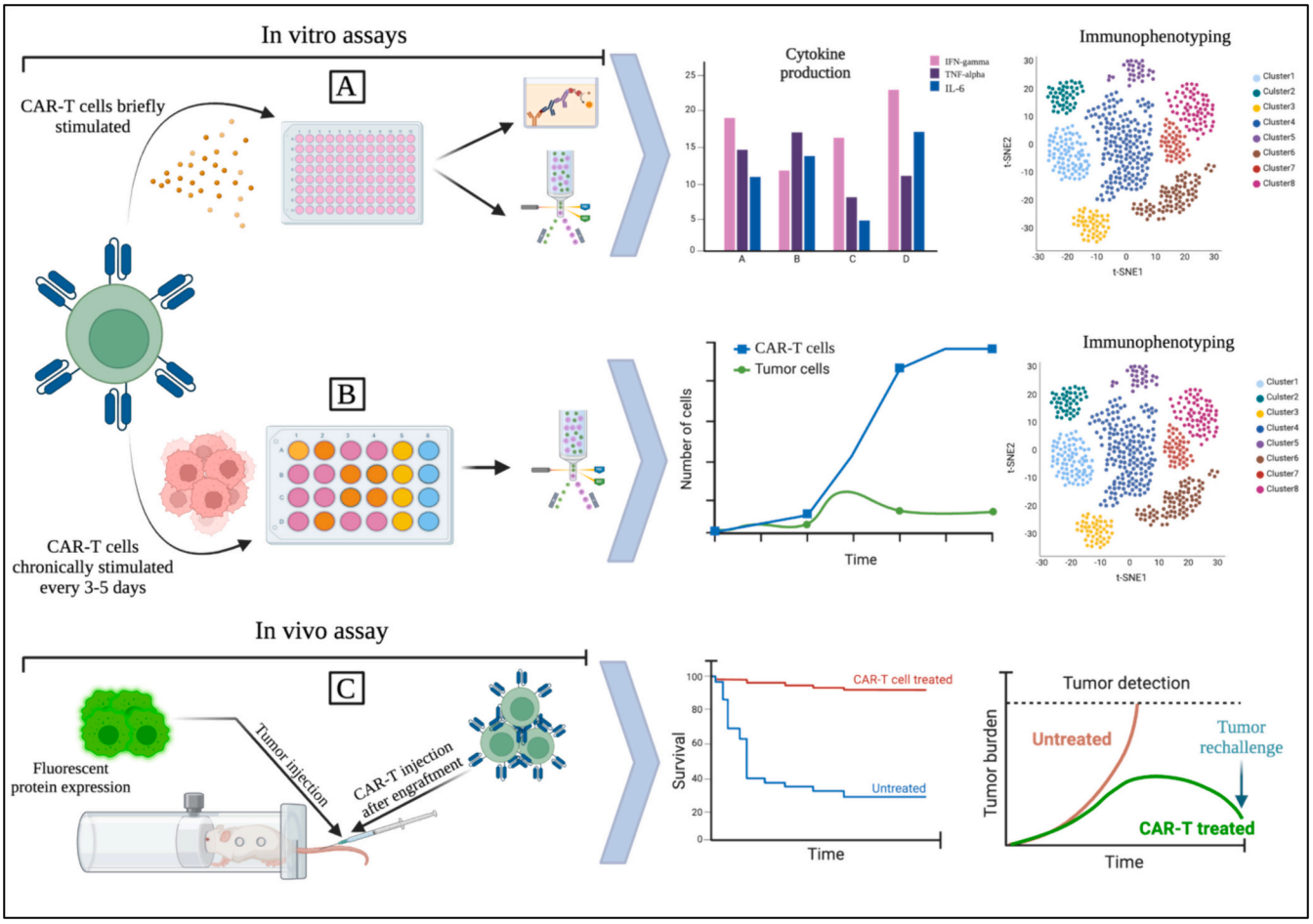

Fig. 1. Assays for the preclinical evaluation of CAR-T cells. Diagram containing different types of assays to determine CAR-T cell function. In vitro assays can assess the potency of the product upon: A) brief stimulation, where T cells are activated using either PMA/ionomycin, which bypass the T cell receptor by directly activating intracellular signaling pathways, or CD3/CD28 beads, which mimic physiological T cell receptor and co-stimulatory signaling. After that stimulation period, cytokine production is assessed in the cell culture supernatant by ELISA, and intracellularly by flow cytometry, where immunophenotyping can be performed at the same time; B) chronic stimulation, when the CAR-T cells are exposed multiple times at given intervals over the course of weeks. CAR-T cell proliferation is measured, and immunophenotyping can be used to assess the phenotype of those cells after multiple tumor encounters. In vivo assays (C) utilizing immunocompromised mice to evaluate human tumor and CAR-T cells are useful to assess safety and long-term remission, especially if a rechallenge is administered after tumor clearance, showing the presence of memory cells that can sustain response. (Created with BioRender.com).

relatively complex, and they vary significantly between different laboratories. For instance, co-cultures with CAR T-cells can be performed with cell lines or tumor cells in the presence or absence of cytokines in the media. Other variables are the effector-to-target (E:T) ratios, and the duration of co-culture, which can go from 4 to 6 h, or longer [43,47–51]. Usually, the main read-out of potency assays is tumor lysis, however, the phenotype of the cells as well as their cytokine profiles can also be assessed.

The quantification of Type-1 cytokines (i.e., IL-2, IL-6, IFN-γ, TNF-α) in co-culture supernatants is usually considered an indicator of cell activation and specific activity [51,52], and although it does not directly indicate which cells were responsible for producing them, it is likely associated with immune protective activities coming from the CAR T-cells. In this assay, a short incubation (4 h) of the CAR T-cells with TCR-dependent or -independent molecules can be done (e.g., CD3/CD28 or PMA/Ionomycin, respectively). Another strategy could be a longer stimulation (6–12 h) with artificial antigen-presenting cells (aAPCs), which consist of tumor cell lines engineered to over-express the antigen of interest (i.e., K562-CD19 cells for B cell malignancies [53]), prior to cytokine detection. Additionally, the phenotype of the cells could be assessed by flow cytometry, so the characterization of the product would become more robust.

One assay that provides comprehensive information about the CAR T-cell product, involves the iterative stimulation (3 to 4 times at 2 to 5-day intervals) of CAR T-cells with aAPCs or patient-derived tumor cells at different effector:target (E:T) ratios. This assay, also considered a pressure test, allows for phenotype and cytokine evaluation at various time points, where CAR T-cell products are queried for differentiation capacity and tumor killing while preserving a memory state after multiple challenges [53–55]. This assay can be useful especially in the context of chronic diseases such as CLL, where immune-surveillance and CAR T-cell persistence is required to provide long-term remissions [42].

Importantly, some groups direct their efforts to developing new methods to assess CAR T-cell function [56,57], and there is a hope that if they are used across different products with no significant variability, they could become a standard. However, it is important to consider the type of disease (acute vs. chronic) and the biomarkers identified within their responder or non-responder groups of patients.

3.2. In vivo assays

In vivo models are crucial for evaluating the functionality of CAR T-cells, providing a more realistic and complex environment to assess antitumor efficacy, persistence, and interactions with the tumor microenvironment (TME). These models include immune-compromised or immune-competent systems. Immune-compromised models, such as NSG (NOD/SCID/IL2Rγnull) mice, allow for the engraftment of human tumors without a graft-versus-host event, creating a suitable platform for testing human CAR T-cells. The effectiveness against xenografts has been an important consideration in determining the feasibility of advancing specific CAR designs into clinical development [6]. To replicate clinical scenarios accurately, xenografts are frequently exposed to suboptimal amounts of CAR T-cells (“stress test”), and tumor rechallenges can be conducted to assess the persistence and memory responses mediated by the CAR T products [58], which can be predictors of long-term efficacy.

Immune-competent models, such as those involving genetically engineered mouse models (GEMMs) and humanized mouse (HM) models, offer the advantage of a fully functional immune system, which is essential for understanding the interactions between CAR T-cells and endogenous immune cells [59]. These models help evaluate CAR T-cell responses in a more physiologically relevant context, including the effects of preconditioning regimens, combination therapies, and the suppression of immune inhibitory signals within the TME [58]. GEMMs allow for the study of CAR T-cell activity against early-stage tumors and the co-evolution of antitumor T cells with tumor progression. HM models, which involve the reconstitution of the human immune system in mice, are particularly useful for assessing CAR T-cell function, adverse events, and the potential for cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) [59–63].

The use of in vivo models in CAR T-cell development is indispensable for preclinical studies, as they provide a comprehensive understanding of CAR T-cell functionality, efficacy, and safety. These models facilitate the optimization of CAR T-cell therapies, guiding the development of more effective and safer treatments for clinical applications.

4. Clinical trials data summary

4.1. Pre-clinical development of the currently-approved CAR T-cells

Although cytotoxicity is essential for CAR T-cells to be effective, enhanced cytotoxicity does not necessarily translate to superior clinical efficacy. While the superiority of second-generation CAR T-cells over first-generation CAR T-cells is widely accepted, early data from their development revealed that the introduction of a costimulatory domain in anti-CD19 CAR T-cells did not increase efficacy in short-term cytotoxicity assays [5,6,64,65]. This indicates that factors beyond immediate cytotoxicity are critical for the overall clinical success of CAR T-cell therapies. Importantly, the superiority of these second-generation CAR T-cells became evident when interrogating sustained functionality in long-term cytotoxicity and proliferation assays, as well as cytokine production [5,6,64,65].

The preclinical research at the University of Pennsylvania that led to the development of Tisa-Cel focused on long-term proliferation. The 4-1BB costimulatory domain was selected over the CD28 costimulatory domain due to its superior long-term antitumor efficacy, as demonstrated in a long-term in vivo murine model using primary ALL cells with follow-up periods exceeding five months [6]. On the other hand, the CD28 costimulatory domain was selected during preclinical development at the National Cancer Institute, NIH, due to its high transduction efficiency and robust cytokine production [3]. This research ultimately resulted in the development of the commercial product Axi-Cel. Exploring further the differences between CAR T-cells incorporating a CD28 or 4-1BB costimulatory domain (28ζ-CAR or BBζ-CAR), Kawalekar et al. confirmed the ability of the 4-1BB costimulatory domain to promote a central memory phenotype in $CD8^+$ T cells, associated with fatty acid oxidation and mitochondrial biogenesis, while the CD28 domains favors effector memory differentiation, associated with glycolysis. [66] In clinical application, the outcomes of these preclinical tests are validated: the 28ζ-CAR T product Axi-Cel shows fast proliferation and higher rates or toxicities (which have been associated with IFNγ secretion) [67], while the BBζ-CAR T product Tisa-Cel demonstrates long-term persistence [42]. The third successfully developed anti-CD19 CAR T-cell product is the BBζ-CAR T-cell Liso-Cel. Its development at the Fred Hutchinson Cancer Research Center was based on preclinical research demonstrating the synergistic effect of $CD4^+$ T cells and central memory $CD8^+$ T cells. This combination resulted in optimal proliferation upon stimulation (both in vitro and in vivo) and superior long-term tumor control, with higher survival rates observed over follow-up periods of up to 16–18 weeks [68]. However, recent preclinical work has demonstrated the critical role of $CD4^+$ T cells in supporting $CD8^+$ T-cell function, as manufacturing $CD8^+$ CAR T cells without $CD4^+$ cells results in a hypofunctional phenotype and reduced anti-tumor activity [69].

Regarding anti-BCMA CAR T-cells, the BB2121 construct was selected from among the various anti-BCMA CAR constructs due to its high transduction efficiency, greater IFNγ production, and enhanced cytotoxic abilities. Further experiments demonstrated that T-cells engineered with this construct could induce tumor clearance and improve survival in a murine model, with follow-up periods extending up to 85 days. In contrast, chemotherapeutic treatment using bortezomib could only reduce the tumor burden in this model, with tumor progression observed after treatment discontinuation [70].

Overall, the preclinical development of most of these successful CAR T-cell products included long-term evaluation of efficacy. Only the preclinical development of the 28ζ-CAR Axi-Cel focused on acute efficacy and less on long-term persistence, which was later observed in clinical practice as well. This observation provides a good signal that well-designed assays used during pre-clinical evaluation can robustly predict clinical outcomes. However, while some key determinants of efficacy may be shared across CAR T-cell products and diseases, other important factors may vary depending on the specific context, and the pipeline of assays may need to be tailored accordingly.

