Journal of Biomedical Research

Allogeneic CAR-T cell therapies: Overcoming clinical challenges and therapeutic potential against tumors

Raniya Khadiullina, Aigul Valiullina, Elvina Gilyazova, Yulia Skibo, Sabir Mukhametshin, Emil Bulatov

Cite this article as:
Raniya Khadiullina, Aigul Valiullina, Elvina Gilyazova, Yulia Skibo, Sabir Mukhametshin, Emil Bulatov. Allogeneic CAR–T cell therapies: Overcoming clinical challenges and therapeutic potential against tumors[J]. *Journal of Biomedical Research*, In press. doi: 10.7555/JBR.40.20260137

View online: https://doi.org/10.7555/JBR.40.20260137

Available online at www.jbr-pub.org.cn

Open Access at PubMed Central

Journal of Biomedical Research, 2026 40(0): 1–19

JBR

Review Article

Allogeneic CAR-T cell therapies: Overcoming clinical challenges and therapeutic potential against tumors

Raniya Khadiullina[1], Aigul Valiullina[1], Elvina Gilyazova[1], Yulia Skibo[1], Sabir Mukhametshin[1], Emil Bulatov[1,2,✉]

[1]*Institute of Fundamental Medicine and Biology, Kazan Federal University, 420008, Kazan, Russia;*
[2]*Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 117997 Moscow, Russia.*

Abstract

Chimeric antigen receptor T-cell (CAR-T) therapy represents a major advance in cellular immunotherapy and has demonstrated substantial clinical benefit in relapsed or refractory B-cell malignancies. However, autologous CAR-T therapy remains constrained by manufacturing complexity, high cost, variability in product quality, and treatment delays that may compromise outcomes in rapidly progressing disease. Allogeneic "off-the-shelf" CAR-T cell approaches have emerged as a potential strategy to address these limitations by enabling standardized manufacturing, rapid availability, and scalable production. Nevertheless, these theoretical advantages must be carefully balanced against significant challenges, including alloreactivity, immune rejection, complex genome engineering requirements, and regulatory constraints. This review provides a critical and balanced overview of the advantages and limitations of allogeneic CAR-T cell therapy, with a particular focus on applications in solid tumors. We discuss key biological barriers, including tumor microenvironment-mediated immunosuppression, and evaluate current engineering strategies aimed at enhancing efficacy, along with emerging clinical data. Collectively, while allogeneic CAR-T therapies hold considerable promise, substantial scientific, technical, and regulatory challenges must be addressed before their widespread clinical implementation.

Keywords: chimeric antigen receptor, CAR-T therapy, allogeneic, off-the-shelf CAR-T cells, graft-versus-host disease, solid tumors

Introduction

Chimeric antigen receptor T-cell (CAR-T) therapy is one of the most advanced areas of modern cellular immunotherapy. CAR-T cells are genetically engineered lymphocytes designed to redirect antigen-specific cytotoxicity toward tumor cells[1]. The CAR structure integrates an extracellular antigen-binding domain, a transmembrane region, and intracellular signaling modules that together enable antigen-specific T-cell activation independent of major histocompatibility complex (MHC) recognition[2]. Antigen binding is most commonly mediated by a single-chain variable fragment (scFv), while CD3ζ provides the primary activation signal, and costimulatory domains such as CD28 or 4-1BB enhance proliferation, cytokine production, and *in vivo* persistence[3]. The structural configuration of these domains critically determines CAR-T cell functionality, stability, and antitumor efficacy[4]. The

✉Corresponding author: Emil Bulatov, E-mail: chembio.kazan@gmail.com, ORCID: 0000-0003-2961-0032.

Received: 25 February 2026; Revised: 07 June 2026; Accepted: 11 June 2026; Available online: 20 June 2026

CLC number: R730.51, Document code: A

The authors reported no conflict of interests.

https://doi.org/10.7555/JBR.40.20260137

most recognized targets for CAR-T cells are B-cell maturation antigen (BCMA) in multiple myeloma and the B-cell antigen CD19 in various B-cell hematologic malignancies[5-6]. Seven CAR-T cell products have been approved by the US Food and Drug Administration (FDA) for the treatment of patients with relapsed or refractory B cell malignancies[5-6]. However, despite advances in the treatment of hematologic malignancies, the efficacy of CAR-T therapy against solid tumors remains insufficient, limited by several factors, including an immunosuppressive and hypoxic tumor microenvironment, antigenic heterogeneity, and other barriers that impede CAR-T cell infiltration into tumor tissue and promote T-lymphocyte anergy[7-10].

CAR-T cell therapies in advanced development, including those currently available in clinical practice, are autologous; they involve the extraction of peripheral blood cells from the patient, which are then engineered into CAR-T cells and subsequently reinfused into the same patient. Despite their effectiveness, autologous CAR-T therapies face significant limitations[8]. The customized manufacturing process is not only expensive and labor-intensive but also highly variable, leading to inconsistencies in treatment outcomes. Patient variability due to prior therapies and individual medical histories can impact cell expansion and functionality. Additionally, logistical challenges, such as delays in cell production and transportation, can be particularly detrimental for patients with rapidly progressing disease[8]. Allogeneic CAR-T platforms have emerged as a strategy to overcome these limitations by enabling rapid, standardized, and scalable cell production[11]. Despite their promising potential, allogeneic CAR-T therapies for tumors face several serious challenges. One of the main challenges is the risk of graft-versus-host disease (GvHD) and host-versus-graft rejection (HvGR), serious complications that can arise when using donor cells and require careful monitoring. In addition, the immunosuppressive microenvironment of solid tumors often creates unfavorable conditions for CAR-T cell activity, underscoring the need for innovative strategies to overcome these barriers and improve therapeutic efficacy[12].

This review provides a critical and integrated analysis of "off-the-shelf" allogeneic CAR-T cell therapies, focusing on their therapeutic potential and key limitations. Particular attention is given to their application in solid tumors, including the biological barriers that restrict efficacy and the emerging strategies to overcome them, such as advanced genetic engineering and rational combination approaches.

Allogeneic CAR-T cell therapy

Comparative analysis of autologous and allogeneic CAR-T cell therapy: differences and similarities

CAR-T cell therapies can be broadly classified into autologous and allogeneic approaches based on the source of T lymphocytes. Autologous CAR-T cells are generated from a patient's own T cells, whereas allogeneic products are derived from healthy donors (***Fig. 1***)[8]. Despite the demonstrated clinical efficacy of autologous CAR-T therapy, its broader implementation is constrained by several biological and logistical limitations (***Table 1***). Patient-derived T cells are often functionally impaired due to tumor-associated immunosuppression and prior cytotoxic therapies. This can lead to T-cell exhaustion, characterized by reduced proliferation, impaired cytotoxicity, and limited *in vivo* persistence[13]. In addition, restricted availability of functional starting material and challenges in *ex vivo* expansion may lead to manufacturing failure. The individualized nature of production also entails prolonged manufacturing timelines, which may be critical for patients with rapidly progressing disease[14], as well as complex and resource-intensive quality control requirements. Furthermore, high treatment costs and reliance on specialized centers limit the accessibility of autologous CAR-T therapy, particularly for patients in resource-limited or geographically remote settings[15-16].

Advantages of allogeneic CAR-T cell therapy

The use of healthy donor T cells enables the selection based on favorable characteristics such as age. CAR-T cells derived from younger donors may exhibit a more favorable phenotype, lower expression of exhaustion markers, and improved long-term cytotoxic potential[17]. Autologous CAR-T therapies are associated with high costs (approximately $ 373 000–475 000 per treatment) due to individualized manufacturing and complex logistics[18]. In contrast, allogeneic "off-the-shelf" approaches may reduce costs through scalable production, with estimated manufacturing expenses as low as $ 4 000–10 000 per dose[19]. However, these estimates remain preliminary, as most allogeneic CAR-T products are still in early clinical development[16]. A further important advantage is that allogeneic CAR-T cells can be pre-manufactured and cryopreserved in biobanks, allowing more rapid treatment initiation without the need for patient-specific cell harvesting and modification[8,20]. Furthermore, "off-the-shelf" CAR-T cell therapy has the potential to expand access beyond

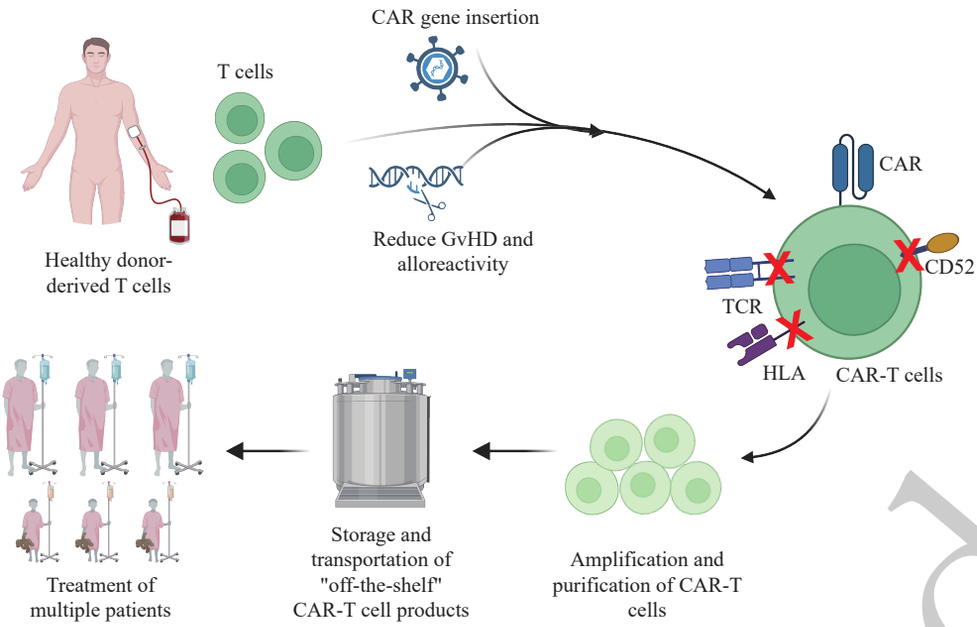

Fig. 1 **Schematic Representation of Allogeneic CAR-T Cell Manufacturing.** Donor-derived T cells are genetically engineered to express a CAR and reduce alloreactivity, followed by cell expansion, purification, storage, and use as an "off-the-shelf" therapy for multiple patient. Created with BioRender.com. Abbreviations: CD52, cluster of differentiation 52; GvHD, graft-versus-host disease; HLA, human leukocyte antigen; TCR, T-cell receptor.

Table 1 **Comparative characteristics of autologous and allogeneic CAR-T cells.**

Characteristics	Autologous CAR-T	Allogeneic CAR-T
Origin	Made from the patient's own T-cells	Made from donor T-cells (usually healthy)
Immune response	Minimal risk of rejection since cells are "self"	Higher risk of GvHD and HvGR
Manufacturing process	Long process (weeks), requires cell collection from the patient	Faster (can use pre-prepared cells)
Genetic modification	Often tailored to the specific patient	Can be standardized for various patients
Cost	Generally higher due to individualized production	Potentially lower with mass production
Risk of side effects	Risk of toxicity (*e.g.*, cytokine release syndrome, neurotoxicity)	Similar risks, but may have additional risks due to rejection, *e.g.*, graft-versus-host disease
Clinical application	Widely used, especially for certain cancers	In clinical trials, but with promising potential
Availability	Limited availability due to the need for patient cell collection	More accessible if produced at scale

Abbreviaitons: CAR-T, antigen receptor T-cell; GvHD, graft-versus-host disease; HvGR, host-versus-graft rejection.

highly specialized centers, provided that appropriate infrastructure for cell administration is available[21]. The possibility of producing allogeneic CAR-T cells at scale may enable batch-level quality control, in contrast to the individualized testing required for autologous products[11]. However, the extent to which these advantages can be realized in clinical practice remains contingent upon overcoming existing technical and regulatory challenges.

