Revista Colombiana de
Hematología y Oncología
Biología molecular tumoral: de la básica a la clínica • Volumen 13 - Número 1
Suplemento 1

REVISIÓN

CAR-T Therapy: molecular fundamentals, clinical challenges, and emerging perspectives

Terapia CAR-T: fundamentos moleculares, retos clínicos y nuevas perspectivas

»Bonell Patiño-Escobar[1]

[1] Department of Laboratory Medicine, University of California San Francisco, San Francisco, CA, USA.

Recibido el 1 de octubre de 2025. Aceptado el 23 de diciembre de 2025

https://doi.org/10.51643/22562915.829

Abstract

Introduction: chimeric antigen receptor (CAR)-T cell therapy has transformed the treatment of hematologic malignancies, establishing a new paradigm for personalized cellular immunotherapy. Despite remarkable clinical success, its broader application remains limited by biological, logistical, and safety-related challenges.

Methods: this review synthesizes current evidence on the molecular foundations of CAR-T cell biology, integrating insights from preclinical studies and clinical trials. We analyze receptor design, co-stimulatory signaling, manufacturing strategies, and emerging engineering approaches aimed at improving efficacy and safety.

Results: advances in CAR design and manufacturing have led to multiple FDA approvals in B-cell leukemias, lymphomas, and multiple myeloma. However, key obstacles persist, including antigen escape, T-cell exhaustion, limited persistence, neurotoxicity, and on-target/off-tumor effects. Emerging strategies—such as genome editing, allogeneic and in vivo CAR-T generation, transcriptional and metabolic reprogramming, and synthetic biology circuits (including SynNotch, SNIPR, and logic-gated CARs)—are demonstrating promise in overcoming these limitations. In parallel, conformation-specific target discovery and the use of natural ligands are expanding the scope of actionable antigens.

Discussion: collectively, these innovations are reshaping CAR-T therapy into a more modular,

*** Autor para correspondencia:** Bonell Patiño Escobar, MD. Department of Laboratory Medicine, University of California San Francisco, San Francisco, CA, USA.
Dirección: 185 Berry Street Bldg B, #2410, San Francisco CA 94107.
Correo electrónico: orlando.patinoescobar@ucsf.edu
https//doi.org/10.51643/22562915.829

programmable, and controllable platform. By addressing resistance mechanisms and toxicity, next-generation CAR designs aim to improve durability and safety while enabling more precise immune activation.

Conclusion: continued integration of molecular engineering, systems biology, and synthetic immunology is poised to expand the therapeutic reach of CAR-T cells beyond hematologic malignancies, opening new opportunities in solid tumors and immune-mediated diseases.

Keywords: single-chain antibodies; cytokine release syndrome; immunotherapy; tumor microenvironment; cell- and tissue-based therapy.

Resumen

Introducción: la terapia con células T con receptor de antígeno quimérico (CAR-T) ha revolucionado el tratamiento de las neoplasias hematológicas, estableciendo un nuevo estándar de atención en la inmunoterapia celular personalizada. No obstante, pese a su notable éxito clínico, su aplicación generalizada continúa limitada por desafíos logísticos, biológicos y de seguridad.

Métodos: en esta revisión se sintetizan los fundamentos moleculares de la biología de las células CAR-T a partir de evidencia preclínica y clínica. Se analizan los avances en el diseño de los receptores, la señalización coestimuladora y los procesos de fabricación, así como los mecanismos implicados en la resistencia terapéutica y la toxicidad.

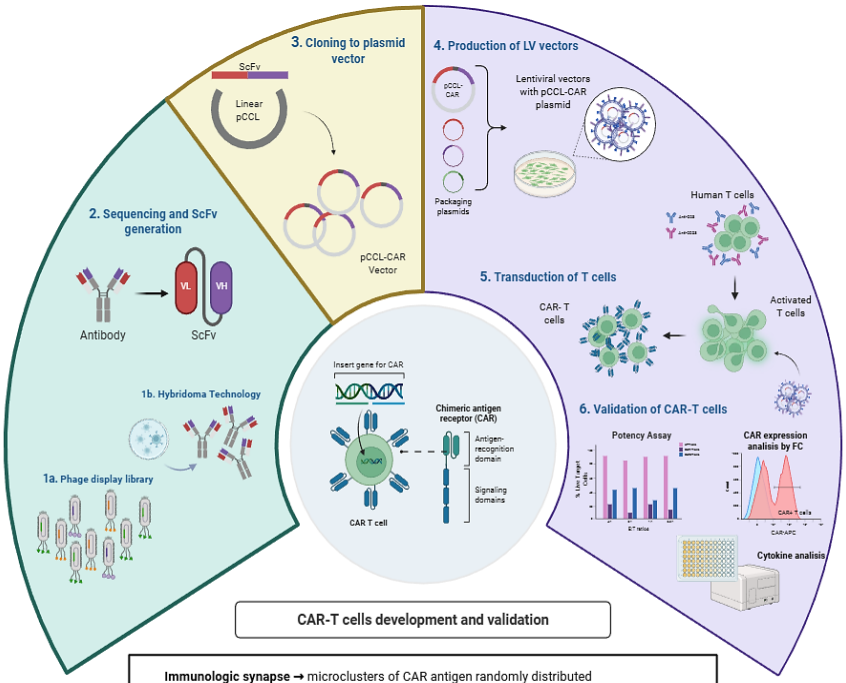
Resultados: las mejoras iterativas en el diseño y la manufactura de CAR-T han incrementado significativamente la eficacia y la seguridad, conduciendo a múltiples aprobaciones de la FDA en leucemias de células B, linfomas y mieloma múltiple. Sin embargo, persisten limitaciones clave, incluyendo los largos tiempos de fabricación, el escape antigénico, el agotamiento de las células T, la persistencia limitada, la neurotoxicidad y la toxicidad *on-target/off-tumor*. Estrategias emergentes como la edición genómica, la generación de CAR-T alogénicas e *in vivo*, la reprogramación transcripcional y metabólica, los circuitos de biología sintética (SynNotch, SNIPR y CAR con compuertas lógicas), así como el descubrimiento de dianas conformacionales específicas y el uso de ligandos naturales, están mostrando un potencial prometedor para superar estas barreras.