4.2. Pre-clinical development of new CAR T-cell products

Lessons can also be drawn from unsuccessful clinical trials as well. The 28ζ anti-BCMA CAR T product KITE-585 exhibited limited expansion and lack of efficacy in a phase I clinical trial for relapsed/refractory multiple myeloma. Although the full preclinical data have not been published, the authors retrospectively assessed their preclinical findings and identified several factors that might have contributed to the "artificial" positive results in preclinical assessments. These factors included the use of IL-2 in the assays, high alloreactivity in the preclinical models, and higher BCMA expression in cell lines compared to primary myeloma cells [71]. This underscores the importance of a robust pipeline of reliable preclinical assays to evaluate new CAR T-cell products before moving forward to clinical trials.

CAR T-cells targeting CD22 or both CD19 and CD22 have been developed by several groups, but several challenges have arisen in their clinical application. Fry et al. led a clinical trial evaluating a new BBζ-CD22-targeted CAR T-cells in 21 children and young adults with B-ALL, including 15 relapsing after anti-CD19 CAR T-cell therapy [72]. They observed complete remission in 73 % of patients receiving $\geq 1 \times 10^6$ CD22-CART cells/kg, including patients with CD19dim or CD19neg disease. However, the median duration of remission was only 6 months, with most relapses associated with diminished CD22 surface expression. Further preclinical evaluation confirmed that anti-CD22 CAR T-cells could only delay the growth of NALM6 cells modified to express lower levels of CD22, while the original NALM6 cells were eradicated [72]. Using the same scFv in a CAR construct with a longer linker, Singh et al. observed disappointing results in a phase 1 clinical trial in pediatric B-ALL, with a response in only 4/8 patients, who ultimately experienced a $CD22^+$ relapse [73]. Comparing the impact of a long or short linker with this CD22 scFv, they observed that the construct with a short linker was inducing more tonic signaling secondary to homodimerization, resulting in a higher phosphorylation of Pi3K and MAPK pathways, with enhanced proliferation during manufacturing, more durable synapse formation with target cells, and higher secretion of cytokines early after antigen exposure. In vivo, this also resulted in a higher CAR T-cell expansion (at day 15) and better tumor control (with a follow-up of 50 days) [73]. Following this observation, they developed a new anti-CD22 CAR construct with a short linker, that is currently evaluated in an amended version of the clinical trial (NCT02650414; no published results yet). In a recent single center, dose-escalation phase 1 trial (NCT04088890), 38 large B-cell lymphoma patients with median 4 lines of therapy who had relapsed after CD19 targeted CAR T-cell therapy were treated with CD22 directed CAR T-cell therapy. The CR and PR rates were 53 % and 16 % respectively with a median duration of response of 27.8 months [74]. While the results are promising and provides a therapeutic option following relapse after CD19 CAR T-cell therapy, this is a phase 1 dose finding study and further research is warranted to measure the long-term efficacy.

In the preclinical development of a dual CD19/CD22-targeting CAR by Autolus, substantial efforts were dedicated to optimizing the CAR design. However, the assays designed to evaluate the efficacy of this CAR T-cell product predominantly focused on short-term efficacy measures, such as cytotoxicity, IFNγ production, and proliferation up to four days in vitro, along with an in vivo model with only a 15-day follow-up period. Although these CAR T-cells demonstrated a notable acute effect, evidenced by a high rate of complete remission (CR + CRi) at one month in relapsed/refractory (R/R) B-ALL, they exhibited low persistence compared to Tisa-Cel in the ELIANA trial. Consequently, the 1-year overall survival (OS) and event-free survival (EFS) rates were relatively modest at 60 % and 32 %, respectively [75].

In the preclinical development of another bivalent CD19/CD22 CAR T-cell product at the NIH, significant optimization of the CAR was achieved, including the comparison of different tandem/loop structures. However, in vivo assays suggested a reduced ability to clear tumor cells expressing only the CD22 target [76]. Subsequently, the phase 1 trial using this construct in B-ALL and LBCL failed to achieve outcomes superior to CD19-alone targeting CARs, with $CD19^{-/dim}$ relapses in 50 % and 29 % of patients with B-ALL and LBCL, respectively, and no $CD22^{-/dim}$ relapse [77]. To interrogate the relative potency of the signal delivered via the CD19 scFv versus the CD22 scFv, authors compared the response to CD22-only stimulation and CD19-only stimulation using samples from manufactured cell products. They observed a reduced activation (CD69 expression) and cytokine secretion (TNFα, IFNγ and IL2) in response to NALM6 cells expressing CD22 only, compared to NALM6 cells expressing CD19 only. When comparing the activity of the CD22 scFv in bispecific CD19–22 CAR and in monospecific CD22 CAR, they observed similar levels of activation but lower secretion of TNF-α and IL-2 [77]. Other bispecific CARs such as CD19/CD20 or CD19/BCMA have been evaluated in phase 1–2 clinical trials in NHL and multiple myeloma respectively while trispecific (CD19/CD20/CD22) CARs have been evaluated in preclinical models of large cell lymphoma. [78–80]

Long-term tumor clearance and tumor control upon rechallenge are crucial parameters, but the importance of CAR T-cell persistence should not be underestimated. In the preclinical development of NKG2D-based CAR T cells, the cells demonstrated their long-term efficacy in vivo in a murine model of ovarian cancer, including a rechallenge with tumor cells after 225 days. However, the NKG2D-based CAR T cells were not persisting, and this long-term anti-tumor immunity was mediated by a host immune response [81]. The clinical trials evaluating NKG2D-based CAR T cells in myeloid malignancies and multiple myeloma have revealed low response rates, with no sign of long-term tumor control [82,83]. These results may also stem from the significant differences between the immune environment in murine models and in patients. In myeloid malignancies, the strong immunosuppressive microenvironment might limit the efficacy of this product and hinder the development of a secondary endogenous immune response.

Overall, these observations underscore the significance of conducting comprehensive evaluations of new CAR T-cell products. This includes not only optimizing the CAR structure when designing a new CAR construct, but also assessing functionality through robust in vitro and in vivo assays. These assays should evaluate both acute and chronic CAR T-cell functions, encompassing proliferation, cytotoxicity, and cytokine production following acute and chronic stimulation, the latter mimicking the sustained antigen exposure of these CAR T-cells when infused into patients. In murine models, long-term monitoring of CAR T-cell persistence and tumor control, ideally incorporating tumor rechallenge in surviving mice, can offer valuable insights into the long-term efficacy of the CAR T-cell product. Finally, further work may be needed to better mimic the inflammatory and immunosuppressive environment present in many malignant diseases, as its impact on CAR T-cell efficacy and toxicity might be underestimated in current preclinical models.

5. Enhancing CAR T-cell therapeutic efficacy: lessons from preclinical studies and early clinical evaluations

The pursuit of enhanced efficacy with CAR T-cell therapy has resulted in extensive preclinical studies employing different approaches ranging from manufacturing strategies to innovative CAR designs. Some

of these approaches are highlighted in Table 2. The lessons from these pre-clinical studies help in a better understanding of the various dynamics governing the CAR T-cell efficacy and could help refine the strategies and subsequently optimize the approach in clinical settings.

5.1. Manufacturing strategies to obtain an improved memory function

Numerous correlative studies have consistently identified the proportion of early memory (eM) or stem cell memory (SCM) T cells as a robust signature associated with CAR T-cell proliferation, efficacy, and persistence [15,37,40,43,84,85]. In alignment with these findings, Biasco et al. demonstrated that CAR T_{SCM} cells undergo a clonal burst shortly after infusion and are the primary contributors to the clonal pool at both early and late timepoints [86]. Building upon these insights, several research groups are now developing CAR T-cell manufacturing processes aimed at enriching for these eM/SCM T-cells. In addition to the FDA-approved Liso-Cel, which is enriched in CD8 central memory (CM) T-cells [87], other CAR T-cell products enriched in early memory T-cells are currently under development.

Enriching the starting material in T_{CM} through CD25/CD14/CD45RA depletion and CD62L selection has proven ineffective in B-NHL and B-ALL treated with anti-CD19 28ζ CAR T-cells, with poor persistence ($\leq$28 days) and low response rates [88,89]. Interestingly, in the preclinical evaluation of this process, the engraftment of T_{CM}-enriched CAR T-cells in NSG mice was measured only up to day 24, with no report of subsequent evaluation of persistence or in vivo tumor control. These disappointing results prompted a modification of the process to enrich in both naive ($T_{N)}$ and T_{CM} cells (through CD62L selection, without CD45RA depletion) [89]. In adult patients with B-ALL treated, $T_{N/CM}$ enrichment before anti-CD19 CAR T-cell manufacturing resulted in a high complete remission/complete remission with incomplete hematologic recovery (CR/CRi) rate of 87 %, with 95 % of these patients achieving minimal residual disease (MRD) negativity. Responders had a median relapse-free survival (RFS) of 17.1 months, and some patients exhibited long-term CAR T-cell persistence. However, consolidation with allogeneic hematopoietic stem cell transplantation, which was performed in 53 % of responding patients, was still associated with superior RFS, indicating that these CAR T-cells were still not providing a sufficient long-term control of the disease [89]. Another CAR T-cell product enriched in naive and memory cell (positive selection of $CD62L^+$ cells), with a BBζ-based bispecific anti-CD19/CD20 CAR was developed at UCLA and showed promising results in a phase 1 clinical trial in patients with relapsed/refractory B-NHL after $\geq$2 prior lines of therapy (ORR 90 %, CR 70 %, median PFS 18 months) [90]. The development of this new CAR structure included optimization of the extracellular spacer and linker sequence to obtain an optimal activation in response to CD20 as well as CD19 stimulation [91]. Interestingly, the preclinical manufacturing of this CAR T-cell product was performed on $CD8^+$ selected cells [91]. When moving to the clinical CAR T-cell product, the $CD62L^+$ selection without this $CD8^+$ selection resulted in an enrichment in monocytes, and the protocol had to be amended to include a CD14/CD25 depletion when $\geq$5 % of $CD62L^+$ cells were $CD14^+$ and/or $CD25^+$ [90].

The T-memory stem cell subset has been described as crucial for CAR T -cell efficacy, and their expansion during manufacturing has been favored using IL-7 and IL-15 rather than IL-2 [84]. After observing the poor persistence and efficacy of anti-Lewis Y CAR T cells in a clinical trial for metastatic solid tumors and noting the low abundance of stem-like T cells in the infusion product, Meyran et al. reported the preclinical optimization of a CAR T-cell product enriched in stem-like T cells. This was achieved by selecting naive T-cells using negative selection process (magnetic depletion of non-naive T-cells) and employing a shortened manufacturing process with IL-7 and IL-15 instead of IL-2 [92]. They demonstrated that this process preserved a more stem-like phenotype at the end of manufacturing, and that these CAR T-cells exhibited superior proliferation not only after acute stimulation but also after a 30-day

Table 2
Strategies used to improve CAR T-cell efficacy in preclinical models or clinical trials of hematological malignancies.