Limitations of allogeneic CAR-T cell therapy

Allogeneic CAR-T cell therapy has emerged as a scalable alternative to autologous approaches in oncology. However, its implementation in clinical practice faces several challenges related to alloreactivity derived from both donor and recipient cells. In particular, the use of CAR-T cells obtained from an incompatible donor may lead to the development of GvHD[22]. Conversely, the patient's immune system may recognize the transplanted cells as foreign, leading to HvGR and thereby limiting the therapeutic efficacy of allogeneic "off-the-shelf" CAR-T cells[8].

Beyond immune-related barriers, the commonly cited advantages of reduced cost, greater homogeneity, and scalability must be carefully

weighed against substantial technical and economic challenges. The manufacturing of allogeneic CAR-T products relies on complex gene-editing strategies, which are both costly and technically demanding. In addition, variability in donor material and editing efficiency can compromise product consistency, while stringent safety and quality requirements further constrain large-scale production. Although advances in manufacturing may eventually alleviate some of these limitations, they currently represent key bottlenecks to the clinical translation of allogeneic CAR-T therapies.

GvHD

GvHD occurs when the T-cell receptor (TCR) expressed on the surface of allogeneic CAR-T cells recognizes healthy patient tissues, representing a serious, potentially life-threatening complication[11,23]. GvHD involves inflammatory damage to multiple tissues, including the skin, gastrointestinal tract, and liver in its acute form[22]. In the chronic setting, it can lead to fibrosis affecting organs such as the eyes, mouth, joints, and genital tract[23]. Various approaches have been proposed to minimize the risk of GvHD[24]. The most straightforward of these is selecting a donor with a fully matched human leukocyte antigen (HLA) profile. However, given the low probability of finding such a match, this approach requires the establishment of an extensive bank of cell products, which diminishes the advantages of "off-the-shelf" therapy[11]. As illustrated in Fig. 2, alternative strategies to reduce GvHD risk include the genetic knockout or knockdown of TCR genes, the use of cells with known TCR specificity, or cells that do not express the TCRα/β complex[24].

(1) TCR deletion

Gene editing serves as a prevalent approach to mitigate the risk of GvHD. Various technologies for the genetic modification of CAR-T cells are under investigation, including the use of transcription activator-like effector nuclease (TALEN), CRISPR/Cas9, zinc-finger nuclease (ZFN), and base editing (BE)[25-26]. Gene-editing strategies therefore focus on targeting the *TRAC* and *TRBC* loci to disrupt αβ TCR expression[24]. The *TRAC* gene, which codes for the α-chain, has only one constant region, making it a more accessible editing target than *TRBC*, which contains two constant regions (*TRBC1* and *TRBC2*)[16].

Torikai *et al*[27] initially investigated the feasibility of disrupting TCR expression in CAR-T cells by employing ZFNs to knock out *TRAC*/*TRBC* in CAR-T cells directed against CD19. The deletion of *TRAC*/*TRBC* in CAR-T cells was demonstrated to inhibit TCR expression while preserving CAR

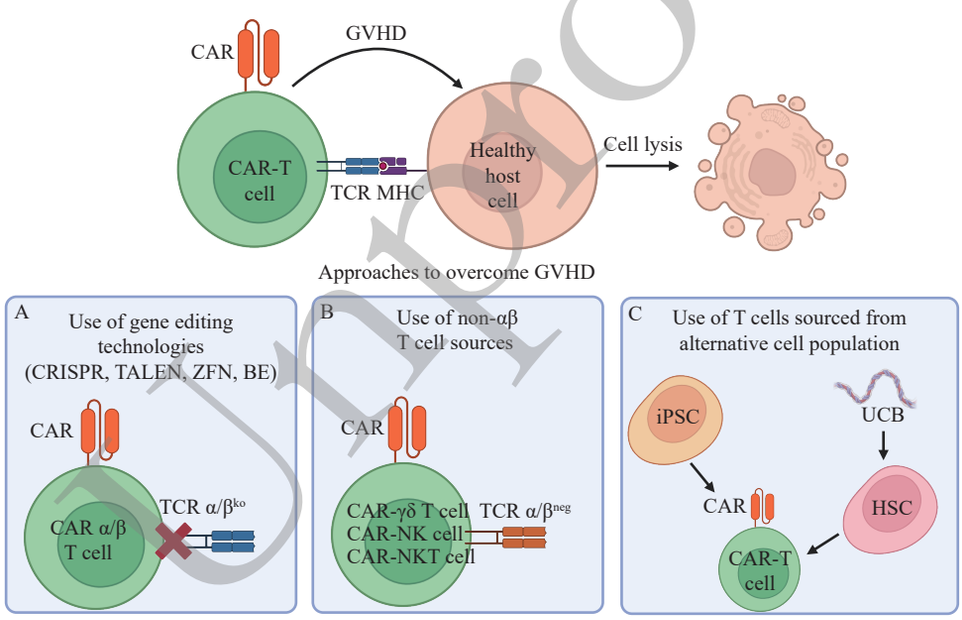

Fig. 2 **Approaches to overcome GvHD.** A. Genome editing technologies like CRISPR-Cas9, ZFNs, BE, and TALENs disrupt *TRAC* or *TRBC*, preventing TCR-mediated alloreactivity and recognition of pMHC molecules on host cells. B. Use non-α/β T cell populations as a source for allogeneic CAR-T cell production. C. Use of T cells sourced from alternative cell populations. Created with BioRender.com. Abbreviations: BE, base editing; CAR, chimeric antigen receptor; CRISPR, clustered regularly interspaced short palindromic repeat; GvHD, graft-versus-host disease; HSC, hematopoietic stem cell; iPSC, induced pluripotent stem cells; pMHC, peptide-major histocompatibility complex; NK, natural killer; NKT, natural killer T; TALEN, transcription activator-like effector nuclease; TCR, T cell receptor; UCB, umbilical cord blood; ZFN, zinc finger nuclease.

specificity for CD19[27]. Subsequently, similar studies were conducted using TALENs and CRISPR-Cas9 as gene-editing tools. These studies demonstrated the efficacy of allogeneic "off-the-shelf" CAR-T cells in treating B-lineage malignancies while avoiding the induction of GvHD[28-29]. An intriguing strategy to improve the controlled prevention of GvHD involves the direct introduction of a CAR transgene into the *TRAC* locus. This modification not only reduces GvHD risk but also allows for consistent and controlled CAR expression driven by the endogenous *TCR* promoter, which has been demonstrated to reduce differentiation and exhaustion of CAR-T cells[30].

Despite these advances, important differences exist between gene-editing platforms in terms of efficiency, safety, and scalability. TALENs offer high specificity but are less suited for multiplex editing, whereas ZFNs are constrained by complex design[31]. CRISPR/Cas9 enables efficient multi-gene editing and facilitates the generation of universal CAR-T cells. However, it raises concerns regarding off-target effects and genomic instability[31]. Base editing may reduce genotoxicity by avoiding double-strand DNA breaks, although its clinical application remains limited[32].

Currently, several active clinical trials are assessing the efficacy of TCR deletion in the development of allogeneic CAR-T cells. For instance, allogeneic CAR-T therapies developed using TALENs, such as ALLO-501 (NCT03939026)[33], UCART19 (NCT02746952)[34], and ALLO-715 (NCT04093596)[35], have shown no incidence of GvHD. Likewise, clinical trials utilizing CRISPR-Cas9 for TCR deletion, such as CTX110 (NCT04035434)[36], WU-CART007 (NCT04984356)[37], and TyU19 (NCT05859997)[38], have similarly reported no GvHD events. However, these studies are predominantly small and short-term, and do not address long-term persistence or comparative efficacy, despite consistently demonstrating reduced GvHD following TCR disruption[11]. Moreover, extensive genome editing may impair T-cell fitness, suggesting that increased engineering complexity does not necessarily translate into improved clinical outcomes[39].

(2) Use of non-α/β T cell populations

A second approach to minimizing the risk of GvHD is to use non-α/β T cells (***Fig. 2***). Particularly relevant candidates for allogeneic CAR therapy are γδ T cells, natural killer (NK) cells, natural killer T (NKT) cells, and mucosal-associated invariant T (MAIT) cells

γδ T-cells are important in tumor immunosurveillance, as demonstrated by the favorable prognostic significance of tumor-infiltrating γδ T-cells in diverse human malignancies[40-41]. Due to their MHC-independent antigen recognition, γδ T cells exhibit a low risk of GvHD[42]. Early clinical data from ADI-001, an allogeneic γδ T-cell-based CAR-T product targeting CD20, demonstrated a favorable safety profile and preliminary antitumor activity in relapsed/refractory B-cell malignancies (NCT04735471)[43]. Given the favorable prognosis associated with tumor-infiltrating γδ T cells in several malignancies, along with their tissue-resident properties and low alloreactive potential, γδ T cells are being actively investigated for solid tumor immunotherapy[8,41].

Another candidate cell type is NK cells, which are effector cells of the innate immune system that play a crucial role in eliminating both virus-infected and malignant cells. NK cells constitute 5%–15% of human peripheral blood leukocytes and are characterized by the expression of CD56 and the absence of CD3 and the T-cell receptor[44]. NK cells exhibit functional similarities to cytotoxic $CD8^+$ T cells, employing analogous cytotoxic mechanisms to eliminate target cells. However, in contrast to T cells, NK cells do not depend on MHC-mediated antigen recognition; rather, they rely on innate recognition mechanisms. This enables them to circumvent tumor antigen escape mechanisms and mediate cytotoxic activity against tumor cells without inducing GvHD, although their *in vivo* persistence is generally limited[45]. Numerous preclinical studies have demonstrated the antitumor activity of CAR-engineered NK cells against hematologic malignancies (*e.g.*, CD19 and CD20) as well as solid tumors (*e.g.*, WT1 and GD2), including ovarian and endometrial cancers and glioblastoma[46-48].

NKT cells are a subpopulation of lymphocytes that express both NK cell markers and T cell differentiation antigens[49]. NKT cells are divided into two types based on the structure of their TCR. Type I cells express the canonical α chain associated with a limited repertoire of β chains and are termed invariant NKT (iNKT) cells. iNKT cells recognize glycolipid antigens presented by CD1d, a nonpolymorphic HLA class I-like molecule[50]. Li *et al*[51-53] created universal CAR-iNKT cells by using TCR engineering and HLA gene editing on hematopoietic stem cells (HSCs). These cells exhibited three key advantages: high purity, resistance to allorejection, and potent antitumor activity against a broad range of both hematological malignancies and solid tumors. In preclinical mouse models of acute myeloid leukemia, allogeneic CD33-

directed CAR-iNKT cells demonstrated robust bone marrow homing and efficiently eliminated bone marrow-resident malignant blast cells, including the therapy-resistant CD33-low/negative leukemia stem and progenitor cell population[54]. Initial clinical trials have demonstrated encouraging outcomes in assessing the efficacy of allogeneic CD19-targeted CAR-NKT therapy in acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL)[55]. Since CAR-iNKT cells do not rely on classical MHC molecules and CD1d is non-polymorphic, the risk of GvHD is reduced; therefore, CAR-iNKT cells may represent a viable option for the development of "off-the-shelf" cell therapies[56].