Discusión: en conjunto, estos avances están transformando la terapia CAR-T en una plataforma más modular, programable y controlable, con mayor capacidad para abordar los mecanismos de resistencia y reducir la toxicidad asociada.

Conclusión: estas innovaciones anuncian una nueva era de inmunoterapias celulares más seguras y versátiles, con el potencial de expandir el impacto de la ingeniería de CAR más allá de las neoplasias hematológicas, incluyendo tumores sólidos y enfermedades inmunomediadas.

Palabras clave: anticuerpos de cadena única; síndrome de liberación de citoquinas; inmunoterapia adoptiva; microambiente tumoral; tratamiento basado en trasplante de células y tejidos.

Graphical abstract



Key points

- Chimeric antigen receptor (CAR) engineered cellular immunotherapy offers the potential for precise targeting and elimination of tumor cells, providing a tailored approach to cancer treatment.
- As clinical trials of CAR-T cells progress, there is increasing examination of significant adverse effects such as central neurotoxicity, cytokine release syndrome (CRS), hematopoietic suppression, and infections. Furthermore, the efficacy of CAR-T therapies in targeting solid tumors remains a significant challenge. Consequently, scientists are exploring alternative immunotherapeutic approaches, such as CAR-engineered natural killer cells (CAR-NK) and CAR-macrophages (CAR-M), which utilize diverse cell types, including autologous, allogeneic, xenogeneic, and transgenic cells, to trigger distinct anti-cancer immune responses.
- T cell activation necessitates two critical signals. The first is an antigen-specific signal, which is triggered when the T cell receptor (TCR) recognizes a specific peptide presented by the major histocompatibility complex (MHC) on antigen-presenting cells. The second is a co-stimulatory signal, typically mediated by the interaction between CD28 on T cells and its ligands, B7.1 (CD80) or B7.2 (CD86), on antigen-presenting cells. Antigen recognition and binding are facilitated by the two highly variable chains that make up the TCR. One fraction, γδT cells, consists of a γ chain and a δ chain, while the majority of mature T cells, called αβT cells, are made up of an α and a β chain. CAR holds specific advantages over TCR. Unlike the αβT cell, which requires MHC-dependent recognition of antigens, CAR can operate independently of MHC, targeting both protein and non-protein molecules expressed on the cell surface to activate T cell effector functions

Introduction and molecular fundamentals

Chimeric antigen receptor (CAR)-T cell therapy has emerged over the past decade as one of the most transformative approaches in cancer treatment[1]. It represents the convergence of basic immunology, genetic engineering, and advances in cellular bioprocessing[1,2]. The fundamental premise is to harness the natural ability of T lymphocytes to recognize and kill abnormal cells and enhance it by introducing a synthetic receptor specifically designed to target tumor-associated antigens[1–4].

Under physiological conditions, antitumor immunity by T lymphocytes depends on recognition of antigens through the T cell receptor (TCR). The TCR, composed of α and β chains associated with the CD3 complex, recognizes peptide fragments derived from tumor proteins presented on major histocompatibility complex (MHC) molecules. These peptides may be displayed by professional antigen-presenting cells (APCs), which prime and activate T cells, or directly by tumor cells through their own MHC class I expression[5]. However, many tumors evade this immune surveillance by downregulating MHC expression, secreting immunosuppressive cytokines, or expressing inhibitory ligands that block T cell activation[5,6]. These mechanisms limit the effectiveness of natural immune responses and provide the rationale for alternative strategies.

Chimeric antigen receptors were designed to overcome these barriers. Their architecture usually integrates an antibody-derived single-chain variable fragment (scFv) with the intracellular signaling domains of T lymphocytes[7]. This structure combines the antigen-recognition specificity of antibodies with the activation machinery of T cells[1,2,5,7]. A CAR typically consists of: a) an extracellular antigen-binding domain, most often an scFv; b) a hinge/spacer that modulates reach and synapse geometry; c) a transmembrane domain that anchors the receptor; and d) an intracellular domain containing CD3ζ, which initiates TCR-like signaling. More advanced CAR designs incorporate co-stimulatory domains derived from molecules such as CD28 or 4-1BB, which reinforce expansion and persistence of the engineered cells[1,3,8]. Hinge length and composition (e.g., IgG1 CH2–CH3 vs. CD8α hinges) and the choice of transmembrane segment (CD28 vs. CD8α) can significantly influence antigen access, tonic signaling, and sensitivity to antigen density, key determinants of efficacy and toxicity[9].

The design of CARs has evolved through multiple "generations". First-generation CARs contained only the CD3ζ chain, resulting in incomplete activation and limited persistence in vivo. Second-generation CARs, which form the basis of most currently approved therapies, add a single co-stimulatory domain (CD28 or 4-1BB). Third-generation CARs combine two co-stimulatory domains to balance expansion and persistence; however, some constructs exhibit excessive tonic signaling if not finely tuned. Fourth-generation CARs, also called TRUCKs (T cells redirected for universal cytokine killing) or "armored CARs", encode inducible payloads (e.g., IL-12, IL-18, IL-7/CCL19) that remodel the tumor microenvironment, recruit host immunity, and potentially sustain CAR-T fitness[1]. A fifth-generation CAR has recently been proposed, integrating three synergistic signals: TCR/CD3ζ activation, CD28 costimulation, and cytokine-mediated JAK–STAT3/5 signaling[1,2].