Approach	Description
Incorporation of co-stimulatory domains	Addition of co-stimulatory domains like CD28, 4-1BB, OX40, or ICOS to enhance T-cell activation and persistence [178,179].
Optimization of CAR construct	Optimizing the components of CAR construct to improve antigen recognition and binding affinity [180,181].
Cytokine secretion	Arming the CAR T-cells to secrete cytokines to enhance T-cell proliferation, persistence, cytotoxicity and overcome an immunosuppressive TME [105,106,112].
Suicide genes	Incorporation of suicide genes for safety control and elimination of CAR T cells [182].
Trafficking chemokines/ chemokine receptors	Addition of chemokine or chemokine receptors to enhance migration towards tumor sites and improve CAR T-cell infiltration [120,183].
Resistance to immunosuppression	Engineering CAR T cells to resist inhibitory signals from the tumor microenvironment or express immune checkpoint inhibitors [184].
miRNA modulation	Integrating miRNA in the CAR construct to enhance proliferation and effector functions [185].
Switchable CAR T cells	Designing CAR T cells with inducible activation or deactivation mechanisms for enhanced safety and control [186].
Transcriptional regulation of CAR expression	Controlling CAR expression levels through inducible promoters or regulatory elements to optimize efficacy [187].
Modulation of CAR affinity	Controlling the CAR affinity to the target antigen with low affinity CAR constructs showing reduced trogocytosis and better effector functions [188,189].
Dual-targeting CAR T cells	Designing CAR T cells targeting multiple tumor antigens to enhance specificity or to overcome tumor heterogeneity and antigen escape [190].
Targeting novel antigens	Designing CAR constructs targeting antigens other than CD19, CD20 and CD22 to combat antigen negative relapses following targeted therapies [191–193].
Tumor microenvironment modulation	Modification of CAR T cells to resist inhibitory signals or express factors enhancing tumor infiltration [154,155].
Enhancing resistance to T cell exhaustion	Incorporating factors that counteract T-cell exhaustion, such as blocking PD-1, TIM-3 or TIGIT signaling pathways [194,195].
Metabolic programming	Modulating the different metabolic pathways like glycolysis, cholesterol and fatty acid metabolism in the CAR T-cells allowing for sustained effector function and survival [141,144].
Optimizing the culture	Optimizing the CAR T-cell culture conditions such as serum supplements and PH to improve the CAR T-cell phenotype and function [196,197].
Gene expression regulation	Overexpression or downregulation of specific genes leading to enhanced CAR T-cell function [198].
Gene specific vector integration	Integration of the lentiviral vector in specific genes such as *TET2* enhancing the CAR T-cell potency [53,199].
Non-viral gene delivery methods	Utilizing non-viral methods such (CRISPR)/Cas9 for site specific integration and enhanced tumor specific cytotoxicity [200,201].
Combination therapies	Combining CAR T-cell therapy with other treatments like checkpoint inhibitors, BTK inhibitors, to synergistically enhance efficacy [202,203].
In vivo CAR T cell manufacturing	In vivo CAR transduction using T cell directed vectors has the potential to overcome the limitations in terms of cost and infrastructure requirements of ex vivo manufacturing [170,204].
In vivo CAR restimulation	Restimulation of the CAR T-cells in vivo using a vaccine like approach to enhance their persistence and effector function [205,206].

(*continued on next page*)

Table 2 (*continued*)

Approach	Description
Memory phenotype CAR T cells	Engineering CAR T cells to exhibit a memory phenotype, promoting long-term persistence and recall responses [207].
Optimizing the starting material	Enrichment of naïve and memory stem cell populations in the apheresis product [90,208].
Shortening the manufacturing time	Reducing the culture duration to maintain a less differentiated phenotype in the manufactured CAR T-cells [94].

chronic stimulation in vitro. Additionally, the optimized CAR T-cells showed enhanced expansion and tumor control at low doses in NSG mice [92]. This product still needs to demonstrate its superiority in clinical trials. Furthermore, despite the potential for lower dosing compared to conventional CAR T-cells, the limited availability of naive T-cells in heavily treated patients may still constrain feasibility of this process.

Another approach to preserve a less differentiated phenotype is to reduce the manufacturing time [93]. YTB323 is an anti-CD19 BBz CAR T-cell product (with the same CAR construct used for Tisa-Cel) with a short – expansion-less – manufacturing process using the T-Charge™ platform. The preclinical evaluation showed higher IL-2 and IFNγ secretion upon CD19-specific activation, superior efficacy in repeat-stimulation assay, as well as better expansion and tumor control at lower doses in vivo [94]. In a phase 1 clinical trial, this product showed very good overall response rates of 80 % at the optimal dose (dose level 2) in patients with LBCL ≥2 lines of prior therapy (including HSCT) [94]. With a median follow-up of 10 months, responses appeared durable (CR rates of 63 % at 3 months and 69 % at 6 months, median duration of response not reached) [95].

Finally, other methods to enrich CAR T-cells in early memory T-cells have been assessed in preclinical studies. Some groups have used small molecules or cytokines during manufacturing to preserve or enhance the stem-like or memory function of the CAR T-cell product. These include the use of IL-7, IL-21, and the glycogen synthase-3β inhibitor TWS119 [96], AKT inhibition [97], and PI3Kδ/γ inhibition [98]. Additionally, some groups have pursued further genetic modifications in the T cells to induce durable changes that favor the persistence of a stem cell-like pool, such as FOXO1 overexpression [99,100].

5.2. Armored CAR-T cells: preclinical insights and therapeutic strategies

To improve the therapeutic benefits of CAR T-cells, many innovative CAR designs have been developed to enhance the antitumor efficiency and overcome treatment resistance related to inadequate expansion, infiltration, and persistence of CAR T-cells. [101–104] In this regard, fourth-generation CARs (referred to as "armored" CARs) that incorporate cytokines (IL-7, IL-15, IL-18, IL-21, Il-25, IL-33) are being developed to improve CAR T-cell persistence, tumor infiltration and effector functions [105–110]. (Fig. 2) The co-expressed cytokines can help maintain an early memory phenotype in the CAR T-cells, overcome the immunosuppressive microenvironment (CLL/NHL) and reduce the level of apoptosis of CAR T-cells apart from enhanced proliferation and effector functions. [111–113] While the incorporation of cytokines improves the T-cell function, the potential risk of adverse events could

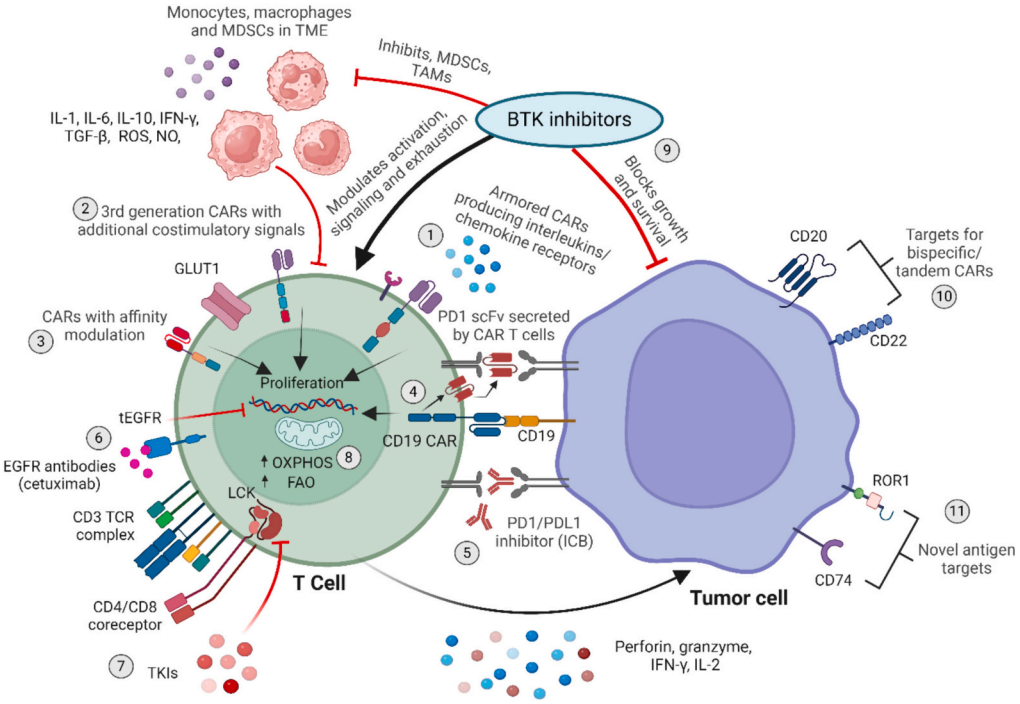

Fig. 2. Overview of optimization strategies to enhance CAR T-cell efficacy. Various strategies have been explored in preclinical models to optimize the effectiveness of CAR T-cell therapy for hematological malignancies. These include: 1) Engineering "armored" CAR T-cells that can produce cytokines or express chemokine receptors to enhance cytotoxicity, memory function, or tumor site migration. 2) Developing second and third generation CAR constructs with additional costimulatory domains to boost T-cell activation and persistence. 3) Modulating the affinity of CAR receptors to reduce exhaustion, trogocytosis, and improve effector functions. 4) Blocking PD1-PDL1 axis using secreted PD1 scFV or 5) anti-PD1 monoclonal antibodies to make the CAR T-cells more resistant to exhaustion. 6) Incorporating suicide genes (irreversible) or 7) use of pharmacological agents such as TKI (reversible) to control CAR T-cell exhaustion and minimize adverse events. 8) Manipulating metabolic pathways, such as increasing oxidative phosphorylation and fatty acid oxidation, to improve CAR T-cell persistence and memory function. 9) Combining CAR T-cell therapy with BTK inhibitors to synergistically enhance CAR T-cell function. 10) Targeting multiple antigens or 11) novel antigens to prevent antigen escape and improve therapeutic efficacy. (Created with BioRender.com).
BTK – Bruton tyrosine kinase. CAR – chimeric antigen receptor. CD – cluster differentiation. IL- interleukins. MDSC – myeloid derived suppressor cells. NO – nitric oxide. ROS – reactive oxygen species. scFv – single chain fragment variable. TAM – tissue associated macrophages. TCR – T cell receptor. TME – tumor microenvironment.

preclude its clinical adoption, although some studies have designed the CAR construct to address this issue [114]. Therefore, it is important to have robust preclinical assays and animal models to evaluate these CARs. Yoshikawa et al. used a chimeric cytokine receptor G6/7R that captures IL-6 from the myeloid cells via extracellular IL-6 receptor reducing the toxicity while the constitutively active IL7 signaling improves the CAR T-cell proliferation and effector function [115]. In another study, hypoxia-controlled secretion (*HIF1α*) of IL12 resulted in regression of large DLBCL in animal models without toxicity [116]. Both the studies used xenograft animal models followed up for 2 months or more after infusion with the latter also claiming "effective cure" with 100 % mice surviving until the termination of the experiment with all the doses assessed in the study.

One of the potential reasons for the failure of CAR T-cells in solid tumors and lymphoma has been the inability of the CAR T-cells to effectively migrate to the tumor site after infusion. Researchers have tried to improve the tumor site homing by incorporating the expression of a chemokine receptors or chemokines in the CAR construct that guides the immune cells to the tumor site [117,118]. (Fig. 2) The feasibility of incorporating chemokines/chemokine receptors in the CAR construct has been demonstrated in preclinical settings and evaluated in clinical trials [119]. Although the preclinical studies do effectively assess the short term, and, in some studies, long term effector function as well the safety, it is important to remember the limitation of animal models in recapitulating the tumor microenvironment in humans with different cellular and non-cellular components. Hence the result from the preclinical studies is not always reproduced when it moves to a clinical trial, and it is imperative to evaluate the CAR T-cell function with different models and appropriate readouts that would best represent the scenario in a patient. Recently, anti-CD19 CAR T-cells expressing IL-7 and CCL19 have demonstrated enhanced tumor targeting and antitumor activity compared to conventional CD19 CAR T-cells with no dose limiting toxicities such as grade 4 CRS. [120] While the preclinical data from tumor animal models was promising with 100 % mice ($n = 6$) survival and 5 out of 6 tumor-bearing mice achieving long-term tumor-free survival up to 95 days post-treatment, the results from the clinical trial were not very different from the other studies. The overall response rate was 79.5 % at 3 months (CR – 56.4 %; PR – 23.1 %) and the median progression-free survival was 13 months with a median follow up of 32 months. In another study, 1928z/IL-12 CAR T-cells derived from umbilical cord blood retained a central memory-effector phenotype with a significantly enhanced survival of CD19+ tumor bearing mice. Using appropriate preclinical models, the authors demonstrated that clinically relevant doses could be achieved from umbilical cord derived CAR T-cells; however, relevant models to test the safety were not assessed in the study as noted by the authors [121]. Like lymphoma, the TME plays an important role in myeloma disease progression and treatment outcomes. CAR T-cells targeting the myeloma-associated antigens BCMA and B-cell activating factor (BAFF-R) failed to eliminate myeloma when these antigens were weakly expressed, whereas IL-18-secreting CAR T-cells targeting these antigens promoted myeloma clearance. The IL-18-secreting CAR T-cells developed an effector-like T-cell phenotype, promoted interferon-gamma production, reprogrammed the myeloma bone marrow microenvironment through type I/II interferon signaling, and activated macrophages to mediate anti-myeloma activity [122].