MAIT cells are a distinct subset of innate-like T lymphocytes that rapidly respond to bacterial and fungal infections at mucosal barriers, in the liver, and in the bloodstream[57]. These cells recognize microbial-derived vitamin B_2 (riboflavin) metabolites presented by the non-classical MHC class I-related protein 1 (MR1) and, upon activation, rapidly secrete pro-inflammatory cytokines (*e.g.*, IFN-γ, TNF-α) and exert potent cytotoxicity to eliminate infected cells[57]. MAIT cells are attracting growing interest for the development of allogeneic CAR-MAIT cell therapy due to their innate effector properties, chemotherapy resistance, and low risk of GvHD[58]. They express IL-12R, IL-18R, and IL-23R receptors, which enable their activation by inflammatory cytokines independently of the TCR. Furthermore, MAIT cells carry activating NK cell receptors (NKG2D, CD161) that provide alternative mechanisms for tumor recognition, including in an MR1-independent manner[59]. Dogan *et al*[60] developed CAR-MAIT cells targeting CD19 and HER2 to evaluate their efficacy relative to traditional CAR-T cells. Their findings revealed that CAR-MAIT cells exhibit comparable or even superior cytotoxicity while demonstrating reduced secretion of pro-inflammatory cytokines *in vitro* compared with conventional CAR-T cells, suggesting a lower risk of cytokine release syndrome[60]. These findings suggest that MAIT cells may represent a potentially safer and more efficacious alternative to conventional CAR-based treatments. Nonetheless, their safety and efficacy *in vivo* and in allogeneic settings require validation, necessitating additional research[61].

(3) Use of T cells obtained from alternative cell populations

Another approach to preventing GvHD is to use alternative cell populations that ultimately differentiate into T cells and can be used for allogeneic CAR-T therapy. Such populations include umbilical cord blood cells and induced pluripotent stem cells.

Umbilical cord blood (UCB), a childbirth product, is a promising source for cellular immunotherapy due to its abundant immune cells and low donor morbidity[62]. T cells generated from UCB demonstrate a diminished incidence and severity of GvHD as well as decreased alloreactivity, likely attributable to their naive nature and lower cytokine output upon stimulation[63]. Liu *et al*[64] demonstrated that CAR-T cells sourced from cord blood exhibited better anticancer activity and prolonged tumor-suppressive effects in a leukemia mouse model compared with CAR-T cells obtained from patients with relapsed/refractory acute lymphoblastic leukemia. UCB can also be used to obtain hematopoietic stem cells, which can be differentiated into T cells that subsequently undergo transduction to express a CAR[65]. Given the numerous benefits of UCB-derived T cells, they represent a feasible source for the production of "off-the-shelf" allogeneic CAR-T cells for patients with hematologic malignancies or solid tumors.

Induced pluripotent stem cells (iPSCs) are derived from mature human tissues, such as skin or blood, and possess the ability to differentiate into various cell types. iPSCs offer product homogeneity, facilitating the introduction of genetic modifications using gene-editing technologies. In addition, iPSCs allow for the production of cells on a large scale, which is especially important for the generation of "off-the-shelf" cell products[66]. Recent studies have demonstrated the feasibility of generating allogeneic CAR-T cells from iPSCs. Examples include 1928z-T-iPSC-T, CAR iPSC-T cells, and T-iPSCs[67-68]. These findings suggest that iPSC-derived CAR-T cells may represent a scalable platform for "off-the-shelf" immunotherapy, pending further clinical validation.

HvGR

To maximize the efficacy of allogeneic CAR-T cells, it is also important to minimize the risk of HvGR. As discussed above, GvHD can be effectively mitigated through TCR disruption or the use of alternative cell sources[24]. In contrast, overcoming HvG-mediated rejection remains more challenging and represents a key factor limiting CAR-T cell persistence. Standard lymphodepletion regimens using cyclophosphamide and fludarabine can transiently delay host immune rejection and promote initial CAR-T cell engraftment[69]. However, these effects are short-lived and insufficient to provide sustained suppression of HvGR[70].

Immune rejection of allogeneic CAR-T cells is primarily mediated by recipient T-cell recognition of donor HLA molecules. To address this, CAR-T cells are commonly engineered to evade immune detection, for example, through deletion of *HLA* class I and II to reduce $CD8^+$ and $CD4^+$ T cell-mediated clearance[71]. One of the most widely used approaches is the inactivation of the gene encoding β2-microglobulin (*B2M*), which abolishes HLA class I surface expression and thereby reduces allorecognition by host T cells[72-73]. However, HLA class I molecules also serve as essential inhibitory ligands for natural killer (NK) cells[74]. Thus, loss of HLA class I renders CAR-T cells susceptible to NK cell-mediated lysis *via* "missing-self" recognition. To address this challenge, additional engineering strategies have been developed to mitigate NK cell-mediated clearance, including overexpression of non-classical HLA molecules such as HLA-E or HLA-G[75]. For example, Jo *et al*[70] developed immune-evasive universal CAR-T cells (ΔTRAC CAR ΔB2M HLAE) by integrating CAR and HLA-E —a non-polymorphic NK cell inhibitor—into the *TRAC* and *B2M* loci, respectively. These CAR cells exhibited antitumor efficacy and resistance to both alloreactive T cells and NK cells derived from both healthy donors and patients with acute myeloid leukemia[70]. Other approaches to preventing NK cell rejection are also being explored, including overexpression of CD47, a "don't eat me" signal that was first shown to protect against killing by macrophages but was recently found to also prevent killing by NK cells expressing the corresponding SIRPα receptor[76]. Knockout of *CD58* and *CD54* also prevents NK cell targeting of HLA class I-negative cells[77].

Taken together, these findings indicate that HLA disruption alone is insufficient, and that combinatorial strategies incorporating NK-inhibitory signaling are required to enhance CAR-T cell persistence. Such approaches are currently being evaluated in clinical trials (*e.g.*, NCT04035434, NCT04502446, NCT04960579, NCT05239143)[78-80]. Another strategy to reduce host-mediated rejection is to abrogate *CD52* expression on allogeneic CAR-T cells and use an anti-CD52 conditioning antibody (alemtuzumab) to sustain host's lymphodepletion[81]. This allows for long-term persistence of allogeneic CAR-T cells without the risk of HvGR and is currently being actively studied in clinical trials[34-35,82].

Collectively, these approaches highlight that effective control of HvGR requires multi-layered immune evasion strategies. Despite significant progress, achieving durable persistence of allogeneic CAR-T cells remains a major challenge, and further optimization of genome engineering and immune modulation will be critical for improving long-term clinical outcomes.

Allogeneic CAR-T cell therapy for solid tumors

CAR-T cell therapy has demonstrated limited efficacy in solid tumors compared with hematological malignancies, reflecting the convergence of multiple tumor-intrinsic and microenvironmental barriers, including antigen heterogeneity, impaired trafficking and infiltration, limited persistence, and progressive loss of effector function within the tumor microenvironment (TME)[12,83]. As noted above, the intrinsic fitness of T cells is a central determinant of CAR-T cell durability and antitumor activity. This is further compromised by profound T-cell depletion following chemotherapy, which is often more pronounced in patients with solid tumors than in those with leukemia[84], thereby constraining therapeutic efficacy. In this context, allogeneic CAR-T cell platforms may offer a conceptual advantage by providing access to functionally competent donor-derived T cells with enhanced proliferative capacity and persistence in hostile microenvironments[85]. Moreover, allogeneic approaches enable the scalable generation of multi-antigen-targeting or multivalent CAR-T cell products, thereby directly addressing tumor antigen heterogeneity[86-87].

The TME constitutes a major barrier to CAR-T cell efficacy and can be conceptualized as a multilayered and interconnected network of immunosuppressive mechanisms. These include suppressive cellular populations, soluble and metabolic inhibitory factors, and inhibitory receptor signaling pathways, all of which collectively contribute to CAR-T cell dysfunction and exhaustion[88-90].

At the cellular level, the TME is enriched with immunosuppressive populations, such as myeloid-derived suppressor cells (MDSCs), regulatory T (Treg) cells, and tumor-associated macrophages (TAMs)[88]. These cells actively promote tumor progression and suppress antitumor immunity through the production of inhibitory cytokines, including IL-4, IL-10, and transforming growth factor-β (TGF-β)[89]. Importantly, these populations not only directly inhibit T-cell function but also reinforce other immunosuppressive circuits within the TME.

Soluble and metabolic factors further exacerbate CAR-T cell dysfunction. TGF-β is a central immunosuppressive cytokine that inhibits T-cell proliferation and effector activity while promoting the expansion of regulatory immune subsets[91]. In

parallel, hypoxia —a defining feature of solid tumors—drives metabolic reprogramming, leading to the accumulation of extracellular adenosine[92]. Engagement of the A2A receptor (A2AR) on CAR-T cells increases intracellular cyclic AMP (cAMP) levels and activates protein kinase A (PKA), ultimately suppressing effector function[93]. Notably, hypoxia-driven adenosine accumulation, together with cytokines such as TGF-β and IL-10 produced by TAMs and MDSCs, establishes a reinforcing network of metabolic and cytokine-mediated immunosuppression.

A third layer of regulation is mediated by inhibitory immune checkpoint pathways. Molecules such as programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), lymphocyte activation gene-3 (LAG-3), and T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) are upregulated in response to chronic antigen stimulation and the immunosuppressive milieu, resulting in T-cell exhaustion and diminished therapeutic efficacy[90]. These pathways are functionally integrated with other components of the TME, as TGF-β signaling enhances checkpoint expression, while suppressive immune cells sustain inhibitory receptor signaling.

Given the interconnected nature of these resistance mechanisms, effective CAR-T cell engineering strategies must target multiple layers of TME-mediated suppression. One major approach involves disruption of inhibitory receptor signaling, including the use of antibodies targeting PD-1, CTLA-4, and TIM-3, as well as the incorporation of dominant-negative or switch receptors. In parallel, CRISPR/Cas9-mediated gene editing has been employed to abrogate checkpoint expression, including PD-1 and CTLA-4[83-85]. For example, Wang *et al*[94] demonstrated the feasibility and safety of allogeneic CAR-T cells with simultaneous disruption of PD-1 and TCR in solid tumor settings. In addition to gene knockout approaches, emerging CAR designs incorporate PD-1/CD28 switch receptors, which convert inhibitory PD-1 signaling into a co-stimulatory activation signal, thereby enhancing CAR-T cell function and persistence under immunosuppressive conditions within the tumor microenvironment[95].

To overcome soluble immunosuppression, CAR-T cells have been engineered to resist TGF-β signaling. Disruption of TGF-β receptor II (TGFBR2) enhances antitumor activity in both *in vitro* and *in vivo* models[96], while expression of dominant-negative TGFBR2 variants improves persistence and resistance to exhaustion[97-98]. More recently, inverted cytokine receptor strategies have been developed to convert inhibitory signals into stimulatory ones. For instance, TGF-β signaling can be rewired into an IL-15-mediated activation signal, markedly enhancing antitumor efficacy in preclinical models[99]. Clinical evaluation of allogeneic CAR-T cells with TGFBR2 disruption is currently underway[78], as discussed in the following section.

Targeting metabolic constraints represents an additional strategy. Deletion of *ADORA2A* enables CAR-T cells to resist adenosine-mediated suppression and maintain functionality under hypoxic conditions[100]. Consistent with the need to simultaneously address multiple barriers, Hatfield et al[101] developed allogeneic CAR-T cells incorporating six genetic modifications to prevent allorejection (*B2M*, *CIITA*), eliminate GvHD (*CD3E*), and overcome both metabolic (*ADORA2A*) and immunological (*CD274*, *TGFB1*) suppression. This multi-layered engineering approach resulted in enhanced tumor clearance and improved survival in humanized models[101]. Additional strategies include the genetic disruption of intracellular negative regulators such as *PTPN6*, *PTEN*, *CBLB*, *NR4A1*, and *ZC3H12A*/*RC3H1*, as well as engineering CAR-T cells to secrete activating molecules, including bispecific T-cell engagers (BiTEs) and cytokines such as IL-12[12,102-103]. Collectively, these approaches aim to reprogram CAR-T cells to withstand the complex, interconnected immunosuppressive networks of the TME, rather than targeting individual pathways in isolation, thereby enhancing therapeutic efficacy in solid tumors.