At the molecular level, CAR activation assembles a signaling complex reminiscent of the physiological immune synapse. Lck-mediated phosphorylation of CD3ζ ITAMs triggers ZAP-70 recruitment and downstream MAPK/NF-κB/NFAT cascades. Co-stimulatory domains shape this signaling "tone", CD28 drives brisk effector differentiation and rapid early expansion, while

4-1BB enriches oxidative phosphorylation and mitochondrial biogenesis, often translating into greater long-term persistence[3,5]. However, chronic signaling in the absence of appropriate rest can induce epigenetic fixation of an exhausted state, with upregulation of NR4A/TOX programs and diminished cytotoxicity[3,10].

The clinical impact of these innovations is undeniable. From the first reports in the 1990s to the present day, CAR-T therapies have dramatically altered the prognosis of diseases once considered untreatable, such as relapsed/refractory acute lymphoblastic leukemia and diffuse large B-cell lymphoma[2,8,11,12]. More recently, success has extended to multiple myeloma with the approval of ciltacabtagene autoleucel (cilta-cel)[13] and even to select solid tumors such as synovial sarcoma[14]. These advances reflect not only the power of technology but also the speed at which laboratory discoveries can be translated into clinical practice.

In this narrative literature review, we examine the basic molecular foundations of CAR-T therapy, which integrates the antibody-like specificity of engineered receptors with the cytotoxic capacity of T lymphocytes. This strategy has overcome several intrinsic limitations of the physiological TCR; however, major challenges remain, particularly regarding safety, efficacy in solid tumors, delayed manufacturing, limited persistence and expansion, and the long-term durability of responses. These challenges, explored in the following sections, represent the current focus of research and will shape the future of personalized cellular therapy.

CAR-T cell therapy: current landscape and FDA approvals

Chimeric antigen receptor T cells (CAR-T cells) are autologous or, in investigational settings, allogeneic T lymphocytes that have been genetically modified to express synthetic receptors designed to target specific tumor-associated antigens. Unlike the physiological TCR, which requires peptide presentation on MHC molecules, CARs recognize surface antigens in an MHC-independent manner. This property allows CAR-T therapy to circumvent one of the main mechanisms of tumor immune evasion: loss or downregulation of MHC expression[3–5].

The process of generating a CAR-T product begins with leukapheresis to collect the patient's peripheral blood mononuclear cells. T cells are then isolated and genetically engineered ex vivo using viral vectors (such as lentivirus, retrovirus, or Adeno-associated virus [AAV]) or, more recently, non-viral platforms like transposons and CRISPR-based approaches. These modified cells are expanded in culture under conditions that promote viability and functionality, cryopreserved, and ultimately reinfused into the patient following lymphodepleting chemotherapy. Between engineering and infusion, quality-control assays (vector copy number, identity/purity, sterility, replication-competent virus, potency) and release criteria ensure product consistency and safety[15]. The infusion is designed to establish a population of tumor-specific effector cells capable of expanding in vivo and mediating durable antitumor responses.

CAR-T therapies have been under investigation during the last 30 years[2,8]. After decades of refinement, the U.S. Food and Drug Administration (FDA) granted its first approval in 2017 for tisagenlecleucel (Kymriah), targeting CD19 in pediatric and young adult patients with relapsed or refractory B-cell acute lymphoblastic leukemia[16]. This landmark approval was followed by axicabtagene ciloleucel (Yescarta) for adult patients with diffuse large B-cell lymphoma[17], and subsequently lisocabtagene maraleucel (Breyanzi)[18] and brexucabtagene autoleucel (Tecartus) for mantle cell lymphoma[19]. Across

these products, differences in co-stimulation (CD28 vs 4-1BB), vector systems, manufacturing methods, and strategies contribute to distinct expansion kinetics, toxicity profiles, and durability[3].

The landscape of approvals has continued to expand. In 2021, idecabtagene vicleucel (idecel, Abecma) became the first CAR-T product approved for relapsed or refractory multiple myeloma[20] by targeting B-cell maturation antigen (BCMA). This was followed by ciltacabtagene autoleucel (cilta-cel, Carvykti) in 2022[13,21,22], which demonstrated very high overall response rates and depth of remission in heavily pretreated myeloma patients. In 2024, afamitresgene autoleucel (Tecelra) became the first CAR-T product approved outside hematologic malignancies, specifically for advanced synovial sarcoma[14]. Collectively, as of early 2025, seven CAR-T therapies have been approved by the FDA, with dozens more under clinical evaluation for both hematologic and solid tumors.

Despite these successes, all currently approved CAR-T products are autologous. This approach ensures immunologic compatibility but introduces challenges such as variability in product quality, lengthy manufacturing times (often three to four weeks), and limited access for patients with rapidly progressing disease[15]. These constraints have fueled interest in "off-the-shelf" allogeneic CAR-T therapies derived from healthy donors, which may allow for faster administration and broader accessibility. Early-phase trials are actively exploring this approach, though issues such as graft-versus-host disease (GvHD), host-versus-graft rejection, and host immune clearance remain significant obstacles[2]. Off-the-shelf natural killer (CAR-NK) products are also emerging, with the advantage of minimal GvHD risk and batch manufacturing potential, making them an attractive parallel path to broaden access[23].