5.3. Mitigating CAR T-cell exhaustion: strategies for controlled signaling

One of the reasons for CAR T-cell exhaustion (especially CARs with CD28 costimulatory domain) is the strong tonic and ligand independent constitutive signaling which initially results in results in uncontrolled proliferation, cytokine production and eventually leads to exhaustion. Unlike the T-cell receptors (TCRs) which are controlled by a stringent feedback mechanism, constitutive CAR expression is less susceptible to feedback regulation [123]. Controlling this constitutive CAR signaling not only delays exhaustion but could also reduce the adverse events. Different strategies have been tried to address this issue such as use of suicide genes (truncated EGFR) that could be targeted pharmacologically [124], use of inhibitory CARs that temporarily and reversibly inhibits the CAR T-cell activity [125] or the use of clinically used drugs such as TKIs that could function as a reversible on/off switch for T cell activation and differentiation [126,127]. (Fig. 2) While using a "kill switch" results in irreversible loss of potentially therapeutic CAR T-cells, using reversible switches has the potential to avoid or minimize adverse events like CRS and TLS without compromising the efficacy [128]. Using appropriate in vitro models and flow cytometry assays, Rodgers et al. showed activation and cytotoxicity of their switchable CAR T-cells was dependent on the presence of CD19 antigen. While there were minimal differences in the lytic activity with the different switch/hinge designs in the in-vitro assays, the differences were significantly different in the in-vivo models highlighting the role of using appropriate preclinical models in evaluating the CAR T-cell function. TKIs namely dasatinib abrogate the phosphorylation of multiple key components like lymphocyte specific protein tyrosine kinase (LCK), CD3ζ, and ZAP70 in the CAR signaling domain which was completely reversed following depletion of the drug [126]. While the preclinical data looks promising, there is limited clinical data available for using TKIs with CAR T therapy outside of Ph + ALL (NCT03984968, NCT04603872) and few phase I/II trials are currently underway [129]. Eyquem et al. integrated the CD19-specific CAR to the T-cell receptor α constant (TRAC) locus using the CRISPR/Cas9 genome editing and studied the T cell phenotypes and function in mouse models of ALL [130]. They showed that directing the CAR to the TRAC locus avoided the tonic signaling with internalization and re-expression of the CAR on the surface following repeat exposure to CD19+ B cells. The strategy delayed the T cell differentiation and exhaustion and led to better efficacy in mouse models compared to conventional CD19 CAR T-cells. Webster et al. designed CD19-targeting CAR, which regulates its own function based on the presence of a CD19 antigen expressing cells, by placing the CAR19 constructs under transcriptional control of inducible promoters AP1-NFκB or STAT5. By using repeat stimulation assays and in-vitro and in-vivo models to assess long term persistence the authors showed the outcomes are different when using a proximal (AP1-NFκB) and distal (STAT5) promoters in the construct [131].

5.4. Augmenting CAR T-cell function via checkpoint inhibition

Several Inhibitory receptors have been characterized in T-cells, such as cytotoxic T-lymphocyte–associated protein 4 (CTLA-4), T-cell immunoglobulin domain and mucin domain–containing protein 3 (TIM-3), lymphocyte-activation gene 3 (LAG-3), and programmed death-1 (PD1). These molecules are upregulated following sustained activation of T-cells in chronic disease and cancer, and they promote T-cell dysfunction and exhaustion, thus resulting in the escape of tumor from immunosurveillance. Targeting the checkpoint inhibitors on the T-cells has been an attractive strategy in solid tumors and hematological malignancies [132–135]. (Fig. 2) Different approaches have been tried with the goal of enhancing the CAR T-cell function without triggering the uncontrolled proliferation of non-CAR T-cells leading to autoimmune side effects [132–134,136–138]. Blaeschke et al. created aPD-1-CD28 fusion protein to transform inhibitory signals of leukemic cells (PD-1) into T-cell stimulation (CD28) [136]. When they evaluated the cytotoxicity of the CAR T cells with PD-1-CD28 fusion proteins (19_BB_3z_PD-1_28) it was not different from the conventional second-generation CAR T cells (19_BB_3z) at any of the E:T ratio. However, when target cells were added to anti-CD19 CARs every 3 to 4 days to assess persistent effector function, CAR T-cells with PD-1-CD28 fusion proteins clearly outperformed the conventional CAR T-cells. CAR T-cells engineered to express PD-L1 scFv antibody demonstrated enhanced anti-tumor activity in in vitro and in vivo mouse models by blocking the PD-1/PD-L1 signaling [139]. Although the clinical trials targeting the

checkpoint inhibition axis in B cell malignancies are limited, the promising results show that the outcomes with CAR T-cell therapies can be improved by targeting the immune checkpoint axis [140].

5.5. Enhancing CAR T-cell performance: leveraging the metabolic pathways

The metabolic characteristics of the CAR T-cells depend on the coreceptor included in the CAR construct with 41BB CARs showing increased fatty acid oxidation and mitochondrial biogenesis while CD28 CARs showing increased glycolytic metabolism [66]. This difference also partly explains the difference in phenotype and persistence of the CAR T-cells harboring different costimulatory domains. Repeat stimulation assays using in-vitro models showed that GLUT1 overexpression in a CD19-28ζ CAR promoted the Tscm formation with increased expression of genes associated with memory phenotypes. (Fig. 2) The improved efficacy was demonstrated using in-vivo models as well with the CD19-28ζ CAR with GLUT1 overexpression showing tumor control in NALM6 mice models up to 200 days while there was rapid tumor progression after an initial response with the control CD19-28ζ CAR T-cells [141]. Mitochondrial function (or dysfunction) plays an important role in determining the functional persistence of CAR T-cells and metabolic alterations during CAR T-cell manufacture could influence its effector function [142,143]. Recent work by Gross et al. showed that priming CAR T-cells with galactose during the culture improved the leukemia free survival in animal models by enhancing the mitochondrial activity in the CAR T-cells [144]. Interestingly, the authors first evaluated the mitochondrial parameters of CAR T-cell products from ALL patients enrolled on a clinical trial demonstrating the improved CAR T-cell function with increased mitochondrial function and subsequently attempted a metabolic conditioning by growing cells in galactose primed media. Two recent papers showed that FOXO1 overexpression in CAR T-cells was associated with increased oxidative phosphorylation and metabolic fitness and enhanced the memory phenotype and improved efficacy [99,100].

5.6. Navigating the tumor microenvironment in lymphoma

One of the big challenges in designing CAR T-cell studies in lymphoma has been recapitulating the heterogeneity in the lymphoma TME. Researchers are trying to address this issue by using strategies such as patient derived organoids [145]. The tumor microenvironment with its cellular and non-cellular components is an important determinant of CAR T-cell outcomes in lymphoma [146]. The presence of immunosuppressive myeloid (PMN-MDSC) and monocyte (M-MDSC) derived suppressor cells have been associated with poor outcomes in NHL and their presence in circulation prior to CAR T-cell infusion was associated with decreased axi-cel expansion and poor outcomes [147–149]. Locke et al. assessed the pretreatment tumor characteristics in patients enrolled in ZUMA-7 trial and showed that low CD19 expression in malignant cells correlated with a tumor gene expression signature (GES) consisting of immune-suppressive stromal and myeloid genes, highlighting the inter-relation between malignant cell features and immune contexture substantially impacting axi-cel outcomes [150]. Apart from the inhibitory cytokines and the infiltration of myeloid cells and Tregs, other factors such as fibrotic stroma, phenotype of the non-CAR T-cells tumor derived exosomes in the TME also plays a role in the CAR T-cell proliferation and outcomes [151–153]. Therefore, robust preclinical evaluation of the TME is important while assessing the functional efficacy of CAR T-cells in lymphoma, myeloma, and solid tumors. Apart from the efficacy the TME also plays a role in the development of adverse events. Giavridis et al. showed in animal model that the severity of CRS is mediated not by CAR T cell–derived cytokines, but by IL-6, IL-1 and nitric oxide (NO) produced by recipient macrophages [62]. This suggests that the response and adverse events post infusion is not just dependent of the tumor-CAR T-Cell interaction but also on the microenvironment and the presence of myeloid cells in the TME. Targeting the immunosuppressive microenvironment pharmacologically or by modifying the CAR construct seems promising strategies and are currently explored [154,155]. While these preclinical studies address some of the important TME factors that contribute to CAR T-cell resistance, better preclinical models to mimic the TME are required to truly assess the CAR T-cell efficacy.

6. Preclinical models of allogeneic CAR T-cell therapy: overcoming challenges for clinical translation

Although allogenic CAR T-cells offer several advantages over autologous CAR T-cells in terms of cost, availability for all patients, starting T-cell fitness and the need for bridging chemotherapy, its clinical utility is limited by graft rejection and graft versus host disease (GVHD) [156,157]. HLA-mediated rejection could be avoided by knocking out the *B2M*, but this could result in elimination of the CAR T-cells by the NK cells [158–160]. Preclinical models using CD19, CD123 and BCMA targeting CARs used different strategies to overcome these limitations and showed potent antitumor efficacy on serial rechallenges and in vivo models [161–164]. Although none of the preclinical studies compare the efficacy of allogenic CAR T-cells to autologous CAR T-cells targeting the same tumor antigen, they do demonstrate robust in-vitro anti-tumor activity which is reproduced in immunocompromised and immunocompetent animal models. Recent and ongoing phase 1/2 clinical trials demonstrate antitumor activity and a manageable safety profile with allogenic CAR-T cells (UCART19, UCART20x22) in B-ALL [165–167] and CD7 expressing T cell leukemia/lymphoma and AML [168].

An alternative approach to lowering costs and eliminating the need for intricate manufacturing processes and bridging chemotherapy is the in vivo production of CAR T-cells using both viral vectors and non-viral techniques, such as nanoparticles. Unlike conventional ex vivo methods, which include isolating, genetically modifying, and reinfusing T-cells, in vivo production involves delivering a genetic construct directly into the patient's cells to facilitate CAR T-cell generation. [169]Despite its potential advantages, this strategy faces significant challenges, including the complexity of the delivery system, the risk of host inflammatory responses to viral vectors, and the potential for unintended vector integration into other cells. These issues raise substantial regulatory and safety concerns, necessitating thorough investigation of the long-term effects of this strategy. Researchers are actively developing methods to enhance the precision of vector integration, such as utilizing CD3-targeted viral vectors and advanced nanoparticle technologies [170,171].

7. Conclusion

The remarkable success of CAR T-cells in treating aggressive, treatment refractory B-cell malignancies has captured the attention of clinicians, scientists, industry as well as the patients for this innovative therapeutic approach. As a result, this area has been the subject of significant research over the past decade with many groups exploring ways to optimize the CAR T-cell treatment, improving the efficacy, and minimizing the adverse events. Despite a lot of promising findings in preclinical studies, these results sometimes do not consistently get translated into improved clinical outcomes. This disparity may be attributed partially to biological variations between humans and animal models but underscores the need for reliable preclinical models that accurately replicate human disease. Understanding the TME and the interactions between CAR T-cells, tumor cells and endogenous immune cells from the preclinical models is essential in understanding the efficacy-toxicity profile of newly developed CAR T-cells.

8. Future considerations

Future research in CAR T cell therapy for hematological

malignancies may prioritize several critical areas to enhance remission rates and maintain favorable safety and efficacy profiles. Apart from identifying new targets and strategies to optimize the CAR construct and function, exploring synergistic effects of combining CAR T cell therapy with available chemotherapy or immunotherapy is important. Optimizing the manufacturing process, decentralized manufacturing and improving logistics for timely delivery of CAR T cell products are important considerations moving forward. An essential objective for the future should involve developing strategies to lower costs and broaden global access to CAR T-cell therapy.