Clinical trials of allogeneic CAR-T cell therapy against solid tumors

In contrast to autologous CAR-T cell therapy, which is an established and effective treatment for B-cell malignancies with durable responses, allogeneic CAR-T approaches —particularly for solid tumors —remain in early clinical development. According to ClinicalTrials.gov, only a limited number of early-phase trials have been conducted, with most data derived from small phase I studies primarily designed to assess safety rather than efficacy, and characterized by small patient cohorts and short follow-up periods (***Table 2***).

An early-phase clinical study by Wang *et al*[94] investigated CRISPR/Cas9-edited allogeneic CAR-T cells with simultaneous disruption of PD-1 and TCR in mesothelin-positive solid tumors (NCT03545815). Among 15 treated patients, no dose-limiting toxicities

Table 2 **Clinical trials of allogeneic CAR-T cell therapy against solid tumors (updated from clinicaltrials.gov.)**

ClinicalTrials.gov ID	Product name	Phase	Target antigen	Indication	Study start date	Location
NCT03545815	Anti-mesothelin CAR-T cells	Phase Ⅰ	Mesothelin	Mesothelin-positive multiple solid tumors	March 19, 2018	China
NCT03692429	CYAD-101	Phase Ⅰ	NKG2D	Unresectable metastatic colorectal cancer	November 28, 2018	United States, Belgium
NCT04696731	ALLO-316	Phase Ⅰ	CD70	Clear cell renal cell carcinoma	February 24, 2021	United States
NCT05239143	P-MUC1C-ALLO1	Phase Ⅰ	MUC1-C	Advanced epithelial malignancy	February 15, 2022	United States
NCT05795595	CTX131	Phase Ⅰ and Phase Ⅱ	CD70	Relapsed or refractory solid tumors	March 13, 2023	United States
NCT05302037	CTM-N2D	Phase Ⅰ	NKG2DL	Advanced solid tumors	November 19, 2024	Singapore, Malaysia
NCT06717022	CHT102	Phase Ⅰ	Mesothelin	Mesothelin-positive solid tumors	May 6, 2024	China
NCT06592092	QH104	Phase Ⅰ	B7H3	Meningeal metastases of B7H3^{+} solid tumors	April 1, 2024	China
NCT06730659	CHT101	Phase Ⅰ	CD70	Advanced solid tumors	April 19, 2024	China
NCT06682793	A2B395	Phase Ⅰ and Phase Ⅱ	EGFR	Solid tumors with HLA-A*02 LOH	May 22, 2025	United States

Abbreviaitons: CAR-T, antigen receptor T-cell; LOH, loss of heterozygosity.

or unexpected adverse events were reported, and no severe cytokine release syndrome (CRS) or neurotoxicity was observed. Clinical efficacy was limited, with no objective responses and stable disease achieved in two of 15 patients. Importantly, CAR-T cell expansion peaked at days 7–14 post-infusion and declined rapidly, becoming undetectable beyond one month, indicating limited *in vivo* persistence. These findings highlight a favorable initial safety profile but underscore the challenges of achieving durable responses and sustained CAR-T cell persistence in solid tumors[94]. A related product, CHT102 (NCT06717022), is currently under clinical evaluation, with results expected in 2026[104].

Celyad Oncology developed the non-gene-edited allogeneic CAR-T product CYAD-101 for the treatment of patients with unresectable metastatic colorectal cancer[105]. In the phase I alloSHRINK trial (NCT03692429), CYAD-101 showed a favorable safety profile, with no dose-limiting toxicities or GvHD and predominantly low-grade CRS, without severe neurotoxicity. Preliminary efficacy was modest, with partial responses observed in two of 15 patients[105]. CAR-T cell expansion decreased after repeated infusions, as reflected by a reduction in Cmax, suggesting limited persistence, possibly due to HvGR[106]. The FDA paused the alloSHRINK clinical trial in March 2022 due to insufficient evidence to assess its risk to participants; although the FDA later lifted the pause, development of CYAD-101 has not yet resumed.

ALLO-316 is an allogeneic CAR-T cell therapy designed to recognize and target both CD70-positive tumor cells and host CD70-positive T cells, which contribute to allorejection[107]. In the phase 1a/b TRAVERSE study (NCT04696731), preliminary results in heavily pretreated renal cell carcinoma patients showed an objective response rate of 20%, increasing to 33% in patients with high CD70 expression (≥ 50%). All confirmed responses were ongoing at the data cut-off (2.1–8.4 months), suggesting early durability[108]. CRS occurred in 57% of patients, with grade ≥ 3 events observed in 2%. Immune effector cell-associated neurotoxicity syndrome (ICANS) was less frequent (9% overall; no grade ≥ 3 events)[104]. Overall, ALLO-316 demonstrates early clinical activity with manageable but significant toxicity, warranting further evaluation of persistence and long-term efficacy.

The next product, CTX131, consists of allogeneic anti-CD70 CAR-T cells with knockout of the *TGFBR2* and *ZC3H12A* genes for patients with advanced solid tumors, including renal cell carcinoma. Disruption of *ZC3H12A* improves the functional

stability of CAR-T cells by increasing their expansion potential. Disruption of *TGFBR2* allows CAR-T cells to evade suppression by the TME. Together, these modifications increase efficacy by at least 10-fold[78]. Preclinical studies demonstrated increased expansion, reduced exhaustion, and enhanced cytokine production, resulting in durable tumor regression across multiple xenograft models. Multiplex gene editing did not induce uncontrolled clonal expansion, supporting a favorable preclinical safety profile. No evidence of clonal expansion or cytokine-independent growth was observed, demonstrating that multiplex editing of CTX131 cells is safe for clinical application[78]. However, clinical data remain limited. Although early-phase trials (NCT05795595) have been completed, detailed results on efficacy, persistence, and treatment-related toxicities are not yet available, and the clinical relevance of these preclinical findings remains to be established.

Poseida Therapeutics has developed P-MUC1C-ALLO1, an allogeneic CAR-T cell product targeting MUC1-C-positive epithelial tumors, including breast, pancreatic, and colorectal cancers. The product is generated using a transposon-based vector and Cas-CLOVER™ gene editing to disrupt *TRBC* and reduce alloreactivity[80]. In an early-phase clinical trial (NCT05239143), evaluation in heavily pretreated patients (n = 41) demonstrated a manageable safety profile, with no cases of GvHD or ICANS reported. CRS occurred in 16% of patients, including grade ⩾ 3 events at higher dose levels, while grade ⩾ 3 hematologic toxicities were common (*e.g.*, neutropenia in 88%). However, clinical efficacy remains incompletely defined, with limited data on response durability and *in vivo* persistence, precluding firm conclusions regarding long-term benefit[80].

Cytomed Therapeutics has developed CTM-N2D, an allogeneic NKG2DL-targeting CAR γδ T-cell therapy for advanced solid tumors[109]. Preclinical studies demonstrated enhanced cytotoxicity and tumor regression, with a 132% increase in median survival in mouse models. In the ongoing ANGELICA trial (NCT05302037), patients receive escalating doses following lymphodepletion[110]. The main limitation of this approach is the recognition of NKG2D ligands on healthy cells. Cases of CRS, ICANS, and tumor lysis syndrome have also been reported[111]. The final results of this clinical trial are expected in December 2026.

Clinical trial NCT06592092 is evaluating QH104, an allogeneic B7H3-targeted CAR-γδ T-cell therapy, in patients with leptomeningeal metastases from B7H3-positive solid tumors. As of January 2025, two patients with lung adenocarcinoma received a single intrathecal dose (3×10^7 cells)[112]. No adverse events above grade 3 were reported; one patient experienced a transient low-grade seizure. At day 30, both patients achieved stable disease, with reduction or clearance of tumor cells in the cerebrospinal fluid and clinical improvement. CAR-γδ T cells persisted in the cerebrospinal fluid for approximately one week, without detectable systemic expansion. These preliminary data suggest a favorable safety profile and early signs of activity; however, the very limited sample size and short persistence preclude conclusions regarding response durability and efficacy[112,113]. Additionally, the Tmod platform incorporates an shRNA module targeting *B2M* to reduce MHC class I expression and potentially mitigate HvGR[113]. However, clinical data on efficacy, persistence, and safety—including CRS and neurotoxicity—are not yet available. Patients are prospectively identified through the BASECAMP-1 prescreening study (NCT04981119), which uses next-generation sequencing to detect HLA loss of heterozygosity[114].

Across studies, allogeneic CAR-T therapies demonstrate generally acceptable safety profiles, with a low incidence of GvHD, reflecting advances in gene editing and cell engineering. Nevertheless, toxicities such as cytokine release syndrome, immune effector cell-associated neurotoxicity syndrome, and potential risks related to off-target gene editing remain important considerations, although their incidence in allogeneic settings is not yet fully defined. A central limitation is the short *in vivo* persistence of allogeneic CAR-T cells, which likely contributes to reduced durability of clinical responses compared with autologous approaches. In solid tumors, this challenge is further exacerbated by the immunosuppressive tumor microenvironment, which constrains CAR-T cell expansion and functional activity.

Safety and regulatory challenges in allogeneic cell therapy

Despite their clear therapeutic promise, the transition of allogeneic CAR-T therapies into routine clinical practice remains far from straightforward. A number of interconnected challenges spanning manufacturing, genome engineering, regulation, and logistics continue to limit their broader implementation.

From a manufacturing perspective, requirements for allogeneic products are inherently more stringent than for autologous approaches. This is largely due to the scale: a single production run may generate doses for multiple patients, meaning that any error or

contamination event carries amplified consequences. As a result, compliance with Good Manufacturing Practice (GMP) standards is not only essential but also operationally demanding, requiring tight control across the entire workflow, from donor cell procurement to final product release[115]. The incorporation of multiplex gene editing, such as the knockout of *TCR* and *HLA* class I alongside the addition of the CAR construct, complicates the process further. Each novel genetic alteration raises legitimate concerns regarding off-target effects and genomic instability, potentially jeopardizing patient safety[116]. Viral vectors commonly used for gene delivery are particularly susceptible to random integration, which can inadvertently activate proto-oncogenes or disrupt tumor suppressor genes. Although CRISPR/Cas9 and TALENs provide more precise editing, they are not immune to off-target effects, which can lead to unintended genetic changes and increase the risk of malignancy. For example, during the UCAR-T19 gene editing process, the simultaneous targeting of the *TRAC* gene (chromosome 14) and the *CD52* gene (chromosome 1) unexpectedly resulted in the detection of the chromosomal translocation t(1;14) in all batches of UCART19 cells[34]. While the clinical significance of such findings is still being clarified, they highlight the need for careful genomic monitoring[117].

Researchers often view newer approaches, such as base and prime editing, as attractive alternatives due to their ability to avoid double-strand DNA breaks. However, their application in primary T cells remains limited by editing efficiency and targeting scope[118]. In addition, there is growing recognition that extensive editing – regardless of the platform—can come at a biological cost, affecting T-cell functional competence, expansion capacity, and effector activity[39]. Efforts to mitigate these risks are ongoing and include the use of high-fidelity editing systems, targeted insertion into defined loci, and incorporation of safety mechanisms such as inducible kill switches[119,120].

Regulatory challenges add another layer of difficulty to using allogeneic CAR-T therapies in clinical practice. Agencies like the FDA and European Medicines Agency (EMA) require comprehensive data on product characterization, sterility, and potency-and because multiplex-edited CAR-T products are still novel, specific guidelines are still evolving. This creates uncertainty in the approval pathway, leaving developers navigating a shifting landscape[121].