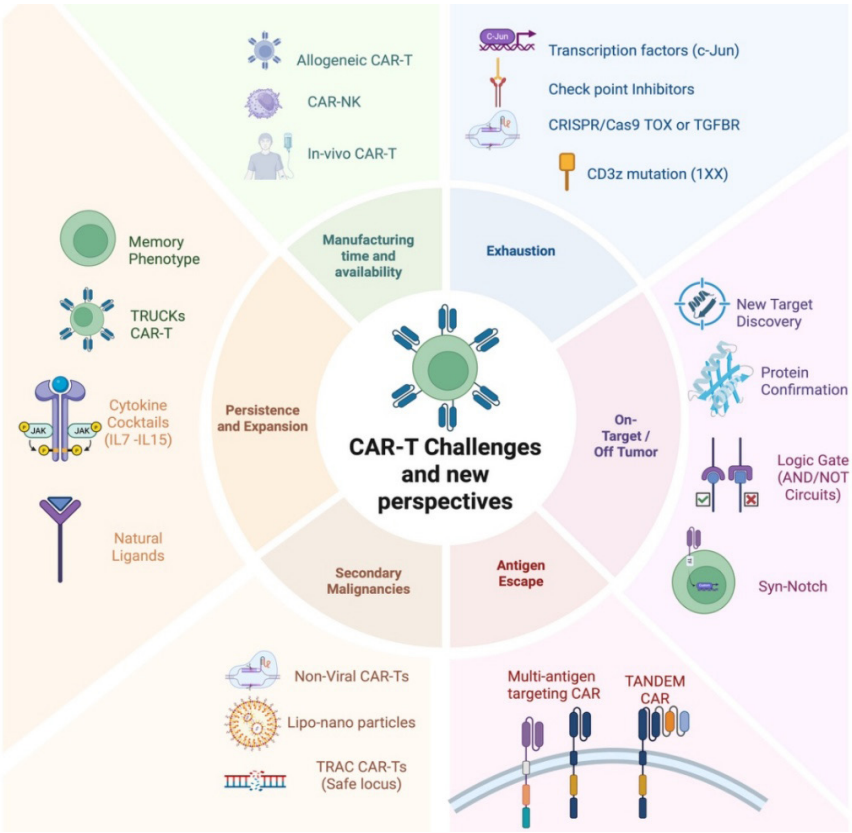
The rapid trajectory of CAR-T approvals reflects both the promise and the complexity of cellular immunotherapy. Each product has contributed unique insights into efficacy, toxicity, and durability, shaping the field's understanding of how CAR design and manufacturing processes influence clinical outcomes. However, the approvals also underscores that CAR-T therapy is not a uniform solution; rather, it is a platform that continues to evolve in response to new challenges, including antigen escape, T cell exhaustion, and safety-related toxicities.

Current challenges of CAR-T Therapy, and emerging perspectives

Although CAR-T therapy has transformed outcomes for many hematologic malignancies, its widespread implementation faces major limitations. These challenges span from logistical barriers in manufacturing to biological hurdles such as antigen escape and T-cell exhaustion (Figure 1).

Figure 1.

Barriers in manufacturing CAR-T cells.



Manufacturing time and availability

The generation of autologous CAR-T products is a complex, multi-step process that requires leukapheresis, T-cell activation, genetic modification, and ex vivo expansion before reinfusion. This procedure typically takes two to four weeks in specialized GMP facilities, a time frame that can be prohibitive for patients with rapidly progressing disease[15]. In addition, manufacturing success depends on the quality and quantity of T cells collected. Patients who are heavily pretreated or lymphopenic often yield poor collections, further delaying or precluding treatment. Bridging therapy (e.g., steroids, targeted agents, localized radiation) is frequently required, adding clinical complexity and risk of immunosuppression that may blunt CAR-T expansion[24].

The logistical challenges extend beyond the laboratory. CAR-T therapy requires highly specialized infrastructure, trained personnel, and coordination across collection centers, manufacturing facilities, and infusion sites. These demands restrict access to academic medical centers and limit treatment availability in many regions worldwide[8]. Real-world data indicate that time-to-infusion and manufacturing attrition (e.g., out-of-spec product, disease progression during the wait) are substantial barriers to equitable access, particularly in community settings[25]. To address these limitations, several strategies are being pursued. One is the development of "off-the-shelf" allogeneic CAR-T cells derived from healthy donors, which could be

manufactured in bulk and stored for on-demand use[15]. Genome editing (e.g., TRAC knockout, β2-microglobulin knockout, CD52 knockout, etc.) can minimize alloreactivity and reduce host rejection, while safety features (e.g., suicide switches) mitigate risks[26–28]. In parallel, off-the-shelf CAR-NK cells are being developed; NK cells do not cause GvHD and are naturally suited for allogeneic application, enabling batch production, cryopreservation, and immediate use[23]. Early-phase studies have shown that CAR-NK products can be safely infused with low rates of cytokine release syndrome (CRS) and neurotoxicity, and they may be combined with cytokine support (e.g., membrane-bound IL-15) or antibody therapy for enhanced persistence [29,30].

An even more radical approach involves in vivo CAR-T generation, where nanoparticles or viral vectors deliver CAR constructs directly into T cells inside the patient. Lymphodepleting conditioning regimens are incompatible with this therapeutic approach, as the presence of pre-existing immune cells is required for efficacy. Some vectors are engineered to enhance cellular uptake, improve target specificity, or modulate the function of the engineered immune cells. In contrast to lentiviral vectors, CAR mRNA constructs are designed to confer only transient expression and activity [31].