Practice points

- Understand the roles and limitations of various preclinical assays and animal models (immune-compromised and immune-competent) in evaluating CAR T-cell functionality and safety is crucial for successful clinical translation.
- Developing predictive biomarkers for safety and efficacy: Investigate the development of standardized assays for predicting efficacy based on preclinical evaluations. The preclinical model should be appropriate to measure both safety and efficacy as improved clinical potency should not be at the risk of increased adverse events.
- Emphasize on the pre-infusion functional assessment alongside phenotypic analysis: Factors influencing clinical responses in patients treated with anti-CD19 CAR T-cells include in vivo expansion, post-treatment persistence, memory phenotype, and genetic expression linked to cellular exhaustion. Non-responders typically exhibit exhausted/senescent CD8+ cells with distinct transcriptional signatures.
- Assessment of pre-clinical development: Understanding the importance and effectiveness of preclinical models in predicting clinical efficacy and safety, including lessons learned from the preclinical studies of unsuccessful trials.
- Emphasize the importance of assessing CAR T-cell persistence and efficacy over extended periods, including potential strategies for enhancing long-term responses. Successful clinical translation depends on robust CAR T-cell responses against repeated tumor challenges and not a one-step cytotoxicity assay.
- Assess the impact of the tumor microenvironment on CAR T-cell efficacy and safety, particularly in lymphoma models, to inform clinical strategies effectively

Research agenda

- Enhancing Memory Function through Manufacturing Strategies: Develop techniques to enrich CAR T-cell products with early memory or stem cell memory T-cells to improve persistence and effectiveness.
- Streamlining Manufacturing Processes: Develop rapid and cost-effective manufacturing protocols that maintain efficacy, evaluating scalability and reproducibility in clinical trials.
- Mitigating CAR T-cell Exhaustion and Improving Function: Focus on strategies to enhance CAR T-cell efficacy without compromising safety, particularly by addressing exhaustion.
- Investigating Synergies with Other Treatments: Explore the combined effects of CAR T cell therapy with checkpoint inhibitors, tumor-targeted chemotherapy, or treatments affecting the tumor microenvironment or T cells.
- Overcoming Challenges of the Tumor Microenvironment: Develop strategies to combat the immunosuppressive effects of the tumor microenvironment (TME), utilizing patient-derived organoids and relevant animal models to mimic TME complexity and assess CAR T-cell efficacy.
- Translation to Clinic: Emphasize research on strategies that demonstrate promising results in terms of safety and efficacy in preclinical models, focusing on their applicability to clinical settings.

Declaration of competing interest

Dr. Melenhorst has patents related to Biomarkers and manufacturing of chimeric antigen receptor-engineered T cells. The remaining authors declare no conflicts of interest.

References

[1] Porter DL, Hwang WT, Frey NV, Lacey SF, Shaw PA, Loren AW, et al. Chimeric antigen receptor T cells persist and induce sustained remissions in relapsed refractory chronic lymphocytic leukemia. Sci Transl Med 2015;7(303). 303ra139.
[2] Kalos M, Levine BL, Porter DL, Katz S, Grupp SA, Bagg A, et al. T cells with chimeric antigen receptors have potent antitumor effects and can establish memory in patients with advanced leukemia. Sci Transl Med 2011;3(95). 95ra73.
[3] Kochenderfer JN, Feldman SA, Zhao Y, Xu H, Black MA, Morgan RA, et al. Construction and preclinical evaluation of an anti-CD19 chimeric antigen receptor. J Immunother 2009;32(7):689–702.
[4] Sadelain M, Brentjens R, Rivière I. The basic principles of chimeric antigen receptor design. Cancer Discov 2013;3(4):388–98.
[5] Kowolik CM, Topp MS, Gonzalez S, Pfeiffer T, Olivares S, Gonzalez N, et al. CD28 costimulation provided through a CD19-specific chimeric antigen receptor enhances in vivo persistence and antitumor efficacy of adoptively transferred T cells. Cancer Res 2006;66(22):10995–1004.
[6] Milone MC, Fish JD, Carpenito C, Carroll RG, Binder GK, Teachey D, et al. Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. Mol Ther 2009;17(8):1453–64.
[7] Zhang N, Wu J, Wang Q, Liang Y, Li X, Chen G, et al. Global burden of hematologic malignancies and evolution patterns over the past 30 years. Blood Cancer J 2023;13(1):82.
[8] Fitzmaurice C, Allen C, Barber RM, Barregard L, Bhutta ZA, Brenner H, et al. Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. JAMA Oncol 2017;3(4):524–48.
[9] Cappell KM, Sherry RM, Yang JC, Goff SL, Vanasse DA, McIntyre L, et al. Long-term follow-up of anti-CD19 chimeric antigen receptor T-cell therapy. J Clin Oncol 2020;38(32):3805–15.
[10] Cappell KM, Kochenderfer JN. Long-term outcomes following CAR T cell therapy: what we know so far. Nat Rev Clin Oncol 2023;20(6):359–71.
[11] Singh N, Lee YG, Shestova O, Ravikumar P, Hayer KE, Hong SJ, et al. Impaired death receptor signaling in leukemia causes antigen-independent resistance by inducing CAR T-cell dysfunction. Cancer Discov 2020;10(4):552–67.
[12] Orlando EJ, Han X, Tribouley C, Wood PA, Leary RJ, Riester M, et al. Genetic mechanisms of target antigen loss in CAR19 therapy of acute lymphoblastic leukemia. Nat Med 2018;24(10):1504–6.
[13] Sotillo E, Barrett DM, Black KL, Bagashev A, Oldridge D, Wu G, et al. Convergence of acquired mutations and alternative splicing of CD19 enables resistance to CART-19 immunotherapy. Cancer Discov 2015;5(12):1282–95.
[14] Wijewarnasuriya D, Bebernitz C, Lopez AV, Rafiq S, Brentjens RJ. Excessive costimulation leads to dysfunction of adoptively transferred T cells. Cancer Immunol Res 2020;8(6):732–42.
[15] Fraietta JA, Lacey SF, Orlando EJ, Pruteanu-Malinici I, Gohil M, Lundh S, et al. Determinants of response and resistance to CD19 chimeric antigen receptor (CAR) T cell therapy of chronic lymphocytic leukemia. Nat Med 2018;24(5):563–71.
[16] Haradhvala NJ, Leick MB, Maurer K, Gohil SH, Larson RC, Yao N, et al. Distinct cellular dynamics associated with response to CAR-T therapy for refractory B cell lymphoma. Nat Med 2022;28(9):1848–59.
[17] Sarén T, Ramachandran M, Gammelgård G, Lövgren T, Mirabello C, Björklund Å, et al. Single-cell RNA analysis reveals cell-intrinsic functions of CAR T cells correlating with response in a phase II study of lymphoma patients. Clin Cancer Res 2023;29(20):4139–52.
[18] Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med 2017;377(26):2531–44.
[19] 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.
[20] Houot R, Bachy E, Cartron G, Gros FX, Morschhauser F, Oberic L, et al. Axicabtagene ciloleucel as second-line therapy in large B cell lymphoma ineligible for autologous stem cell transplantation: a phase 2 trial. Nat Med 2023;29(10):2593–601.
[21] Jacobson CA, Chavez JC, Sehgal AR, William BM, Munoz J, Salles G, et al. Axicabtagene ciloleucel in relapsed or refractory indolent non-Hodgkin lymphoma (ZUMA-5): a single-arm, multicentre, phase 2 trial. Lancet Oncol 2022;23(1):91–103.
[22] Shah BD, Ghobadi A, Oluwole OO, Logan AC, Boissel N, Cassaday RD, et al. KTE-X19 for relapsed or refractory adult B-cell acute lymphoblastic leukaemia: phase 2 results of the single-arm, open-label, multicentre ZUMA-3 study. Lancet 2021;398(10299):491–502.
[23] Wayne AS, Huynh V, Hijiya N, Rouce RH, Brown PA, Krueger J, et al. Three-year results from phase I of ZUMA-4: KTE-X19 in pediatric relapsed/refractory acute lymphoblastic leukemia. Haematologica 2023;108(3):747–60.