Finally, long-term safety remains incompletely defined. While risks such as GvHD and HvGR are relatively well characterized, the consequences of extensive genome editing are less well understood. Off-target effects and editing-induced genomic instability may lead to uncontrolled cellular proliferation and increase the risk of malignant transformation, although definitive clinical evidence remains limited. Accordingly, long-term patient monitoring is considered essential, despite the practical challenges associated with its implementation at scale[39].

Taken together, these issues illustrate that the development of allogeneic CAR-T therapies is not only a scientific endeavor but also a multidisciplinary coordination challenge. Progress in this field will likely depend on continued alignment between academia, industry, regulators, and clinical centers, alongside efforts to standardize manufacturing approaches and streamline regulatory pathways. Only through such coordinated advances can the full potential of allogeneic CAR-T therapies be realized in routine clinical settings.

Combining allogeneic CAR-T cell therapy with other therapies

Combination strategies are increasingly being explored to overcome resistance mechanisms that are not fully addressed by CAR-T cell engineering alone[122]. CAR-T cell therapy can be combined with various therapeutic modalities, including chemotherapy, radiotherapy, oncolytic viruses, checkpoint inhibitors, cancer vaccines, cytokines, and bispecific T-cell engagers (BiTEs)[123].

Co-administration of chemotherapy with CAR-T cells can reduce immunosuppressive cell populations and promote lymphodepletion, thereby enhancing CAR-T cell expansion and persistence *in vivo*[124]. The combination of cyclophosphamide (Cy) and fludarabine (Flu) is a standard lymphodepleting chemotherapy protocol used in conjunction with allogeneic CAR-T cells[81]. For example, a clinical trial NCT04637763 showed the safety, potential efficacy, and immunogenicity of CB-010, a CRISPR-edited allogeneic anti-CD19 CAR-T cell with a *PDCD1* knockout, in adults with relapsed/refractory B-cell non-Hodgkin lymphoma following lymphodepletion with cyclophosphamide and fludarabine[125].

One of the most promising strategies for enhancing the efficacy of CAR-T cell therapy is its combination with immune checkpoint inhibitors (ICIs). The combination of CAR-T cells and ICIs can enhance both the expansion and persistence of CAR-T cells in

the tumor microenvironment[126]. Carneiro *et al*[127] studied the effect of agenT-797, an allogeneic CAR-iNKT cell, alone or in combination with PD-1 inhibitors (pembrolizumab or nivolumab) in a phase 1 clinical trial in patients with advanced solid tumors, including pancreatic cancer, non-small cell lung cancer, colorectal cancer, and several other solid tumors, demonstrating the safety and tolerability of agenT-797 across various malignancies. In a Phase II clinical trial, researchers will study the use of agenT-797 in combination with botensilimab, a novel Fc-enhanced CTLA-4 inhibitor, and balstilimab (anti-PD-1) in esophageal and gastric adenocarcinoma (NCT06251973). Researchers anticipate the trial results in 2027.

Oncolytic virotherapy is an innovative immunotherapeutic strategy for cancer treatment. Oncolytic viruses selectively replicate within neoplastic cells, eliciting tumor-specific inflammatory responses that destroy tumor cells while sparing normal tissue[128]. The combination of oncolytic viruses with CAR-T cells has demonstrated enhanced antitumor activity in preclinical solid tumor models. Oncolytic viruses induce lysis of tumor cells, leading to the release of tumor antigens that subsequently activate CAR-T cells. Additionally, oncolytic viruses can modify the cellular makeup of the tumor microenvironment, leading to diminished local immunosuppression and an enhanced cytotoxic response from CAR-T cells[129]. Park *et al*[130] conducted immunotherapy using a novel poxvirus-based chimeric oncolytic virus called onCARlytics (Imugene Limited), which was engineered to express an inactive truncated CD19 antigen (CD19t) for tumor-selective delivery, in combination with off-the-shelf allogeneic CyCART-19 T cells targeting $CD19^+$ solid tumors. This combined approach effectively promoted in vivo tumor regression in human tumor xenograft models[130].

Maldini *et al*[131] used the immunosuppressants rapamycin and tacrolimus to mitigate host immunologic rejection of HLA-mismatched CAR-T cells, which required genetic disruption of *FKBP1A* in allogeneic CAR-T cells to establish drug resistance[131]. Allogeneic CAR-T cells, coupled with rapamycin and tacrolimus, displayed strong anticancer efficacy in relevant preclinical models.

In conclusion, the advent of alloCAR-T cell therapy represents an important advance in cancer immunotherapy. Its combination with additional immunomodulatory agents can enhance the antitumor immune response and effectively overcome resistance mechanisms that often hinder treatment. Further research in this area may lead to the development of new therapeutic options that could potentially improve clinical outcomes for cancer patients.

In Vivo CAR engineering

The main modalities of CAR-based immunotherapy are autologous, allogeneic, and *in vivo* CAR engineering. *In vivo* CAR engineering offers an innovative approach by facilitating the direct production of CAR-expressing cells within the patient, thus surmounting the logistical and manufacturing challenges associated with *ex vivo* techniques[132]. Diverse strategies have been established, encompassing lipid nanoparticle (LNP)-encapsulated nucleic acids, polymeric nanoparticles loaded with nucleic acids, lentiviral or adeno-associated virus delivery systems, and bioinstructive implantable scaffolds[132]. Each strategy has its own advantages and limitations in terms of cell specificity, systemic effects, and the risk of insertional mutagenesis.

LNP-mRNA technology utilizes a well-established clinical manufacturing framework, demonstrated by the extensive production and FDA authorization of Coronavirus Disease 2019 (COVID-19) vaccines, supporting its applicability to *in vivo* CAR engineering[133]. Preclinical data demonstrate that LNPs conjugated to antibodies or scFvs against CD3, CD4, CD5, CD7, and CD8 can be engineered to selectively target distinct immune cell populations[134]. For example, CD3-targeted LNPs have demonstrated enhanced *in vivo* efficacy, achieving up to 90% B cell depletion in mice *via* dose-dependent CAR expression and efficient cytokine release while exhibiting little hepatic toxicity[134]. In humanized NSG mouse models of Burkitt lymphoma, a LNP system co-delivering a CD19 CAR and IL-6-targeted short hairpin RNA attained 80% CAR transfection of human T cells, with CAR-T cells persisting for over 90 days and mediating significant tumor suppression while reducing the risk of CRS[135]. Nonetheless, obstacles persist, such as off-target effects (*e.g.*, macrophage transfection) and manufacturing complexities related to antibody conjugation. These challenges may diminish particle yield, stability, and long-term storage viability[136].

Biodegradable poly(β-aminoester) (PBAE)-based polymeric nanoparticles electrostatically complex with nucleic acids (DNA or mRNA), enhancing their delivery to target cells, such as T cells and macrophages[137]. Lyophilization enables the generation and preservation of nanoparticles in a stable dry powder format, improving scalability and cost-efficiency[138]. PBAE nanoparticles, engineered

for CAR delivery, have exhibited therapeutic efficacy in preclinical models of leukemia, prostate cancer, and hepatitis B-induced hepatocellular carcinoma, achieving tumor regression similar to traditional adoptive CAR-T cell therapy[139]. Nonetheless, mRNA-based therapeutics require multiple administrations due to transient CAR expression, whereas DNA-based platforms present a risk of insertional mutagenesis, underscoring the necessity for additional research into long-term biocompatibility and safety prior to clinical application[140]. The Implantable Multifunctional Alginate Scaffold for T Cell Engineering and Release (MASTER) exemplifies a hybrid approach to *in vivo* and *ex vivo* CAR-T cell engineering. Initial T-cell isolation is necessary, although processing time is minimized to one day[141]. The alginate scaffold creates a confined milieu that facilitates interactions among T cells, viral vectors, cytokines, and chemokines, thereby enhancing CAR transduction efficacy and reducing systemic side effects. An alginate-based scaffold infused with CD3/CD28 antibodies and IL-2 is loaded with autologous T cells and retroviruses prior to implantation. In a humanized mouse model of $CD19^+$ lymphoma, this technique produced CD19 CAR-T cells that demonstrated efficacy comparable to traditional *ex vivo* cells in tumor growth control, exhibiting enhanced expansion and persistence[142].

In vivo CAR T-cell therapy primarily investigates two classes of viral vectors: replication-defective lentiviruses (the most common platform) and adeno-associated viruses. Lentiviruses, with a single-stranded RNA genome, integrate into the host genome for long-term transgene expression, but their *in vivo* infection efficiency is often limited by low target-cell affinity[143]. To enhance targeting, lentiviral vectors can be pseudotyped with viral envelopes (*e.g.*, Nipah, measles, or Sindbis) or engineered to display ligands (*e.g.*, scFvs or designed ankyrin repeat proteins) fused to envelope proteins for specific T-cell subtype targeting[143]. Michaels *et al*[144] created VivoVec, a cocal glycoprotein-pseudotyped, CD3-redirected lentivirus that encodes a CD19 CAR and a rapamycin-activated synthetic cytokine receptor. In humanized NSG mice, the administration of 10^7 transduction units (TU) resulted in the detection of CAR T cells and nearly full B-cell depletion by day 11. Doses between 2.7×10^7 and 2.7×10^8 TU produced a dose-dependent increase of CAR T-cells (reaching up to 30 CAR T cells/μL blood on day 11) and nearly full tumor control for around 50 days[144]. Interius BioPharma's UB-VV111 delivers a CD19-directed CAR and a RACR module that work together to control the growth and survival of CAR-T cells[145]. Preliminary findings from a Phase I study (NCT06528301) in lymphoma patients suggest encouraging sustained CAR expression and peripheral B-cell depletion, accomplished without lymphodepleting preconditioning and devoid of dose-limiting toxicities. However, a small sample size and short follow-up period limit these findings[145]. *In vivo* technology, which enables the generation of CAR-T cells directly in the patient's body, offers a practical alternative to traditional ex vivo methods. Shorter treatment cycles and fewer infrastructure requirements make this approach more accessible, especially in resource-limited settings. However, implementing this technology into routine practice faces several hurdles, ranging from developing reliable delivery vectors to scaling up production and ensuring consistent batch quality.

Conclusions and perspective

Since 2017, the FDA has approved seven commercially available CAR-T cell products, all derived from autologous T cells. While autologous CAR-T cell therapy demonstrates high efficacy, it has several limitations, including insufficient numbers of functional T cells in the patient, long manufacturing times, high cost, and the limited availability of specialized centers. Although allogeneic CAR-T cell therapy has not yet reached the clinical maturity of autologous CAR-T therapy, its “off-the-shelf” availability, potential for repeated dosing, and scalable manufacturing remain important advantages. Recently, numerous clinical trials have been conducted to evaluate the safety and efficacy of allogeneic CAR-T cells. To prevent GvHD and HvGR, researchers have used various approaches, such as TCR modification, the use of non-α/β T cells, the use of T cells obtained from alternative cell populations, and the deletion of *B2M* and *CD52*. Gene editing has become a priority strategy in clinical trials, as scientists aim to create a universal allogeneic CAR therapy platform. Advances in gene editing technologies have improved the safety profile of allogeneic cell therapy by enabling more precise and controlled genome engineering. The ability to simultaneously introduce multiple genetic modifications into a single cell significantly increases the therapeutic potential of this approach, although it requires long-term monitoring of the safety of the genetically modified products used in patients. A comprehensive assessment of therapeutic efficacy and the durability of the treatment effect requires longer

observation periods and a larger number of clinical trial participants.