Recent preclinical studies with the evolved AAV capsid Ark313 demonstrated highly efficient in vivo transduction and CRISPR-based editing of circulating and tissue-resident T cells, including targeted integration into the TRAC locus through homology-directed repair (HDR) and homology-independent targeted integration (HITI)[32]. In these models, T cells could be programmed in vivo to express therapeutic CARs, bypassing ex vivo manipulation and collapsing vein-to-vein times from weeks to a single infusion[31]. Complementary clinical-stage strategies, including enveloped delivery vehicles that engineer human T cells in vivo are being evaluated and suggest feasibility and rapid kinetics. Hamilton et al., unlike to traditional vectors such as adeno-associated viruses, which depend on naturally evolved capsid tropisms for cargo delivery, Cas9-packaging enveloped delivery vehicles (Cas9-EDVs) utilize defined antibody–antigen interactions to achieve transient and selective transfer of genome-editing components to specific cell types. Antibody-guided Cas9-EDVs preferentially mediate genome editing in target cells while minimizing effects on bystander cells in mixed populations, both ex vivo and in vivo. Through multiplexed targeting of human T cells, Cas9-EDVs enable the in vivo generation of genome-edited chimeric antigen receptor T cells in humanized mice, representing a programmable and versatile delivery platform with broad therapeutic potential [33]. More recently Xu et al., reported 4 patients with diagnosis of multiple myeloma, were treated with a BCMA CAR-T (ESO-T01), a nanobody-directed, immune-shielded lentiviral vector designed for in vivo T-cell engineering, incorporating a humanized anti–BCMA single-domain antibody CAR. To overcome the broad tropism characteristic of lentiviral vectors, key residues in the vesicular stomatitis virus glycoprotein G were mutated. The viral envelope was further engineered to overexpress CD47, a well-known "Don't eat me" molecule, thereby reducing clearance by the mononuclear phagocytic system, and to display an anti–T-cell receptor nanobody for selective T-cell targeting. Moreover, major histocompatibility complex class I molecules were deleted to minimize immunogenicity[34] Those in vivo approaches would allow a more widely access to patients, minimizing time to transduced T-cels, without the need of complex infrastructure for CAR-T cell manufacturing since it could be centralized to the facilities which provide the delivery vehicles as "off the shelf" therapy.

CAR-T Cell Exhaustion

A major barrier to long-term efficacy is the development of T-cell exhaustion, a dysfunctional state characterized by reduced proliferation, impaired cytokine secretion, and loss of cytotoxicity. Exhaustion can arise from chronic antigen stimulation, tonic signaling from the CAR itself, or exposure to inhibitory ligands in the tumor microenvironment[3]. Even strong ligands in scFv-based CAR-T cells, when exhibiting excessively high affinity, have been described as a cause of exhaustion through antigen-independent aggregation[35,36]. This finding has led to a growing focus on optimizing ligand affinity to achieve potent antigen binding without inducing tonic signaling and subsequent exhaustion[35]. Epigenetically, exhausted CAR-T cells acquire stable chromatin landscapes and transcriptional programs dominated by the NR4A and TOX families, which are difficult to reverse once established[10,37]. Functionally, exhausted T cells lose robust effector activity, express multiple inhibitory receptors, and display an altered transcriptional program, ultimately leading to inefficient control of tumors[38].

Differences in co-stimulatory domains also affect exhaustion profiles. CAR-T cells with CD28 domains expand rapidly but often decline in persistence, whereas those with 4-1BB domains show slower proliferation but improved long-term persistence and longer-term survival, and exerted enhanced anti-tumor effects at lower infusion doses[39]. CD28-based CARs once expanded, differentiated into effector memory cells, and had increased glycolytic metabolism-features related to PI3K/AKT/Glycolytic pathways, associated with robust and rapid expansion but short-lived responses and reduced in vivo persistence[39,40]. 4-1BB, in contrast, enhances NF-κB signaling, especially the non-canonical pathway, which is critical for survival and sustained expansion[39]. Multiple preclinical and clinical studies show 4-1BB CAR-Ts cells maintain central memory phenotypes and avoid exhaustion compared to CD28 counterparts[41]. Sustained co-stimulatory signaling, however, can paradoxically drive exhaustion if not properly balanced[42]. Beyond co-stimulation, the antigen context matters; low antigen density and imperfect synapse geometry can trigger sub-threshold chronic signaling, nudging CAR-T cells toward dysfunction[35,39,43–46].

While CD28-based CAR-T cells exhibit rapid activation, expansion, and potent effector function, their strong and sustained signaling can accelerate exhaustion, particularly due to overlapping CD28 and CD3ζ pathways and structural constraints inherent to second-generation CARs[47]. To overcome this, Feucht et al. engineered a series of CARs with calibrated activation potential by selectively mutating the immunoreceptor tyrosine-based activation motifs (ITAMs) within CD3ζ. Among these, the 1XX design (retaining only the most membrane-proximal ITAM) proved superior, promoting balanced effector and memory differentiation, reduced exhaustion, and enhanced persistence compared to the conventional 1928ζ CAR[48]. This study demonstrated that tuning CAR signaling strength at the ITAM level can optimize therapeutic efficacy by minimizing tonic activation while maintaining robust anti-tumor activity, thereby redefining the design principles of CD28-based CAR architecture.

Mechanistic and engineering strategies to prevent or reverse CAR-T exhaustion include: a) enforcing transcription factors such as c-Jun expression to counteract AP-1 imbalance and preserve effector function, including in solid tumor models[36,49,50]; b) disrupting NR4A or TOX programs to sustain functionality[10,51,52]; c) combining with checkpoint inhibitors (anti-PD-1, anti-LAG-3) to relieve extrinsic inhibitory signaling[53,54] or manipulation on the TGF-b which

is highly expressed in the tumor microenvironment, attenuating the immune response by suppressing T cell activation and proliferation. TGF-b signaling blockade have demonstrated enhanced antitumor function of CAR T cells through the knockdown of TGFBR[55]; d) metabolic reprogramming to enhance mitochondrial mass, spare respiratory capacity, and oxidative phosphorylation[55]. TGF-b-mediated mTOR inhibition preserves the cellular metabolism of precursors of exhausted T cells, which both limits and sustains long-term T cell responses[56], and e) transient pharmacologic "resting" with kinase inhibitors such as Dasatinib to reduce tonic signaling during manufacturing or early post-infusion[57–59].