[24] Wang M, Munoz J, Goy A, Locke FL, Jacobson CA, Hill BT, et al. KTE-X19 CAR T-cell therapy in relapsed or refractory mantle-cell lymphoma. N Engl J Med 2020;382(14):1331–42.
[25] 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.
[26] Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. N Engl J Med 2019;380(1):45–56.
[27] Fowler NH, Dickinson M, Dreyling M, Martinez-Lopez J, Kolstad A, Butler J, et al. Tisagenlecleucel in adult relapsed or refractory follicular lymphoma: the phase 2 ELARA trial. Nat Med 2022;28(2):325–32.
[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(10254):839–52.
[29] Abramson JS, Solomon SR, Arnason J, Johnston PB, Glass B, Bachanova V, et al. Lisocabtagene maraleucel as second-line therapy for large B-cell lymphoma: primary analysis of the phase 3 TRANSFORM study. Blood 2023;141(14):1675–84.
[30] Sehgal A, Hoda D, Riedell PA, Ghosh N, Hamadani M, Hildebrandt GC, et al. Lisocabtagene maraleucel as second-line therapy in adults with relapsed or refractory large B-cell lymphoma who were not intended for haematopoietic stem cell transplantation (PILOT): an open-label, phase 2 study. Lancet Oncol 2022;23(8):1066–77.
[31] Siddiqi T, Maloney DG, Kenderian SS, Brander DM, Dorritie K, Soumerai J, et al. Lisocabtagene maraleucel in chronic lymphocytic leukaemia and small lymphocytic lymphoma (TRANSCEND CLL 004): a multicentre, open-label, single-arm, phase 1-2 study. Lancet 2023;402(10402):641–54.
[32] Wang M, Siddiqi T, Gordon LI, Kamdar M, Lunning M, Hirayama AV, et al. Lisocabtagene maraleucel in relapsed/refractory mantle cell lymphoma: primary analysis of the mantle cell lymphoma cohort from TRANSCEND NHL 001, a phase I multicenter seamless design study. J Clin Oncol 2024;42(10):1146–57.
[33] Munshi NC, Anderson Jr 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(8):705–16.
[34] Berdeja JG, Madduri D, Usmani SZ, Jakubowiak A, Agha M, Cohen AD, et al. Ciltacabtagene autoleucel, a B-cell maturation antigen-directed chimeric antigen receptor T-cell therapy in patients with relapsed or refractory multiple myeloma (CARTITUDE-1): a phase 1b/2 open-label study. Lancet 2021;398(10297):314–24.
[35] Laetsch TW, Maude SL, Rives S, Hiramatsu H, Bittencourt H, Bader P, et al. Three-year update of tisagenlecleucel in pediatric and young adult patients with relapsed/refractory acute lymphoblastic leukemia in the ELIANA trial. J Clin Oncol 2023;41(9):1664–9.
[36] Neelapu SS, Jacobson CA, Ghobadi A, Miklos DB, Lekakis LJ, Oluwole OO, et al. 5-year follow-up supports curative potential of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1). Blood 2023;141(19):2307–15.
[37] Westin JR, Oluwole OO, Kersten MJ, Miklos DB, Perales MA, Ghobadi A, et al. Survival with axicabtagene ciloleucel in large B-cell lymphoma. N Engl J Med 2023;389(2):148–57.
[38] Martin T, Usmani SZ, Berdeja JG, Agha M, Cohen AD, Hari P, et al. Ciltacabtagene autoleucel, an anti-B-cell maturation antigen chimeric antigen receptor T-cell therapy, for relapsed/refractory multiple myeloma: CARTITUDE-1 2-year follow-up. J Clin Oncol 2023;41(6):1265–74.
[39] Gauthier J, Bezerra ED, Hirayama AV, Fiorenza S, Sheih A, Chou CK, et al. Factors associated with outcomes after a second CD19-targeted CAR T-cell infusion for refractory B-cell malignancies. Blood 2021;137(3):323–35.
[40] Locke FL, Rossi JM, Neelapu SS, Jacobson CA, Miklos DB, Ghobadi A, et al. Tumor burden, inflammation, and product attributes determine outcomes of axicabtagene ciloleucel in large B-cell lymphoma. Blood Adv 2020;4(19):4898–911.
[41] Turtle CJ, Hay KA, Hanafi LA, Li D, Cherian S, Chen X, et al. Durable molecular remissions in chronic lymphocytic leukemia treated with CD19-specific chimeric antigen receptor-modified T cells after failure of Ibrutinib. J Clin Oncol 2017;35(26):3010–20.
[42] Melenhorst JJ, Chen GM, Wang M, Porter DL, Chen C, Collins MA, et al. Decade-long leukaemia remissions with persistence of CD4(+) CAR T cells. Nature 2022;602(7897):503–9.
[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(12):1878–87.
[44] Good Z, Spiegel JY, Sahaf B, Malipatlolla MB, Ehlinger ZJ, Kurra S, et al. Post-infusion CAR TReg cells identify patients resistant to CD19-CAR therapy. Nat Med 2022;28(9):1860–71.
[45] Capelli C, Cuofano C, Pavoni C, Frigerio S, Lisini D, Nava S, et al. Potency assays and biomarkers for cell-based advanced therapy medicinal products. Front Immunol 2023;14:1186224.
[46] Bravery CA, Carmen J, Fong T, Oprea W, Hoogendoorn KH, Woda J, et al. Potency assay development for cellular therapy products: an ISCT review of the requirements and experiences in the industry. Cytotherapy 2013;15(1):9–19.
[47] Brentjens RJ, Riviere I, Park JH, Davila ML, Wang X, Stefanski J, et al. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. Blood 2011;118(18):4817–28.
[48] Magnani CF, Gaipa G, Lussana F, Belotti D, Gritti G, Napolitano S, et al. Sleeping beauty-engineered CAR T cells achieve antileukemic activity without severe toxicities. J Clin Invest 2020;130(11):6021–33.
[49] Lamble AJ, Myers RM, Taraseviciute A, John S, Yates B, Steinberg SM, et al. Preinfusion factors impacting relapse immunophenotype following CD19 CAR T cells. Blood Adv 2023;7(4):575–85.
[50] Bai Z, Woodhouse S, Zhao Z, Arya R, Govek K, Kim D, et al. Single-cell antigen-specific landscape of CAR T infusion product identifies determinants of CD19-positive relapse in patients with ALL. Sci Adv 2022;8(23). eabj2820.
[51] Ambrose C, Su L, Wu L, Dufort FJ, Sanford T, Birt A, et al. Anti-CD19 CAR T cells potently redirected to kill solid tumor cells. PloS One 2021;16(3):e0247701.
[52] An N, Tao Z, Li S, Xing H, Tang K, Tian Z, et al. Construction of a new anti-CD19 chimeric antigen receptor and the anti-leukemia function study of the transduced T cells. Oncotarget 2016;7(9):10638–49.
[53] Fraietta JA, Nobles CL, Sammons MA, Lundh S, Carty SA, Reich TJ, et al. Disruption of TET2 promotes the therapeutic efficacy of CD19-targeted T cells. Nature 2018;558(7709):307–12.
[54] Collins MA, Jung IY, Zhao Z, Apodaca K, Kong W, Lundh S, et al. Enhanced costimulatory signaling improves CAR T-cell effector responses in CLL. Cancer Res Commun 2022;2(9):1089–103.
[55] Zhang Y, Patel RP, Kim KH, Cho H, Jo JC, Jeong SH, et al. Safety and efficacy of a novel anti-CD19 chimeric antigen receptor T cell product targeting a membrane-proximal domain of CD19 with fast on- and off-rates against non-Hodgkin lymphoma: a first-in-human study. Mol Cancer 2023;22(1):200.
[56] Atanackovic D, Iraguha T, Omili D, Avila SV, Fan X, Kocoglu M, et al. A novel multicolor fluorescent spot assay for the functional assessment of chimeric antigen receptor (CAR) T-cell products. Cytotherapy 2024;26(4):318–24.
[57] Eugene-Norbert M, Cuffel A, Riou G, Jean L, Blondel C, Dehayes J, et al. Development of optimized cytotoxicity assays for assessing the antitumor potential of CAR-T cells. J Immunol Methods 2024;525:113603.
[58] Si X, Xiao L, Brown CE, Wang D. Preclinical evaluation of CAR T cell function: in vitro and in vivo models. Int J Mol Sci 2022;23(6).
[59] Jin CH, Xia J, Rafiq S, Huang X, Hu Z, Zhou X, et al. Modeling anti-CD19 CAR T cell therapy in humanized mice with human immunity and autologous leukemia. EBioMedicine 2019;39:173–81.
[60] Zhen A, Carrillo MA, Mu W, Rezek V, Martin H, Hamid P, et al. Robust CAR-T memory formation and function via hematopoietic stem cell delivery. PLoS Pathog 2021;17(4):e1009404.
[61] Diaconu I, Ballard B, Zhang M, Chen Y, West J, Dotti G, et al. Inducible caspase-9 selectively modulates the toxicities of CD19-specific chimeric antigen receptor-modified T cells. Mol Ther 2017;25(3):580–92.
[62] 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(6):731–8.
[63] 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(6):739–48.
[64] 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(4):676–84.
[65] Carpenito C, Milone MC, Hassan R, Simonet JC, Lakhal M, Suhoski MM, et al. Control of large, established tumor xenografts with genetically retargeted human T cells containing CD28 and CD137 domains. Proc Natl Acad Sci U S A 2009;106(9):3360–5.
[66] Kawalekar OU, O'Connor RS, Fraietta JA, Guo L, McGettigan SE, Posey Jr AD, et al. Distinct signaling of coreceptors regulates specific metabolism pathways and impacts memory development in CAR T cells. Immunity 2016;44(2):380–90.
[67] Maloney DG, Kuruvilla J, Liu FF, Kostic A, Kim Y, Bonner A, et al. Matching-adjusted indirect treatment comparison of liso-cel versus axi-cel in relapsed or refractory large B cell lymphoma. J Hematol Oncol 2021;14(1):140.
[68] Sommermeyer D, Hudecek M, Kosasih PL, Gogishvili T, Maloney DG, Turtle CJ, et al. Chimeric antigen receptor-modified T cells derived from defined CD8+ and CD4+ subsets confer superior antitumor reactivity in vivo. Leukemia 2016;30(2):492–500.
[69] Lee SY, Lee DH, Sun W, Cervantes-Contreras F, Basom RS, Wu F, et al. $CD8^+$ chimeric antigen receptor T cells manufactured in absence of $CD4^+$ cells exhibit hypofunctional phenotype. J Immunother Cancer 2023;11(11):e007803.
[70] 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(5):585–601.
[71] Cornell RF, Bishop MR, Kumar S, Giralt SA, Nooka AK, Larson SM, et al. A phase 1, multicenter study evaluating the safety and efficacy of KITE-585, an autologous anti-BCMA CAR T-cell therapy, in patients with relapsed/refractory multiple myeloma. Am J Cancer Res 2021;11(6):3285–93.
[72] 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(1):20–8.
[73] Singh N, Frey NV, Engels B, Barrett DM, Shestova O, Ravikumar P, et al. Antigen-independent activation enhances the efficacy of 4-1BB-costimulated CD22 CAR T cells. Nat Med 2021;27(5):842–50.
[74] Frank MJ, Baird JH, Kramer AM, Srinagesh HK, Patel S, Brown AK, et al. CD22-directed CAR T-cell therapy for large B-cell lymphomas progressing after CD19-directed CAR T-cell therapy: a dose-finding phase 1 study. Lancet 2024;404(10450):353–63.

[75] 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(10):1797–805.
[76] 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 Oncol 2018;11:127–37.
[77] 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(8):1419–31.
[78] 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(1):189–96.
[79] 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 Transl Med 2021;13(586).
[80] Shi M, Wang J, Huang H, Liu D, Cheng H, Wang X, et al. Bispecific CAR T cell therapy targeting BCMA and CD19 in relapsed/refractory multiple myeloma: a phase I/II trial. Nat Commun 2024;15(1):3371.
[81] Barber A, Zhang T, Sentman CL. Immunotherapy with chimeric NKG2D receptors leads to long-term tumor-free survival and development of host antitumor immunity in murine ovarian cancer. J Immunol 2008;180(1):72–8.
[82] 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(1):100–12.
[83] Sallman DA, Kerre T, Havelange V, Poire 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(3):e191–202.
[84] Xu Y, Zhang M, Ramos CA, Durett A, Liu E, Dakhova O, et al. Closely related T-memory stem cells correlate with in vivo expansion of CAR.CD19-T cells and are preserved by IL-7 and IL-15. Blood 2014;123(24):3750–9.
[85] Chen GM, Chen C, Das RK, Gao P, Chen CH, Bandyopadhyay S, et al. Integrative bulk and single-cell profiling of premanufacture T-cell populations reveals factors mediating long-term persistence of CAR T-cell therapy. Cancer Discov 2021;11(9):2186–99.
[86] Biasco L, Izotova N, Rivat C, Ghorashian S, Richardson R, Guvenel A, et al. Clonal expansion of T memory stem cells determines early anti-leukemic responses and long-term CAR T cell persistence in patients. Nat Cancer 2021;2(6):629–42.
[87] 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.
[88] Wang X, Popplewell LL, Wagner JR, Naranjo A, Blanchard MS, Mott MR, et al. Phase 1 studies of central memory-derived CD19 CAR T-cell therapy following autologous HSCT in patients with B-cell NHL. Blood 2016;127(24):2980–90.
[89] Aldoss I, Khaled SK, Wang X, Palmer J, Wang Y, Wagner JR, et al. Favorable activity and safety profile of memory-enriched CD19-targeted chimeric antigen receptor T cell therapy in adults with high-risk relapsed/refractory ALL. Clin Cancer Res 2023;29(4):742–53.
[90] 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 Discov 2023;13(3):580–97.
[91] 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(6):498–508.
[92] Meyran D, Zhu JJ, Butler J, Tantalo D, MacDonald S, Nguyen TN, et al. T(STEM)-like CAR-T cells exhibit improved persistence and tumor control compared with conventional CAR-T cells in preclinical models. Sci Transl Med 2023;15(690). eabk1900.
[93] Ghassemi S, Nunez-Cruz S, O'Connor RS, Fraietta JA, Patel PR, Scholler J, et al. Reducing ex vivo culture improves the antileukemic activity of chimeric antigen receptor (CAR) T cells. Cancer Immunol Res 2018;6(9):1100–9.
[94] Dickinson MJ, Barba P, Jager U, Shah NN, Blaise D, Briones J, et al. A novel autologous CAR-T therapy, YTB323, with preserved T-cell Stemness shows enhanced CAR T-cell efficacy in preclinical and early clinical development. Cancer Discov 2023;13(9):1982–97.
[95] Barba P, Kwon M, Briones J, Jaeger U, Bachy E, Blaise D, et al. YTB323 (rapcabtagene autoleucel) demonstrates durable efficacy and a manageable safety profile in patients with relapsed/refractory diffuse large B-cell lymphoma: phase I study update. Blood 2022;140(Supplement 1):1056–9.
[96] Sabatino M, Hu J, Sommariva M, Gautam S, Fellowes V, Hocker JD, et al. Generation of clinical-grade CD19-specific CAR-modified CD8+ memory stem cells for the treatment of human B-cell malignancies. Blood 2016;128(4):519–28.
[97] Mehra V, Agliardi G, Dias Alves Pinto J, Shafat MS, Garai AC, Green L, et al. AKT inhibition generates potent polyfunctional clinical grade AUTO1 CAR T-cells, enhancing function and survival. J Immunother Cancer 2023;11(9).
[98] Funk CR, Wang S, Chen KZ, Waller A, Sharma A, Edgar CL, et al. PI3Kdelta/gamma inhibition promotes human CART cell epigenetic and metabolic reprogramming to enhance antitumor cytotoxicity. Blood 2022;139(4):523–37.
[99] Chan JD, Scheffler CM, Munoz I, Sek K, Lee JN, Huang YK, et al. FOXO1 enhances CAR T cell stemness, metabolic fitness and efficacy. Nature 2024;629(8010):201–10.
[100] Doan AE, Mueller KP, Chen AY, Rouin GT, Chen Y, Daniel B, et al. FOXO1 is a master regulator of memory programming in CAR T cells. Nature 2024;629(8010):211–8.
[101] Chen J, Qiu S, Li W, Wang K, Zhang Y, Yang H, et al. Tuning charge density of chimeric antigen receptor optimizes tonic signaling and CAR-T cell fitness. Cell Res 2023;33(5):341–54.
[102] Li W, Qiu S, Chen J, Jiang S, Chen W, Jiang J, et al. Chimeric antigen receptor designed to prevent ubiquitination and downregulation showed durable antitumor efficacy. Immunity 2020;53(2):456–70. e6.
[103] Wei J, Long L, Zheng W, Dhungana Y, Lim SA, Guy C, et al. Targeting REGNASE-1 programs long-lived effector T cells for cancer therapy. Nature 2019;576(7787):471–6.
[104] 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(20):2470–82.
[105] Brog RA, Ferry SL, Schiebout CT, Messier CM, Cook WJ, Abdullah L, et al. Superkine IL-2 and IL-33 armored CAR T cells reshape the tumor microenvironment and reduce growth of multiple solid tumors. Cancer Immunol Res 2022;10(8):962–77.
[106] Hu B, Ren J, Luo Y, Keith B, Young RM, Scholler J, et al. Augmentation of antitumor immunity by human and mouse CAR T cells secreting IL-18. Cell Rep 2017;20(13):3025–33.
[107] Pegram HJ, Lee JC, Hayman EG, Imperato GH, Tedder TF, Sadelain M, et al. Tumor-targeted T cells modified to secrete IL-12 eradicate systemic tumors without need for prior conditioning. Blood 2012;119(18):4133–41.
[108] Avanzi MP, Yeku O, Li X, Wijewarnasuriya DP, van Leeuwen DG, Cheung K, et al. Engineered tumor-targeted T cells mediate enhanced anti-tumor efficacy both directly and through activation of the endogenous immune system. Cell Rep 2018;23(7):2130–41.
[109] Brentjens RJ, Latouche JB, Santos E, Marti F, Gong MC, Lyddane C, et al. Eradication of systemic B-cell tumors by genetically targeted human T lymphocytes co-stimulated by CD80 and interleukin-15. Nat Med 2003;9(3):279–86.
[110] 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(Supplement 1):4612–4.
[111] Stach M, Ptackova P, Mucha M, Musil J, Klener P, Otahal P. Inducible secretion of IL-21 augments anti-tumor activity of piggyBac-manufactured chimeric antigen receptor T cells. Cytotherapy 2020;22(12):744–54.
[112] Hurton LV, Singh H, Najjar AM, Switzer KC, Mi T, Maiti S, et al. Tethered IL-15 augments antitumor activity and promotes a stem-cell memory subset in tumor-specific T cells. Proc Natl Acad Sci U S A 2016;113(48). E7788-E97.
[113] Li L, Li Q, Yan ZX, Sheng LS, Fu D, Xu P, et al. Transgenic expression of IL-7 regulates CAR-T cell metabolism and enhances in vivo persistence against tumor cells. Sci Rep 2022;12(1):12506.
[114] Zhang Y, Zhuang Q, Wang F, Zhang C, Xu C, Gu A, et al. Co-expression IL-15 receptor alpha with IL-15 reduces toxicity via limiting IL-15 systemic exposure during CAR-T immunotherapy. J Transl Med 2022;20(1):432.
[115] Yoshikawa T, Ito Y, Wu Z, Kasuya H, Nakashima T, Okamoto S, et al. Development of a chimeric cytokine receptor that captures IL-6 and enhances the antitumor response of CAR-T cells. Cell Rep Med 2024:101526.
[116] Zhou W, Miao J, Cheng Z, Wang Z, Wang J, Guo H, et al. Hypoxia-regulated secretion of IL-12 enhances antitumor activity and safety of CD19 CAR-T cells in the treatment of DLBCL. Mol Ther Oncol 2023;30:216–26.
[117] Li H, Harrison EB, Li H, Hirabayashi K, Chen J, Li QX, et al. Targeting brain lesions of non-small cell lung cancer by enhancing CCL2-mediated CAR-T cell migration. Nat Commun 2022;13(1):2154.
[118] Schomer NT, Jiang ZK, Lloyd MI, Klingemann H, Boissel L. CCR7 expression in CD19 chimeric antigen receptor-engineered natural killer cells improves migration toward CCL19-expressing lymphoma cells and increases tumor control in mice with human lymphoma. Cytotherapy 2022;24(8):827–34.
[119] 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(25):6392–402.
[120] Lei W, Zhao A, Liu H, Yang C, Wei C, Guo S, et al. Safety and feasibility of anti-CD19 CAR T cells expressing inducible IL-7 and CCL19 in patients with relapsed or refractory large B-cell lymphoma. Cell Discov 2024;10(1):5.
[121] Pegram HJ, Purdon TJ, van Leeuwen DG, Curran KJ, Giralt SA, Barker JN, et al. IL-12-secreting CD19-targeted cord blood-derived T cells for the immunotherapy of B-cell acute lymphoblastic leukemia. Leukemia 2015;29(2):415–22.
[122] Ng BD, Rajagopalan A, Kousa AI, Fischman JS, Chen S, Massa AR, et al. IL-18-secreting multi-antigen targeting CAR T-cells eliminate antigen-low myeloma in an immunocompetent mouse model. Blood 2024:171–86.
[123] Wu L, Wei Q, Brzostek J, Gascoigne NRJ. Signaling from T cell receptors (TCRs) and chimeric antigen receptors (CARs) on T cells. Cell Mol Immunol 2020;17(6):600–12.
[124] Wang X, Chang WC, Wong CW, Colcher D, Sherman M, Ostberg JR, et al. A transgene-encoded cell surface polypeptide for selection, in vivo tracking, and ablation of engineered cells. Blood 2011;118(5):1255–63.
[125] Fedorov VD, Themeli M, Sadelain M. PD-1- and CTLA-4-based inhibitory chimeric antigen receptors (iCARs) divert off-target immunotherapy responses. Sci Transl Med 2013;5(215). 215ra172.