Allogeneic CAR-T therapy represents a scalable but currently incomplete solution, with efficacy in solid tumors still constrained by biological and immunological barriers. Preclinical studies suggest that donor-derived CAR-T cells may retain greater proliferative capacity and functional activity within immunosuppressive tumor microenvironments than heavily pretreated autologous T cells. Given the complexities associated with CAR-T cell therapy for solid tumors, the greater flexibility for multiplex editing afforded by allogeneic products may be particularly advantageous. For example, modifications potentially aimed at eliminating immunosuppression in the tumor microenvironment, such as genetic knockout of *TGFBR2* and/or *PDCD1*, may be more readily achieved in allogeneic CAR-T cell products than in autologous ones. Future progress will depend on the integration of multiplex genome engineering with strategies that ensure durable persistence in immunosuppressive tumor environments.

The diverse *in vivo* CAR-engineering strategies address several key limitations associated with both autologous and allogeneic CAR-T therapies. While allogeneic approaches offer the key advantage of off-the-shelf availability and rapid administration, they face challenges such as HvGR and GvHD; in contrast, *in vivo* methods enable the direct generation of CAR-T cells within the patient's body, significantly reducing systemic toxicity and eliminating the need for complex *ex vivo* manufacturing and cryopreservation. Future synergy between these complementary modalities may improve treatment durability and accessibility: leveraging in vivo techniques (*e.g.*, LNP–mRNA delivery) for rapid initial tumour control via transient CAR expression, followed by allogeneic infusions to provide durable immune memory and sustained remission.

Funding

This study was funded by the Russian Science Foundation (Grant No. 24-74-00092 to A. Kh. Valiullina).

Acknowledgments

None.

Authors' Contribution

Aigul Valiullina: Conceptualization, Funding acquisition, Project administration, Writing — reviewing and editing. **Emil Bulatov**: Conceptualization, Supervision, Writing —reviewing and editing. **Elvina Gilyazova**: Investigation. **Raniya Khadiullina**: Visualization, Writing —original draft. **Yulia Skibo**: Visualization. **Sabir Mukhametshin**: Validation, Writing—reviewing and editing.

References

[1] Zhao Z, Chen Y, Francisco NM, et al. The application of CAR-T cell therapy in hematological malignancies: Advantages and challenges[J]. *Acta Pharm Sin B*, 2018, 8(4): 539–551.

[2] Nair R, Neelapu SS. The promise of CAR T-cell therapy in aggressive B-cell lymphoma[J]. *Best Pract Res Clin Haematol*, 2018, 31(3): 293–298.

[3] Hanssens H, Meeus F, De Veirman K, et al. The antigen-binding moiety in the driver's seat of CARs[J]. *Med Res Rev*, 2022, 42(1): 306–342.

[4] Cappell KM, Kochenderfer JN. A comparison of chimeric antigen receptors containing CD28 versus 4-1BB costimulatory domains[J]. *Nat Rev Clin Oncol*, 2021, 18(11): 715–727.

[5] Onea AS, Jazirehi AR. CD19 chimeric antigen receptor (CD19 CAR)-redirected adoptive T-cell immunotherapy for the treatment of relapsed or refractory B-cell non-Hodgkin's lymphomas[J]. *Am J Cancer Res*, 2016, 6(2): 403–424.

[6] Wang T, He T, Ma L, et al. Clinical outcomes of BCMA CAR-T cells in a multiple myeloma patient with central nervous system invasion[J]. *Front Oncol*, 2022, 12: 854448.

[7] Cappell KM, Kochenderfer JN. Long-term outcomes following CAR T cell therapy: What we know so far[J]. *Nat Rev Clin Oncol*, 2023, 20(6): 359–371.

[8] Sasu BJ, Lauron EJ, Schulz T, et al. Allogeneic CAR T cell therapy for cancer[J]. *Annu Rev Cancer Biol*, 2024, 8: 227–243.

[9] Du B, Qin J, Lin B, et al. CAR-T therapy in solid tumors[J]. *Cancer Cell*, 2025, 43(4): 665–679.

[10] Zong J, Li YR. iPSC technology revolutionizes CAR-T cell therapy for cancer treatment[J]. *Bioengineering (Basel)*, 2025, 12(1): 60.

[11] Chen S, van den Brink MRM. Allogeneic "off-the-shelf" CAR T cells: Challenges and advances[J]. *Best Pract Res Clin Haematol*, 2024, 37(3): 101566.

[12] Albelda SM. CAR T cell therapy for patients with solid tumours: Key lessons to learn and unlearn[J]. *Nat Rev Clin Oncol*, 2024, 21(1): 47–66.

[13] Ghassemi S, Nunez-Cruz S, O'Connor RS, et al. Reducing *ex vivo* culture improves the antileukemic activity of chimeric antigen receptor (CAR) T cells[J]. *Cancer Immunol Res*, 2018, 6(9): 1100–1109.

[14] Kourelis T, Bansal R, Berdeja J, et al. Ethical challenges with multiple myeloma BCMA chimeric antigen receptor T

cell slot allocation: A multi-institution experience[J]. *Transplant Cell Ther*, 2023, 29(4): 255–258.

[15] Litvinova Y, Merkur S, Allin S, et al. Availability and financing of CAR-T cell therapies: A cross-country comparative analysis[J]. *Health Policy*, 2024, 149: 105153.

[16] Depil S, Duchateau P, Grupp SA, et al. 'Off-the-shelf' allogeneic CAR T cells: Development and challenges[J]. *Nat Rev Drug Discov*, 2020, 19(3): 185–199.

[17] Lin MY, Yoon H, Nguyen A, et al. Allogeneic CAR T cells derived from younger donor T cells have more desirable T cell phenotype and better in vitro functionality[C]//Poster Presented at the 25th ASGCT Conference. 2022: 16–19.

[18] Geethakumari PR, Ramasamy DP, Dholaria B, et al. Balancing quality, cost, and access during delivery of newer cellular and immunotherapy treatments[J]. *Curr Hematol Malig Rep*, 2021, 16(4): 345–356.

[19] Mansoori S, Noei A, Maali A, et al. Recent updates on allogeneic CAR-T cells in hematological malignancies[J]. *Cancer Cell Int*, 2024, 24(1): 304.

[20] Ahmed N, Wesson W, Mushtaq MU, et al. "Waitlist mortality" is high for myeloma patients with limited access to BCMA therapy[J]. *Front Oncol*, 2023, 13: 1206715.

[21] Lonez C, Breman E. Allogeneic CAR-T therapy technologies: Has the promise been met?[J]. *Cells*, 2024, 13(2): 146.

[22] Sung AD, Chao NJ. Concise review: Acute graft-versus-host disease: Immunobiology, prevention, and treatment[J]. *Stem Cells Transl Med*, 2013, 2(1): 25–32.

[23] Zeiser R, Blazar BR. Acute graft-versus-host disease —biologic process, prevention, and therapy[J]. *N Engl J Med*, 2017, 377(22): 2167–2179.

[24] Abdelhakim H, Abdel-Azim H, Saad A. Role of αβ T cell depletion in prevention of graft versus host disease[J]. *Biomedicines*, 2017, 5(3): 35.

[25] Mo F, Mamonkin M, Brenner MK, et al. Taking T-cell oncotherapy off-the-shelf[J]. *Trends Immunol*, 2021, 42(3): 261–272.

[26] Qasim W. Genome-edited allogeneic donor "universal" chimeric antigen receptor T cells[J]. *Blood*, 2023, 141(8): 835–845.

[27] Torikai H, Reik A, Liu P, et al. A foundation for universal T-cell based immunotherapy: T cells engineered to express a CD19-specific chimeric-antigen-receptor and eliminate expression of endogenous TCR[J]. *Blood*, 2012, 119(24): 5697–5705.

[28] Cooper ML, Choi J, Staser K, et al. An "off-the-shelf" fratricide-resistant CAR-T for the treatment of T cell hematologic malignancies[J]. *Leukemia*, 2018, 32(9): 1970–1983.

[29] Sommer C, Boldajipour B, Kuo TC, et al. Preclinical evaluation of allogeneic CAR T cells targeting BCMA for the treatment of multiple myeloma[J]. *Mol Ther*, 2019, 27(6): 1126–1138.

[30] Eyquem J, Mansilla-Soto J, Giavridis T, et al. Targeting a CAR to the *TRAC* locus with CRISPR/Cas9 enhances tumour rejection[J]. *Nature*, 2017, 543(7643): 113–117.

[31] Moradi V, Khodabandehloo E, Alidadi M, et al. Progress and pitfalls of gene editing technology in CAR-T cell therapy: A state-of-the-art review[J]. *Front Oncol*, 2024, 14: 1388475.

[32] Petri K, D'Ippolito E, Künkele A, et al. Next-generation T cell immunotherapies engineered with CRISPR base and prime editing: Challenges and opportunities[J]. *Nat Rev Clin Oncol*, 2025, 22(12): 902–923.

[33] Neelapu SS, Nath R, Munoz J, et al. ALPHA study: ALLO-501 produced deep and durable responses in patients with relapsed/refractory non-Hodgkin's lymphoma comparable to autologous CAR T[J]. *Blood*, 2021, 138(S1): 3878.

[34] Benjamin R, Jain N, Maus MV, 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[J]. *Lancet Haematol*, 2022, 9(11): e833–e843.

[35] Mailankody S, Matous JV, Chhabra S, et al. Allogeneic BCMA-targeting CAR T cells in relapsed/refractory multiple myeloma: Phase 1 UNIVERSAL trial interim results[J]. *Nat Med*, 2023, 29(2): 422–429.

[36] McGuirk JP, Tam CS, Kröger N, et al. CTX110 allogeneic CRISPR-Cas9-engineered CAR T cells in patients (Pts) with relapsed or refractory (R/R) large B-cell lymphoma (LBCL): Results from the phase 1 dose escalation carbon study[J]. *Blood*, 2022, 140(S1): 10303–10306.

[37] Ghobadi A, Aldoss I, Maude S, et al. P356: Phase 1/2 dose-escalation study of anti-CD7 allogenic CAR-T cell in relapsed or refractory (R/R) T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma (T-ALL/LBL)[J]. *HemaSphere*, 2023, 7(S3): e1789302.

[38] Wang X, Wu X, Tan B, et al. Allogeneic CD19-targeted CAR-T therapy in patients with severe myositis and systemic sclerosis[J]. *Cell*, 2024, 187(18): 4890–4904. e9.

[39] Su J, Zeng Y, Song Z, et al. Genome-edited allogeneic CAR-T cells: The next generation of cancer immunotherapies[J]. *J Hematol Oncol*, 2025, 18(1): 90.

[40] Wu Y, Kyle-Cezar F, Woolf RT, et al. An innate-like Vδ1^{+} γδ T cell compartment in the human breast is associated with remission in triple-negative breast cancer[J]. *Sci Transl Med*, 2019, 11(513): eaax9364.

[41] Zakeri N, Hall A, Swadling L, et al. Characterisation and induction of tissue-resident gamma delta T-cells to target hepatocellular carcinoma[J]. *Nat Commun*, 2022, 13(1): 1372.

[42] Jhita N, Raikar SS. Allogeneic gamma delta T cells as adoptive cellular therapy for hematologic malignancies[J]. *Explor Immunol*, 2022, 2(3): 334–350.

[43] Neelapu SS, Stevens DA, Hamadani M, et al. A phase 1 study of ADI-001: Anti-CD20 CAR-engineered allogeneic gamma delta1 (γδ) T cells in adults with B-cell malignancies[J]. *Blood*, 2022, 140(S1): 4617–4619.

[44] Xie G, Dong H, Liang Y, et al. CAR-NK cells: A promising

cellular immunotherapy for cancer[J]. *eBioMedicine*, 2020, 59: 102975.

[45] Chen S, Zhu H, Jounaidi Y. Comprehensive snapshots of natural killer cells functions, signaling, molecular mechanisms and clinical utilization[J]. *Signal Transduct Target Ther*, 2024, 9(1): 302.