Emerging evidence suggests that naturally occurring T-cell mutations enhancing proximal signaling and cellular fitness can be harnessed to improve engineered T-cell therapies without exacerbating exhaustion, highlighting the potential of genotype-informed donor selection or targeted editing. A notable example is the CARD11–PIK3R3 gene fusion identified in $CD4^+$ cutaneous T-cell lymphoma, which augments CARD11–BCL10–MALT1 complex signaling and enhances the anti-tumor efficacy of therapeutic T cells in several immunotherapy-refractory models in an antigen-dependent manner. Importantly, CARD11–PIK3R3-expressing cells were monitored for up to 418 days post-transfer without signs of malignant transformation, underscoring the potential safety of this approach. Garcia and Daniels et al., demonstrated that leveraging naturally evolved T-cell mutations represents a promising strategy to explore the upper boundaries of T-cell biology and translate evolutionary adaptations from malignant contexts into next-generation cellular therapies, addressing key challenges such as limited proliferation and exhaustion[60].

On-Target / Off-Tumor toxicity and neurotoxicity

One of the inherent risks of CAR-T therapy is its inability to distinguish malignant from normal cells if both express the targeted antigen. This "on-target, off-tumor" recognition can lead to destruction of healthy tissues, sometimes with severe or life-threatening consequences[61]. For example, targeting antigens such as HER2 or GD2 in solid tumors has caused toxicity in normal tissues expressing low levels of these[62–65]. Even in hematologic cancers, some targets such as CD19 or BCMA are also expressed on normal B-lineage cells, leading to predictable but clinically significant effects like B-cell aplasia and hypogammaglobulinemia[66–69]. While B-cell aplasia is managed with immunoglobulin replacement and antimicrobial prophylaxis[68,69], other antigens (e.g., CD123 in certain contexts) have produced intolerable and sometimes fatal toxicities when targeted without additional safeguards[61,70,71]. A related and clinically prominent adverse effect in current FDA-approved CAR-Ts is neurotoxicity (either for CD19 or BCMA), also referred to as immune effector cell–associated neurotoxicity syndrome (ICANS). ICANS can occur early or in a delayed fashion and manifests such as encephalopathy, aphasia, tremors, seizures, or cerebral edema. Some mechanisms likely include endothelial activation, blood–brain barrier disruption, since Single-cell RNA sequencing analysis shows that CD19, primarily considered as a B cell-specific surface antigen, is expressed in human brain mural cells that are critical for blood-brain-barrier integrity, suggesting that this cell population may contribute to the neurotoxicity of CD19-directed immunotherapy including CAR-T, and high cytokine flux rather than direct CAR infiltration alone. Risk factors include high disease burden, brisk expansion/peak CAR-T levels, and severe CRS. Management centers on supportive care, corticosteroids for moderate–severe ICANS, and IL-6 pathway blockade primarily for CRS. Delayed

neurotoxicities, including movement disorders with some BCMA CAR-Ts, have been described and may reflect off-tumor interactions in neural tissue or sustained inflammatory injury[72,73].

A major clinically significant adverse event associated with current FDA-approved CAR-T therapies (targeting either CD19 or BCMA) is neurotoxicity, commonly termed immune effector cell–associated neurotoxicity syndrome (ICANS)[72]. ICANS can arise early or in a delayed manner and is characterized by encephalopathy, aphasia, tremor, seizures, and, in severe cases, cerebral edema[58,73]. Proposed mechanisms include endothelial activation and blood–brain barrier (BBB) disruption, as supported by single-cell RNA sequencing studies showing that CD19, traditionally viewed as a B cell–specific antigen, is also expressed in human brain mural cells (a population essential for BBB integrity) implicating these cells in the neurotoxicity observed with CD19-directed immunotherapies, including CAR-T cells[74]. Additionally, a high cytokine flux, rather than direct CAR-T cell infiltration into the CNS, appears to contribute substantially to neurotoxicity[58,72,73]. Identified risk factors include high tumor burden, rapid CAR-T expansion, and the presence of severe cytokine release syndrome (CRS)[58]. Management relies on supportive care, with corticosteroids indicated for moderate to severe ICANS and IL-6 pathway blockade used primarily for concurrent CRS[58,72]. Furthermore, delayed neurotoxicities, such as movement disorders reported with some BCMA-directed CAR-Ts, have been documented and may reflect off-tumor interactions within neural tissue or persistent inflammatory injury and its management is less clear, with description intrathecal methotrexate-based therapy, intravenous (IV) cyclophosphamide, high-dose IVIG ≥1g/kg and dopamine agonists[61,68,73].

New strategies targeting tumor-specific antigens are needed to improve efficacy and overcome issues like antigen escape and tumor resistance. Current approaches used for target discovery include bulk transcriptome, single-cell RNA sequencing (scRNAseq), cell surface proteomics, and antibody-based proteomics[75]. These methods have successfully identified new therapeutic targets, aiding in the development of new therapies and advancing disease biology research. Additionally, artificial intelligence and machine learning are increasingly used to analyze large datasets from these techniques, further enhancing target discovery[76]. A recent study introduced cross-linked peptide proteomics as a powerful approach to identify conformational differences in proteins shared between tumors and normal tissues. Using this method, Mandal et al. demonstrated that Integrin β2 (ITGB2) (an integrin broadly expressed in normal hematopoietic and non-hematopoietic tissues) adopts a distinct open conformation in acute myeloid leukemia (AML) cells compared to its closed state in healthy cells. Through phage display screening, the authors identified antibodies selectively recognizing the tumor-specific epitope exposed in the open ITGB2 conformation. Incorporating one of these antibodies as the scFv domain of a CAR-T construct yielded potent and selective antitumor activity in preclinical AML models, including humanized mice, without evidence of toxicity in normal tissues. This work highlights a promising strategy for expanding the repertoire of safe immunotherapy targets by exploiting conformation-specific epitopes on otherwise ubiquitously expressed proteins[77].