[126] 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(499).
[127] 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(5):711–7.
[128] Rodgers DT, Mazagova M, Hampton EN, Cao Y, Ramadoss NS, Hardy IR, et al. Switch-mediated activation and retargeting of CAR-T cells for B-cell malignancies. Proc Natl Acad Sci U S A 2016;113(4):E459–68.
[129] Chen LY, Gong WJ, Li MH, Zhou HX, Xu MZ, Qian CS, et al. Anti-CD19 CAR T-cell consolidation therapy combined with CD19+ feeding T cells and TKI for Ph+ acute lymphoblastic leukemia. Blood Adv 2023;7(17):4913–25.
[130] Eyquem J, Mansilla-Soto J, Giavridis T, van der Stegen SJ, Hamieh M, Cunanan KM, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature 2017;543(7643):113–7.
[131] Webster B, Xiong Y, Hu P, Wu D, Alabanza L, Orentas RJ, et al. Self-driving armored CAR-T cells overcome a suppressive milieu and eradicate CD19(+) Raji lymphoma in preclinical models. Mol Ther 2021;29(9):2691–706.
[132] Li S, Siriwon N, Zhang X, Yang S, Jin T, He F, et al. Enhanced cancer immunotherapy by chimeric antigen receptor-modified T cells engineered to secrete checkpoint inhibitors. Clin Cancer Res 2017;23(22):6982–92.
[133] Rafiq S, Yeku OO, Jackson HJ, Purdon TJ, van Leeuwen DG, Drakes DJ, et al. Targeted delivery of a PD-1-blocking scFv by CAR-T cells enhances anti-tumor efficacy in vivo. Nat Biotechnol 2018;36(9):847–56.
[134] Rupp LJ, Schumann K, Roybal KT, Gate RE, Ye CJ, Lim WA, et al. CRISPR/Cas9-mediated PD-1 disruption enhances anti-tumor efficacy of human chimeric antigen receptor T cells. Sci Rep 2017;7(1):737.
[135] 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 Cancer 2022;21(1):185.
[136] Blaeschke F, Stenger D, Apfelbeck A, Cadilha BL, Benmebarek MR, Mahdawi J, et al. Augmenting anti-CD19 and anti-CD22 CAR T-cell function using PD-1-CD28 checkpoint fusion proteins. Blood Cancer J 2021;11(6):108.
[137] Liang Y, Liu H, Lu Z, Lei W, Zhang C, Li P, et al. CD19 CAR-T expressing PD-1/CD28 chimeric switch receptor as a salvage therapy for DLBCL patients treated with different CD19-directed CAR T-cell therapies. J Hematol Oncol 2021;14(1):26.
[138] Tao L, Farooq MA, Gao Y, Zhang L, Niu C, Ajmal I, et al. CD19-CAR-T cells bearing a KIR/PD-1-based inhibitory CAR eradicate CD19(+)HLA-C1(−) malignant B cells while sparing CD19(+)HLA-C1(+) healthy B cells. Cancers (Basel) 2020;12(9).
[139] Cheng K, Feng X, Chai Z, Wang Z, Liu Z, Yan Z, et al. 4-1BB-based CAR T cells effectively reverse exhaustion and enhance the anti-tumor immune response through autocrine PD-L1 scFv antibody. Int J Mol Sci 2023;24(4).
[140] Liu H, Lei W, Zhang C, Yang C, Wei J, Guo Q, et al. CD19-specific CAR T cells that express a PD-1/CD28 chimeric switch-receptor are effective in patients with PD-L1-positive B-cell lymphoma. Clin Cancer Res 2021;27(2):473–84.
[141] Shi Y, Kotchetkov IS, Dobrin A, Hanina SA, Rajasekhar VK, Healey JH, et al. GLUT1 overexpression enhances CAR T cell metabolic fitness and anti-tumor efficacy. Mol Ther 2024;23:2393–405.
[142] Wu H, Zhao X, Hochrein SM, Eckstein M, Gubert GF, Knopper K, et al. Mitochondrial dysfunction promotes the transition of precursor to terminally exhausted T cells through HIF-1alpha-mediated glycolytic reprogramming. Nat Commun 2023;14(1):6858.
[143] Simula L, Fumagalli M, Vimeux L, Rajnpreht I, Icard P, Birsen G, et al. Mitochondrial metabolism sustains CD8(+) T cell migration for an efficient infiltration into solid tumors. Nat Commun 2024;15(1):2203.
[144] Gross G, Alkadieri S, Meir A, Itzhaki O, Aharoni-Tevet Y, Ben Yosef S, et al. Improved CAR-T cell activity associated with increased mitochondrial function primed by galactose. Leukemia 2024;38:1534–40.
[145] Kastenschmidt JM, Schroers-Martin JG, Sworder BJ, Sureshchandra S, Khodadoust MS, Liu CL, et al. A human lymphoma organoid model for evaluating and targeting the follicular lymphoma tumor immune microenvironment. Cell Stem Cell 2024;31(3):410–20 e4.
[146] 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(9):1872–82.
[147] Jain MD, Zhao H, Wang X, Atkins R, Menges M, Reid K, et al. Tumor interferon signaling and suppressive myeloid cells are associated with CAR T-cell failure in large B-cell lymphoma. Blood 2021;137(19):2621–33.
[148] Azzaoui I, Uhel F, Rossille D, Pangault C, Dulong J, Le Priol J, et al. T-cell defect in diffuse large B-cell lymphomas involves expansion of myeloid-derived suppressor cells. Blood 2016;128(8):1081–92.
[149] Lin Y, Gustafson MP, Bulur PA, Gastineau DA, Witzig TE, Dietz AB. Immunosuppressive CD14+HLA-DR(low)/− monocytes in B-cell non-Hodgkin lymphoma. Blood 2011;117(3):872–81.
[150] Locke FL, Filosto S, Chou J, Vardhanabhuti S, Perbost R, Dreger P, et al. Impact of tumor microenvironment on efficacy of anti-CD19 CAR T cell therapy or chemotherapy and transplant in large B cell lymphoma. Nat Med 2024;30(2):507–18.
[151] Hao Y, Chen P, Guo S, Li M, Jin X, Zhang M, et al. Tumor-derived exosomes induce initial activation by exosomal CD19 antigen but impair the function of CD19-specific CAR T-cells via TGF-beta signaling. Front Med 2024;18(1):128–46.
[152] Cheng J, Yan J, Liu Y, Shi J, Wang H, Zhou H, et al. Cancer-cell-derived fumarate suppresses the anti-tumor capacity of CD8(+) T cells in the tumor microenvironment. Cell Metab 2023;35(6):961–78. e10.
[153] Zhao K, Ren C, Tang D, Zhao L, Chen X, Wang Y, et al. The altering cellular components and function in tumor microenvironment during remissive and relapsed stages of anti-CD19 CAR T-cell treated lymphoma mice. Front Immunol 2023;14:1101769.
[154] Lickefett B, Chu L, Ortiz-Maldonado V, Warmuth L, Barba P, Doglio M, et al. Lymphodepletion - an essential but undervalued part of the chimeric antigen receptor T-cell therapy cycle. Front Immunol 2023;14:1303935.
[155] Qiu Y, Liao P, Wang H, Chen J, Hu Y, Hu R, et al. Enhanced tumor immunotherapy by polyfunctional CD19-CAR T cells engineered to secrete anti-CD47 single-chain variable fragment. Int J Biol Sci 2023;19(15):4948–66.
[156] Brudno JN, Kochenderfer JN. Off-the-shelf CAR T cells for multiple myeloma. Nat Med 2023;29(2):303–4.
[157] Sommer C, Boldajipour B, Kuo TC, Bentley T, Sutton J, Chen A, et al. Preclinical evaluation of allogeneic CAR T cells targeting BCMA for the treatment of multiple myeloma. Mol Ther 2019;27(6):1126–38.
[158] Kagoya Y, Guo T, Yeung B, Saso K, Anczurowski M, Wang CH, et al. Genetic ablation of HLA class I, class II, and the T-cell receptor enables allogeneic T cells to be used for adoptive T-cell therapy. Cancer Immunol Res 2020;8(7):926–36.
[159] Lee J, Sheen JH, Lim O, Lee Y, Ryu J, Shin D, et al. Abrogation of HLA surface expression using CRISPR/Cas9 genome editing: a step toward universal T cell therapy. Sci Rep 2020;10(1):17753.
[160] Wang B, Iriguchi S, Waseda M, Ueda N, Ueda T, Xu H, et al. Generation of hypoimmunogenic T cells from genetically engineered allogeneic human induced pluripotent stem cells. Nat Biomed Eng 2021;5(5):429–40.
[161] Degagne E, Donohoue PD, Roy S, Scherer J, Fowler TW, Davis RT, et al. High-specificity CRISPR-mediated genome engineering in anti-BCMA allogeneic CAR T cells suppresses allograft rejection in preclinical models. Cancer Immunol Res 2024;12(4):462–77.
[162] Grauwet K, Berger T, Kann MC, Silva H, Larson R, Leick MB, et al. Stealth transgenes enable CAR-T cells to evade host immune responses. J Immunother Cancer 2024;12(5).
[163] Sugita M, Galetto R, Zong H, Ewing-Crystal N, Trujillo-Alonso V, Mencia-Trinchant N, et al. Allogeneic TCRalphabeta deficient CAR T-cells targeting CD123 in acute myeloid leukemia. Nat Commun 2022;13(1):2227.
[164] Hu X, Manner K, DeJesus R, White K, Gattis C, Ngo P, et al. Hypoimmune anti-CD19 chimeric antigen receptor T cells provide lasting tumor control in fully immunocompetent allogeneic humanized mice. Nat Commun 2023;14(1):2020.
[165] 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(10266):1885–94.
[166] 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(11). e833-e43.
[167] 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(Supplement 1):2110.
[168] Hu Y, Zhou Y, Zhang M, Zhao H, Wei G, Ge W, et al. Genetically modified CD7-targeting allogeneic CAR-T cell therapy with enhanced efficacy for relapsed/refractory CD7-positive hematological malignancies: a phase I clinical study. Cell Res 2022;32(11):995–1007.
[169] Smith TT, Stephan SB, Moffett HF, McKnight LE, Ji W, Reiman D, et al. In situ programming of leukaemia-specific T cells using synthetic DNA nanocarriers. Nat Nanotechnol 2017;12(8):813–20.
[170] Frank AM, Braun AH, Scheib L, Agarwal S, Schneider IC, Fusil F, et al. Combining T-cell-specific activation and in vivo gene delivery through CD3-targeted lentiviral vectors. Blood Adv 2020;4(22):5702–15.
[171] Zhou JE, Sun L, Jia Y, Wang Z, Luo T, Tan J, et al. Lipid nanoparticles produce chimeric antigen receptor T cells with interleukin-6 knockdown in vivo. J Control Release 2022;350:298–307.
[172] Shah BD, Ghobadi A, Oluwole OO, Logan AC, Boissel N, Cassaday RD, et al. Two-year follow-up of KTE-X19 in patients with relapsed or refractory adult B-cell acute lymphoblastic leukemia in ZUMA-3 and its contextualization with SCHOLAR-3, an external historical control study. J Hematol Oncol 2022;15(1):170.
[173] Schuster SJ, Tam CS, Borchmann P, Worel N, McGuirk JP, Holte H, et al. 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 2021;22(10):1403–15.
[174] 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.
[175] Dreyling M, Fowler NH, Dickinson M, Martinez-Lopez J, Kolstad A, Butler J, et al. Durable response after tisagenlecleucel in adults with relapsed/refractory follicular lymphoma: ELARA trial update. Blood 2024;143(17):1713–25.
[176] Neelapu SS, Chavez JC, Sehgal AR, Epperla N, Ulrickson M, Bachy E, et al. Three-year follow-up analysis of axicabtagene ciloleucel in relapsed/refractory indolent non-Hodgkin lymphoma (ZUMA-5). Blood 2024;143(6):496–506.
[177] Wang M, Munoz J, Goy A, Locke FL, Jacobson CA, Hill BT, et al. Three-year follow-up of KTE-X19 in patients with relapsed/refractory mantle cell lymphoma, including high-risk subgroups, in the ZUMA-2 study. J Clin Oncol 2023;41(3):555–67.
[178] Moreno-Cortes E, Franco-Fuquen P, Garcia-Robledo JE, Forero J, Booth N, Castro JE. ICOS and OX40 tandem co-stimulation enhances CAR T-cell