[46] Shimasaki N, Fujisaki H, Cho D, et al. A clinically adaptable method to enhance the cytotoxicity of natural killer cells against B-cell malignancies[J]. *Cytotherapy*, 2012, 14(7): 830–840.

[47] Shao R, Li Z, Xin H, et al. Biomarkers as targets for CAR-T/NK cell therapy in AML[J]. *Biomark Res*, 2023, 11(1): 65.

[48] Yang K, Zhao Y, Sun G, et al. Clinical application and prospect of immune checkpoint inhibitors for CAR-NK cell in tumor immunotherapy[J]. *Front Immunol*, 2023, 13: 1081546.

[49] Matsuda JL, Mallevaey T, Scott-Browne J, et al. CD1d-restricted iNKT cells, the 'Swiss-Army knife' of the immune system[J]. *Curr Opin Immunol*, 2008, 20(3): 358–368.

[50] Metelitsa LS. Anti-tumor potential of type-I NKT cells against CD1d-positive and CD1d-negative tumors in humans[J]. *Clin Immunol*, 2011, 140(2): 119–129.

[51] Li YR, Zhou Y, Yu J, et al. Generation of allogeneic CAR-NKT cells from hematopoietic stem and progenitor cells using a clinically guided culture method[J]. *Nat Biotechnol*, 2025, 43(3): 329–344.

[52] Li YR, Li Z, Zhu Y, et al. Overcoming ovarian cancer resistance and evasion to CAR-T cell therapy by harnessing allogeneic CAR-NKT cells[J]. *Med*, 2025, 6(10): 100804.

[53] Li YR, Shen X, Zhu E, et al. Targeting orthotopic and metastatic pancreatic cancer with allogeneic stem cell–engineered mesothelin-redirected CAR-NKT cells[J]. *Proc Natl Acad Sci U S A*, 2025, 122(47): e2517786122.

[54] Li YR, Fang Y, Niu S, et al. Allogeneic CD33-directed CAR-NKT cells for the treatment of bone marrow-resident myeloid malignancies[J]. *Nat Commun*, 2025, 16(1): 1248.

[55] Ramos CA, Courtney AN, Robinson SN, et al. Allogeneic NKT cells expressing a CD19-specific CAR in patients with relapsed or refractory B-cell malignancies: An interim analysis[J]. *Blood*, 2021, 138(S1): 2819.

[56] Chaudhry MS, Karadimitris A. Role and regulation of CD1d in normal and pathological B cells[J]. *J Immunol*, 2014, 193(10): 4761–4768.

[57] Godfrey DI, Koay HF, McCluskey J, et al. The biology and functional importance of MAIT cells[J]. *Nat Immunol*, 2019, 20(9): 1110–1128.

[58] Tourret M, Talvard-Balland N, Lambert M, et al. Human MAIT cells are devoid of alloreactive potential: Prompting their use as universal cells for adoptive immune therapy[J]. *J Immunother Cancer*, 2021, 9(10): e003123.

[59] Petley EV, Koay HF, Henderson MA, et al. MAIT cells regulate NK cell-mediated tumor immunity[J]. *Nat Commun*, 2021, 12(1): 4746.

[60] Dogan M, Karhan E, Kozhaya L, et al. Engineering human MAIT cells with chimeric antigen receptors for cancer immunotherapy[J]. *J Immunol*, 2022, 209(8): 1523–1531.

[61] Li YR, Zhu Y, Fang Y, et al. Emerging trends in clinical allogeneic CAR cell therapy[J]. *Med*, 2025, 6(8): 100677.

[62] Balassa K, Rocha V. Anticancer cellular immunotherapies derived from umbilical cord blood[J]. *Expert Opin Biol Ther*, 2018, 18(2): 121–134.

[63] Kwoczek J, Riese SB, Tischer S, et al. Cord blood–derived T cells allow the generation of a more naïve tumor-reactive cytotoxic T-cell phenotype[J]. *Transfusion*, 2018, 58(1): 88–99.

[64] Liu D, Hong W, Qiu K, et al. Umbilical cord blood: A promising source for allogeneic CAR-T cells[J]. *Front Oncol*, 2022, 12: 944248.

[65] Van Caeneghem Y, De Munter S, Tieppo P, et al. Antigen receptor-redirected T cells derived from hematopoietic precursor cells lack expression of the endogenous TCR/CD3 receptor and exhibit specific antitumor capacities[J]. *Oncoimmunology*, 2017, 6(3): e1283460.

[66] Shi Y, Inoue H, Wu JC, et al. Induced pluripotent stem cell technology: A decade of progress[J]. *Nat Rev Drug Discov*, 2017, 16(2): 115–130.

[67] Jing R, Scarfo I, Najia MA, et al. EZH1 repression generates mature iPSC-derived CAR T cells with enhanced antitumor activity[J]. *Cell Stem Cell*, 2022, 29(8): 1181–1196. e6.

[68] Wang Z, McWilliams-Koeppen HP, Reza H, et al. 3D-organoid culture supports differentiation of human CAR^+ iPSCs into highly functional CAR T cells[J]. *Cell Stem Cell*, 2022, 29(4): 515–527. e8.

[69] Bot A, Rossi JM, Jiang Y, et al. Cyclophosphamide and fludarabine conditioning chemotherapy induces a key homeostatic cytokine profile in patients prior to CAR T cell therapy[J]. *Blood*, 2015, 126(23): 4426.

[70] Jo S, Das S, Williams A, et al. Endowing universal CAR T-cell with immune-evasive properties using TALEN-gene editing[J]. *Nat Commun*, 2022, 13(1): 3453.

[71] Ren J, Liu X, Fang C, et al. Multiplex genome editing to generate universal CAR T cells resistant to PD1 inhibition[J]. *Clin Cancer Res*, 2017, 23(9): 2255–2266.

[72] Arce-Gomez B, Jones EA, Barnstable CJ, et al. The genetic control of HLA-A and B antigens in somatic cell hybrids: Requirement for β_2 microglobulin[J]. *Tissue Antigens*, 1978, 11(2): 96–112.

[73] Lee J, Sheen JH, Lim O, et al. Abrogation of HLA surface expression using CRISPR/Cas9 genome editing: A step toward universal T cell therapy[J]. *Sci Rep*, 2020, 10(1): 17753.

[74] Bix M, Liao NS, Zijlstra M, et al. Rejection of class I MHC-deficient haemopoietic cells by irradiated MHC-matched mice[J]. *Nature*, 1991, 349(6307): 329–331.

[75] Kagoya Y, Guo T, Yeung B, 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[J]. *Cancer Immunol Res*, 2020, 8(7): 926–936.

[76] Deuse T, Hu X, Agbor-Enoh S, et al. The SIRPα–CD47 immune checkpoint in NK cells[J]. *J Exp Med*, 2021, 218(3): e20200839.

[77] Hammer Q, Perica K, Mbofung RM, et al. Combined genetic ablation of CD54 and CD58 in CAR engineered cytotoxic lymphocytes effectively averts allogeneic immune cell rejection[J]. *Blood*, 2022, 140(S1): 1165–1166.

[78] Terrett JA, Kalaitzidis D, Dequeant ML, et al. Abstract ND02: CTX112 and CTX131: Next-generation CRISPR/Cas9-engineered allogeneic (allo) CAR T cells incorporating novel edits that increase potency and efficacy in the treatment of lymphoid and solid tumors[J]. *Cancer Res*, 2023, 83(S7): ND02.

[79] Dholaria B, Shune LO, Kocoglu MH, et al. A phase 1 study of P-BCMA-ALLO1, a non-viral, allogeneic BCMA directed CAR-T in relapsed/refractory multiple myeloma (RRMM): Results from optimized lymphodepletion cohort[J]. *Blood*, 2024, 144(S1): 4828.

[80] Oh D, Vu P, Baranda JC, et al. 50P phase I trial of P-MUC1C-ALLO1 allogeneic CAR-T cells in advanced epithelial malignancies[J]. *Immuno-Oncol Technol*, 2024, 24: 100861.

[81] Benjamin R, Graham C, Yallop D, et al. Preliminary data on safety, cellular kinetics and anti-leukemic activity of UCART19, an allogeneic anti-CD19 CAR T-cell product, in a pool of adult and pediatric patients with high-risk CD19+ relapsed/refractory B-cell acute lymphoblastic leukemia[J]. *Blood*, 2018, 132(S1): 896.

[82] Qasim W, Ciocarlie O, Adams S, et al. Preliminary results of UCART19, an allogeneic anti-CD19 CAR T-cell product in a first-in-human trial (PALL) in pediatric patients with CD19+ relapsed/refractory B-cell acute lymphoblastic leukemia[J]. *Blood*, 2017, 130(S1): 1271.

[83] Peng L, Sferruzza G, Yang L, et al. CAR-T and CAR-NK as cellular cancer immunotherapy for solid tumors[J]. *Cell Mol Immunol*, 2024, 21(10): 1089–1108.

[84] Das RK, Vernau L, Grupp SA, et al. Naïve T-cell deficits at diagnosis and after chemotherapy impair cell therapy potential in pediatric cancers[J]. *Cancer Discov*, 2019, 9(4): 492–499.

[85] Chok R, Ramakrishna S. Allogeneic approach unlocks CAR T cell benefits in solid tumors[J]. *Nat Med*, 2025, 31(3): 733–734.

[86] Drougkas K, Karampinos K, Karavolias I, et al. Comprehensive clinical evaluation of CAR-T cell immunotherapy for solid tumors: A path moving forward or a dead end?[J]. *J Cancer Res Clin Oncol*, 2023, 149(6): 2709–2734.

[87] Walsh Z, Ross S, Fry TJ. Multi-specific CAR targeting to prevent antigen escape[J]. *Curr Hematol Malig Rep*, 2019, 14(5): 451–459.

[88] Haist M, Stege H, Grabbe S, et al. The functional crosstalk between myeloid-derived suppressor cells and regulatory T cells within the immunosuppressive tumor microenvironment[J]. *Cancers (Basel)*, 2021, 13(2): 210.

[89] Mirlekar B. Tumor promoting roles of IL-10, TGF-β, IL-4, and IL-35: Its implications in cancer immunotherapy[J]. *SAGE Open Med*, 2022, 10: 20503121211069012.

[90] Kong X. Discovery of new immune checkpoints: Family grows up[M]//Xu J. Regulation of Cancer Immune Checkpoints: Molecular and Cellular Mechanisms and Therapy. Singapore: Springer, 2020: 61–82.

[91] Travis MA, Sheppard D. TGF-β activation and function in immunity[J]. *Annu Rev Immunol*, 2014, 32(1): 51–82.

[92] Xing J, Zhang J, Wang J. The immune regulatory role of adenosine in the tumor microenvironment[J]. *Int J Mol Sci*, 2023, 24(19): 14928.

[93] Newick K, O'Brien S, Sun J, et al. Augmentation of CAR T-cell trafficking and antitumor efficacy by blocking protein kinase A localization[J]. *Cancer Immunol Res*, 2016, 4(6): 541–551.

[94] Wang Z, Li N, Feng K, et al. Phase I study of CAR-T cells with PD-1 and TCR disruption in mesothelin-positive solid tumors[J]. *Cell Mol Immunol*, 2021, 18(9): 2188–2198.

[95] Du H, Gong H, Wang Y, et al. High-affinity PD-1-CD28 switch receptor enhances sustained antitumor activity of CAR-T cells in both lymphoma and Ewing sarcoma[J]. *Blood*, 2025, 146(S1): 4102.

[96] Tang N, Cheng C, Zhang X, et al. TGF-β inhibition via CRISPR promotes the long-term efficacy of CAR T cells against solid tumors[J]. *JCI Insight*, 2020, 5(4): e133977.