Other mechanistic strategies to mitigate on-target/off-tumor effects include tuning CAR affinity to preferentially bind high-density antigens on tumor cells, designing dual-antigen "logic gate" CARs (AND/NOT circuits)[78,79], or employing inducible CARs that can be pharmacologically regulated or CAR T-cells that are responsive to a hypoxic environment, a hallmark of certain tumors[80]. Synthetic biology platforms

such as SynNotch receptors add a staged-activation layer: sensing Antigen A induces a transcription factor to express CAR against Antigen B, confining cytotoxicity to sites where both cues co-localize[81] . More recently, in a similar fashion, domains involved in regulated intramembrane proteolysis and showed that systematic modular engineering can generate a class of receptors that we call synthetic intramembrane proteolysis receptors (SNIPRs) that have tunable sensing and transcriptional response abilities[82]. Those synthetic T cells can be customized to deliver cytokines, antibodies, bi-specific antibodies in response to antigens in a very precise and localized way[81–83]. SNIPER and other multi-input circuits integrate positive and negative antigens, anatomical promoters, or hypoxia-responsive elements to sharpen specificity and reduce collateral toxicity[61,79–82].

Antigen escape

Even when CAR-T cells successfully eliminate tumor cells initially, relapse may occur due to antigen escape. Mechanisms include downregulation of the targeted antigen, splice variants that eliminate the epitope, lineage switching, or selective outgrowth of antigen-negative clones[84–86]. Antigen density thresholds are crucial: subthreshold expression can sustain CAR-T engagement insufficient to kill, yet sufficient to chronically stimulate and exhaust the product[46].

In multiple myeloma, BCMA loss is now recognized as a significant resistance mechanism. Large cohort studies show that monoallelic BCMA deletions are frequent at baseline and often co-occur with high-risk events such as TP53 deletions, predisposing patients to biallelic BCMA loss under therapeutic pressure[86,87]. Clinical observations confirm that relapses after BCMA CAR-T can occur via genetic deletions, promoter methylation, or altered trafficking leading to diminished surface expression[84–87].

To overcome this, multi-antigen targeting is being pursued. CAR-T cells have been engineered to recognize BCMA and GPRC5D, or CD19 and CD22 in B-cell malignancies, reducing the likelihood of tumor escape[88–91]. Phase 1 studies of GPRC5D-targeted CAR-T cells (MCARH109) demonstrated robust activity, including responses in patients previously treated with BCMA therapies, validating the antigen as an effective complementary target[92]. Tandem CARs and bispecific CARs increase the antigen threshold for escape even in solid tumor models[93,94], while sequential/conditional antigen targeting via SynNotch-SNIPER and switch-based CARs allows dynamic returning as tumors evolve[81–83,95].

Secondary malignancies

The possibility of therapy-related secondary cancers, particularly T-cell lymphomas arising after CAR-T treatment, has gained attention. In March 2024, the FDA reported 33 cases of secondary T-cell lymphomas among more than 30,000 treated patients, corresponding to an incidence of <1%[96]. While this risk appears low compared to the survival benefit of CAR-T therapy, it underscores the importance of long-term surveillance and careful vector design to minimize insertional mutagenesis and raises concern about the use of CAR-T in non-malignant diseases such as autoimmune diseases, which is an area of rapid research growing[97,98].

Mechanistically, case reports have linked CAR vector integration near oncogenes such as TP53 with clonal T-cell transformation, supporting insertional mutagenesis as a plausible contributor in rare cases[99]. In a different case, Whole-genome sequencing identified integration of the CAR vector within intron 7 of the TIA1 gene, accompanied by a loss of TIA1 protein expression confirmed by immunohistochemistry. This finding represents at least the second instance of CAR insertion involving

a known or potential tumor suppressor locus. Moreover, heterozygous truncating variants in TET2 and EZH2 (absent in the pre–CAR-T samples) were detected. These alterations, often associated with clonal hematopoiesis and T-cell lymphomagenesis, may have contributed to the observed malignant transformation. Unlike prior reports showing biallelic TET2 inactivation as a driver of T-cell proliferation and infiltration, the current case involved only a single-allele (heterozygous) mutation[100].

To reduce this risk, research is advancing in non-integrating vectors (e.g., mRNA, episomes) and in genome-editing strategies that insert CARs into safe-harbor loci such as T-Cell receptor α constant (TRAC) locus[101,102]. TRAC knock-in CAR-Ts generated by CRISPR-Cas9 not only show uniform CAR expression under the endogenous TCR promoter, reduced tonic signaling, improved phenotype/persistence, but also enhances T-cell potency, with edited cells vastly outperforming conventionally generated CAR T cells[102,103]. Coupling site-specific integration with suicide switches (e.g., iC9) adds a rapid pharmacologic off switch for rare malignant transformation or severe toxicity[104].

Persistence and expansion

Durable remission depends on the ability of CAR-T cells to persist long-term and maintain functional activity. However, persistence is variable across patients and products and CAR-T generations. Factors influencing persistence include co-stimulatory domain selection, manufacturing conditions (activation strength, cytokine milieu), memory subset composition, antigen burden/kinetics, and hostile tumor microenvironment[1,3]. Trafficking and tissue residency also matter, poor penetration into privileged sites (e.g., CNS, hypoxic cores) can limit sustained control[105].