cytotoxicity and promotes T-cell persistence phenotype. Front Oncol 2023;13:1200914.
[179] Pule MA, Straathof KC, Dotti G, Heslop HE, Rooney CM, Brenner MK. A chimeric T cell antigen receptor that augments cytokine release and supports clonal expansion of primary human T cells. Mol Ther 2005;12(5):933–41.
[180] Ochi T, Maruta M, Tanimoto K, Kondo F, Yamamoto T, Kurata M, et al. A single-chain antibody generation system yielding CAR-T cells with superior antitumor function. Commun Biol 2021;4(1):273.
[181] Ma R, You F, Tian S, Zhang T, Tian X, Xiang S, et al. Enhanced efficacy of CD19/CD22 bispecific CAR-T cells with EAAAK linker on B-cell malignancies. Eur J Haematol 2024;112(1):64–74.
[182] 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;5:235.
[183] Foeng J, Comerford I, McColl SR. Harnessing the chemokine system to home CAR-T cells into solid tumors. Cell Rep Med 2022;3(3):100543.
[184] Cherkassky L, Morello A, Villena-Vargas J, Feng Y, Dimitrov DS, Jones DR, et al. Human CAR T cells with cell-intrinsic PD-1 checkpoint blockade resist tumor-mediated inhibition. J Clin Invest 2016;126(8):3130–44.
[185] Zhang J, Zhu J, Zheng G, Wang Q, Li X, Feng Y, et al. Co-expression of miR155 or LSD1 shRNA increases the anti-tumor functions of CD19 CAR-T cells. Front Immunol 2021;12:811364.
[186] Pennell CA, Campbell H, Storlie MD, Bolivar-Wagers S, Osborn MJ, Refaeli Y, et al. Human CD19-specific switchable CAR T-cells are efficacious as constitutively active CAR T-cells but cause less morbidity in a mouse model of human CD19(+) malignancy. J Immunother Cancer 2022;10(12).
[187] Uchibori R, Teruya T, Ido H, Ohmine K, Sehara Y, Urabe M, et al. Functional analysis of an inducible promoter driven by activation signals from a chimeric antigen receptor. Mol Ther Oncol 2019;12:16–25.
[188] Olson ML, Mause ERV, Radhakrishnan SV, Brody JD, Rapoport AP, Welm AL, et al. Low-affinity CAR T cells exhibit reduced trogocytosis, preventing rapid antigen loss, and increasing CAR T cell expansion. Leukemia 2022;36(7):1943–6.
[189] 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 Adv 2023;7(9):1725–38.
[190] Xie B, Li Z, Zhou J, Wang W. Current status and perspectives of dual-targeting chimeric antigen receptor T-cell therapy for the treatment of hematological malignancies. Cancers (Basel) 2022;14(13).
[191] Chan WK, Williams J, Sorathia K, Pray B, Abusaleh K, Bian Z, et al. A novel CAR-T cell product targeting CD74 is an effective therapeutic approach in preclinical mantle cell lymphoma models. Exp Hematol Oncol 2023;12(1):79.
[192] Chu F, Cao J, Liu J, Yang H, Davis TJ, Kuang SQ, et al. Chimeric antigen receptor T cells to target CD79b in B-cell lymphomas. J Immunother Cancer 2023;11(11).
[193] Luo Y, Qie Y, Gadd ME, Manna A, Rivera-Valentin R, To T, et al. Translational development of a novel BAFF-R CAR-T therapy targeting B-cell lymphoid malignancies. Cancer Immunol Immunother 2023;72(12):4031–47.
[194] Lee YH, Lee HJ, Kim HC, Lee Y, Nam SK, Hupperetz C, et al. PD-1 and TIGIT downregulation distinctly affect the effector and early memory phenotypes of CD19-targeting CAR T cells. Mol Ther 2022;30(2):579–92.
[195] Wang Z, Li N, Feng K, Chen M, Zhang Y, Liu Y, et al. Phase I study of CAR-T cells with PD-1 and TCR disruption in mesothelin-positive solid tumors. Cell Mol Immunol 2021;18(9):2188–98.
[196] Prochazkova M, Dreyzin A, Shao L, Garces P, Cai Y, Shi R, et al. Deciphering the importance of culture pH on CD22 CAR T-cells characteristics. J Transl Med 2024;22(1):384.
[197] Sanyanusin M, Tudsamran S, Thaiwong R, Tawinwung S, Nishio N, Takahashi Y, et al. Novel xeno-free and serum-free culturing condition to improve piggyBac transposon-based CD19 chimeric antigen receptor T-cell production and characteristics. Cytotherapy 2023;25(4):397–406.
[198] Prinzing B, Zebley CC, Petersen CT, Fan Y, Anido AA, Yi Z, et al. Deleting DNMT3A in CAR T cells prevents exhaustion and enhances antitumor activity. Sci Transl Med 2021;13(620). eabh0272.
[199] Nobles CL, Sherrill-Mix S, Everett JK, Reddy S, Fraietta JA, Porter DL, et al. CD19-targeting CAR T cell immunotherapy outcomes correlate with genomic modification by vector integration. J Clin Invest 2020;130(2):673–85.
[200] Hu Y, Zu C, Zhang M, Wei G, Li W, Fu S, et al. Safety and efficacy of CRISPR-based non-viral PD1 locus specifically integrated anti-CD19 CAR-T cells in patients with relapsed or refractory non-Hodgkin's lymphoma: a first-in-human phase I study. EClinicalMedicine 2023;60:102010.
[201] Zhang J, Hu Y, Yang J, Li W, Zhang M, Wang Q, et al. Non-viral, specifically targeted CAR-T cells achieve high safety and efficacy in B-NHL. Nature 2022;609(7926):369–74.
[202] Gill S, Vides V, Frey NV, Hexner EO, Metzger S, O'Brien M, et al. Anti-CD19 CAR T cells in combination with ibrutinib for the treatment of chronic lymphocytic leukemia. Blood Adv 2022;6(21):5774–85.
[203] Xin X, Zhu X, Yang Y, Wang N, Wang J, Xu J, et al. Efficacy of programmed cell death 1 inhibitor maintenance after chimeric antigen receptor T cells in patients with relapsed/refractory B-cell non-Hodgkin-lymphoma. Cell Oncol (Dordr) 2024;47:1425–40.
[204] Agarwal S, Hanauer JDS, Frank AM, Riechert V, Thalheimer FB, Buchholz CJ. In vivo generation of CAR T cells selectively in human CD4(+) lymphocytes. Mol Ther 2020;28(8):1783–94.
[205] Ma L, Ramasubramanian R, Mehta N, Cossette B, Morgan D, Sukaj I, et al. Directed evolution-based discovery of ligands for in vivo restimulation of CAR-T cells. bioRxiv. 2024.
[206] Tu S, Zhou L, Huang R, Zhou X, Yang J, He Y, et al. Dendritic cell vaccines extend CAR T-cell persistence and improve the efficacy of CD19 CAR T-cell therapy in refractory or relapsed adult B-ALL patients. Am J Hematol 2024;99:1437–40.
[207] Blaeschke F, Stenger D, Kaeuferle T, Willier S, Lotfi R, Kaiser AD, et al. Induction of a central memory and stem cell memory phenotype in functionally active CD4(+) and CD8(+) CAR T cells produced in an automated good manufacturing practice system for the treatment of CD19(+) acute lymphoblastic leukemia. Cancer Immunol Immunother 2018;67(7):1053–66.
[208] Aldoss I, Khaled SK, Wang X, Palmer J, Wang Y, Wagner JR, et al. Favorable activity and safety profile of memory-enriched CD19-targeted chimeric antigen receptor T-cell therapy in adults with high-risk relapsed/refractory ALL. Clin Cancer Res 2023;29(4):742–53.