[97] Kloss CC, Lee J, Zhang A, et al. Dominant-negative TGF-β receptor enhances PSMA-targeted human CAR T cell proliferation and augments prostate cancer eradication[J]. *Mol Ther*, 2018, 26(7): 1855–1866.

[98] Zhu S, Hu J, Lin J, et al. Co-expression of dominant-negative TGF-β receptor 2 enhances the therapeutic efficacy of novel TREM1/DAP12-BB-based CAR-T cells in solid tumours[J]. *Immunology*, 2025, 174(3): 310–321.

[99] Zheng S, Che X, Zhang K, et al. Potentiating CAR-T cell function in the immunosuppressive tumor microenvironment by inverting the TGF-β signal[J]. *Mol Ther*, 2025, 33(2): 688–702.

[100] Giuffrida L, Sek K, Henderson MA, et al. CRISPR/Cas9 mediated deletion of the adenosine A2A receptor enhances CAR T cell efficacy[J]. *Nat Commun*, 2021, 12(1): 3236.

[101] Hatfield SM. Engineering 'off-the-shelf' CAR-T cells resistant to biochemical and immunological barriers in the solid tumor microenvironment 2279[J]. *J Immunol*, 2025, 214(S1): vkaf283.214.

[102] Sun D, Shi X, Li S, et al. CAR-T cell therapy: A breakthrough in traditional cancer treatment strategies (review)[J]. *Mol Med Rep*, 2024, 29(3): 47.

[103] Mai D, Johnson O, Reff J, et al. Combined disruption of T cell inflammatory regulators Regnase-1 and Roquin-1 enhances antitumor activity of engineered human T cells[J]. *Proc Natl Acad Sci U S A*, 2023, 120(12): e2218632120.

[104] Hao J, Song Y, Li H, et al. Phase I open-label single-arm study to evaluate the safety, tolerability and preliminary efficacy of CHT102, an allogeneic mesothelin-targeted

CAR-T Cell, in patients with advanced solid tumors (CHAPTER)[C]//Molecular Therapy. Vol 33. Cell Press 50 Hampshire St, Floor 5, Cambridge, MA 02139 USA, 2025.

[105] Michaux A, Mauën S, Breman E, et al. Clinical grade manufacture of CYAD-101, a NKG2D-based, first in class, non–gene-edited allogeneic CAR T-cell therapy[J]. *J Immunother*, 2022, 45(3): 150–161.

[106] Prenen H, Dekervel J, Anguille S, et al. CYAD-101: An innovative non-gene edited allogeneic CAR-T for solid tumor cancer therapy[J]. *J Clin Oncol*, 2020, 38(S15): 3032.

[107] Srour S, Kotecha R, Curti B, et al. Abstract CT011: A phase 1 multicenter study (TRAVERSE) evaluating the safety and efficacy of ALLO-316 following conditioning regimen in pts with advanced or metastatic clear cell renal cell carcinoma (ccRCC)[J]. *Cancer Res*, 2023, 83(S8): CT011.

[108] Srour SA, Chahoud J, Drakaki A, et al. ALLO-316 in advanced clear cell renal cell carcinoma (ccRCC): Updated results from the phase 1 TRAVERSE study[J]. *J Clin Oncol*, 2025, 43(S16): 4508.

[109] Ang WX, Ng YY, Xiao L, et al. Electroporation of NKG2D RNA CAR improves Vγ9Vδ2 T cell responses against human solid tumor xenografts[J]. *Mol Ther Oncolytics*, 2020, 17: 421–430.

[110] Choo J, Tan WK, Luk L, et al. Abstract CT160: A phase I trial to evaluate allogeneic NKG2DL-targeting chimeric antigen receptor-grafted γδ T cells in subjects with advanced solid tumors or hematological malignancies (the ANGELICA trial)[J]. *Cancer Res*, 2024, 84(S7): CT160.

[111] Mukhametshin SA, Gilyazova EM, Davletshin DR, et al. Allogeneic NKG2D CAR-T cell therapy: A promising approach for treating solid tumors[J]. *Biomedicines*, 2025, 13(9): 2314.

[112] Ma P, Wang S, Ma W, et al. Targeting cancer leptomeningeal metastasis with allogeneic chimeric antigen receptor γδ T-cell therapy[J]. *J Clin Oncol*, 2025, 43(S16): 2533.

[113] Kirtane K, Park JC, Ulrickson M, et al. Logic-gated, allogeneic tmod chimeric antigen receptor T-cell (CAR T) therapy targeting epidermal growth factor receptor (EGFR) in advanced solid tumors with human leukocyte antigen (HLA) loss of heterozygosity (LOH): DENALI-1 trial[J]. *J Clin Oncol*, 2025, 43(S16): TPS2677.

[114] Simeone D, Morelli MP, Hecht JR, et al. 639 BASECAMP-1: Leveraging HLA loss of heterozygosity in solid tumors by NGS to identify patients with relapsed solid tumors for future CEA and MSLN Logic-gated Tmod™ CAR T-cell therapy[J]. *J Immunother Cancer*, 2022, 10(S2): A670.

[115] Sasu BJ, Lauron EJ, Schulz T, et al. Allogeneic CAR T cell therapy for cancer[J]. *Annu Rev Cancer Biol*, 2024, 8: 227–243.

[116] US Food and Drug Administration. Considerations for the development of chimeric antigen receptor (CAR) T cell products[R]. Silver Spring: US Food and Drug Administration, 2024.

[117] Sasu BJ, Opiteck GJ, Gopalakrishnan S, et al. Detection of chromosomal alteration after infusion of gene-edited allogeneic CAR T cells[J]. *Mol Ther*, 2023, 31(3): 676–685.

[118] Diorio C, Murray R, Naniong M, et al. Cytosine base editing enables quadruple-edited allogeneic CART cells for T-ALL[J]. *Blood*, 2022, 140(6): 619–629.

[119] Benjamin R, Graham C, Yallop D, 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[J]. *Lancet*, 2020, 396(10266): 1885–1894.

[120] Singh H, Huls H, Kebriaei P, et al. A new approach to gene therapy using *Sleeping Beauty* to genetically modify clinical-grade T cells to target CD19[J]. *Immunol Rev*, 2014, 257(1): 181–190.

[121] US Food and Drug Administration. Considerations for the development of chimeric antigen receptor (CAR) T cell products[R]. Silver Spring: US Food and Drug Administration, 2024.

[122] Khaliulin M, Valiullina A, Petukhov A, et al. Breaking the shield of solid tumors: A combined approach for enhanced efficacy of CAR-T cells[J]. *Cancer Immunol Immunother*, 2024, 74(1): 3.

[123] Al-Haideri M, Tondok SB, Safa SH, et al. CAR-T cell combination therapy: The next revolution in cancer treatment[J]. *Cancer Cell Int*, 2022, 22(1): 365.

[124] Muranski P, Boni A, Wrzesinski C, et al. Increased intensity lymphodepletion and adoptive immunotherapy —how far can we go?[J]. *Nat Clin Pract Oncol*, 2006, 3(12): 668–681.

[125] Hu B, Nastoupil LJ, Holmes H, et al. A CRISPR-edited allogeneic anti-CD19 CAR-T cell therapy with a PD-1 knockout (CB-010) in patients with relapsed/refractory B cell non-Hodgkin lymphoma (r/r B-NHL): Updated phase 1 results from the ANTLER trial[J]. *J Clin Oncol*, 2024, 42(S16): 7025.

[126] Lv Y, Luo X, Xie Z, et al. Prospects and challenges of CAR-T cell therapy combined with ICIs[J]. *Front Oncol*, 2024, 14: 1368732.

[127] Carneiro B, Garmezy B, Hamm JT, et al. Abstract CT275: Phase 1 clinical update of allogeneic invariant natural killer T cells (iNKTs), agenT-797, alone or in combination with pembrolizumab or nivolumab in patients with advanced solid tumors[J]. *Cancer Res*, 2023, 83(S8): CT275.

[128] Tian Y, Xie D, Yang L. Engineering strategies to enhance oncolytic viruses in cancer immunotherapy[J]. *Signal Transduct Target Ther*, 2022, 7(1): 117.

[129] Ponterio E, Haas TL, De Maria R. Oncolytic virus and CAR-T cell therapy in solid tumors[J]. *Front Immunol*, 2024, 15: 1455163.

[130] Park A, Monroy I, Cook C, et al. 847 CF33-CD19t oncolytic virus (onCARlytics) in combination with off-the-shelf allogeneic CyCART-19 T cells targeting de novo CD19+ solid tumors[J]. *J Immunother Cancer*, 2022, 10(S2): A885.

[131] Maldini CR, Messana AC, Bendet PB, et al. Immunosuppressant therapy averts rejection of allogeneic *FKBP1A*-disrupted CAR-T cells[J]. *Mol Ther*, 2024, 32(10):

3485–3503.

[132] Li YR, Zhu Y, Halladay T, et al. In vivo CAR engineering for immunotherapy[J]. *Nat Rev Immunol*, 2025, 25(10): 725–744.

[133] Wilson B, Geetha KM. Lipid nanoparticles in the development of mRNA vaccines for COVID-19[J]. *J Drug Deliv Sci Technol*, 2022, 74: 103553.

[134] Billingsley MM, Gong N, Mukalel AJ, et al. In vivo mRNA CAR T cell engineering via targeted ionizable lipid nanoparticles with extrahepatic tropism[J]. *Small*, 2024, 20(11): 2304378.

[135] Zhou J, Sun L, Jia Y, et al. Lipid nanoparticles produce chimeric antigen receptor T cells with interleukin-6 knockdown in vivo[J]. *J Controlled Release*, 2022, 350: 298–307.

[136] Li YR, Zhu Y, Halladay T, et al. In vivo CAR engineering for immunotherapy[J]. *Nat Rev Immunol*, 2025, 25(10): 725–744.

[137] Smith TT, Stephan SB, Moffett HF, et al. *In situ* programming of leukaemia-specific T cells using synthetic DNA nanocarriers[J]. *Nat Nanotechnol*, 2017, 12(8): 813–820.

[138] Zielińska A, Carreiró F, Oliveira AM, et al. Polymeric nanoparticles: Production, characterization, toxicology and ecotoxicology[J]. *Molecules*, 2020, 25(16): 3731.

[139] Parayath NN, Stephan SB, Koehne AL, et al. In vitro-transcribed antigen receptor mRNA nanocarriers for transient expression in circulating T cells in vivo[J]. *Nat Commun*, 2020, 11(1): 6080.

[140] Short L, Holt RA, Cullis PR, et al. Direct *in vivo* CAR T cell engineering[J]. *Trends Pharmacol Sci*, 2024, 45(5): 406–418.

[141] Agarwalla P, Ogunnaike EA, Ahn S, et al. Bioinstructive implantable scaffolds for rapid in vivo manufacture and release of CAR-T cells[J]. *Nat Biotechnol*, 2022, 40(8): 1250–1258.

[142] Inamdar VV, Hao S, Stephan SB, et al. Biomaterial-based scaffolds for direct in situ programming of tumor-infiltrating T lymphocytes[J]. *J Controlled Release*, 2024, 370: 310–317.

[143] Baek Y, Seo YR, Kim S, et al. From ex vivo to in vivo: Advances in lentiviral vector engineering for CAR-T therapy[J]. *Immune Netw*, 2026, 26(1): e13.

[144] Michels KR, Sheih A, Hernandez SA, et al. Preclinical proof of concept for VivoVec, a lentiviral-based platform for in vivo CAR T-cell engineering[J]. *J Immunother Cancer*, 2023, 11(3): e006292.

[145] Garcia J, Dehner C, Teoh J, et al. A phase 1, multicenter, open-label study of UB-VV111 in combination with Rapamycin in relapsed/refractory CD19+ B-cell malignancies[J]. *Blood*, 2024, 144(S1): 1750.1.