Products enriched in T memory stem cells (CD45RA+ CCR7+ CD62L+) show superior expansion and long-term control[106,107]. Cytokines such as IL-7 and IL-15 support this phenotype, while fourth-generation TRUCKs engineered to secrete IL-7, IL-15, or IL-21 aim to enhance survival and expansion in vivo[108]. Nano-CARs, built with nanobody (VHH) scaffolds instead of scFvs, offer smaller, stable, less immunogenic binders capable of targeting recessed epitopes and potentially lowering tonic signaling[109,110]. Natural Ligand-based CARs, such as APRIL-CARs that co-target BCMA/TACI[111], and CD27-based constructs that leverage physiologic receptor–ligand biology, are being explored to reduce anti-idiotype responses and improve proliferation[112]. In contrast, several conventional scFv-based CARs utilize murine-derived antibody fragments, which can elicit immunogenicity and the formation of anti-CAR antibodies, though the precise impact of these responses on long-term CAR-T persistence remains unclear[113]. By leveraging endogenous ligand–receptor biology, natural ligand–based CARs may mitigate these immunogenicity concerns while preserving physiological binding characteristics. In preclinical and early translational work, CD27-based anti-CD70 CARs have shown striking in vivo proliferative advantages, on the order of >80-fold expansion compared with some control designs, while maintaining potent activity against myeloma models[112]. Another example of a ligand-based CAR design is the CCL27–CAR-T, which targets CCR10 and demonstrates preclinical efficacy comparable to the best-in-class myeloma CAR-T, ciltacabtagene autoleucel (cilta-cel)[114,115].

To tackle safer, smarter control of CAR-T activity, persistence and expansion. Chen et al. develop a CRISPR knock-in strategy that "rewires" endogenous, tumor-activated promoters (notably NR4A2 and RGS16) to drive payloads like IL-12 or IL-2 only where CAR-T cells encounter tumor cues, boosting efficacy and survival in mouse

models while avoiding peripheral toxicity; the approach outperforms a synthetic NFAT promoter and is compatible with clinical-style manufacturing of human CAR-T cells[116]. In parallel, Edelstein et al. convert natural cytokine receptors into orthogonal MESA biosensors (NatE MESA) that sense soluble TME signals (e.g., VEGF, IL-10) and trigger user-defined transcriptional programs, including logic-gated circuits that can conditionally support CAR-T function, offering a modular way to couple environmental inputs to precise genetic outputs in engineered T cells[117].

Conclusions

CAR-T cell therapy has rapidly progressed from a conceptual innovation in the 1990s to a transformative treatment modality that is reshaping the landscape of cancer care. Its success in hematologic malignancies such as B-cell acute lymphoblastic leukemia, diffuse large B-cell lymphoma, mantle cell lymphoma, and multiple myeloma demonstrates the power of combining the antibody-like specificity of synthetic receptors with the cytotoxic potency of T lymphocytes. The expanding series of FDA approvals, culminating in the first approval for a solid tumor in 2024, underscores the therapeutic potential of this platform and highlights the pace of translational advances in the field.

Nevertheless, significant obstacles remain. The six central challenges, delayed manufacturing and limited availability, T-cell exhaustion, on-target/off-tumor toxicity (including neurotoxicity), antigen escape, therapy-related secondary malignancies, and inconsistent persistence and expansion, illustrate the complexity of engineering living therapies. Each of these barriers has both a mechanistic basis and a clinical impact, shaping outcomes for patients and influencing the safety, accessibility, and durability of CAR-T therapy.

The field's response to these challenges has been a remarkable wave of innovation. Advances in manufacturing and delivery, such as allogeneic products, CAR-NK platforms, and in vivo CAR-T generation, promise to shorten treatment times and broaden patient access. At the molecular level, strategies to prevent exhaustion, including c-Jun overexpression, transcriptional/epigenetic rewiring (NR4A/TOX), metabolic optimization, and intermittent kinase-inhibitor "rest," are aimed at sustaining long-term activity. Synthetic biology approaches, including logic-gated CARs, SynNotch circuits, SNIPER designs, protease-activated "masked" CARs, and drug-tunable CARs, offer powerful tools to reduce toxicity and improve precision. Multi-antigen targeting and adaptive CAR designs address antigen escape and tumor heterogeneity, while safety innovations (suicide switches, TRAC knock-in strategies, and non-integrating vectors) provide reassurance against rare but concerning risks of secondary malignancies. Finally, methods to enhance persistence and expansion, including enrichment for T-stem cell memory, cytokine engineering, nano-CAR scaffolds, and ligand-based CARs (APRIL, CD27, CCL27), hold promise for durable responses across hematologic and solid tumors. Importantly, the future of engineered cell therapy is likely to extend beyond T cells. CAR-modified NK cells and macrophages are being explored as complementary or alternative platforms, with potential advantages in safety, allogeneic compatibility, tissue remodeling, and antigen presentation. These directions illustrate a broader vision in which CAR engineering becomes a versatile framework applied across multiple immune effector cell types.

From a clinical perspective, the integration of artificial intelligence (AI) and machine learning into target discovery, trial design, and real-world response modeling continues to

accelerate progress. By enabling the analysis of high-dimensional datasets from transcriptomics, proteomics, and single-cell technologies, these computational tools help to identify novel targets, deconvolve resistance mechanisms, and predict patient responses, ensuring that the next generation of CAR-T therapies is guided by data-driven precision.

In conclusion, CAR-T therapy exemplifies both the promise and the complexity of personalized medicine in oncology. It has already transformed the standard of care for several hematologic malignancies and continues to evolve toward safer, faster, and more durable applications. The trajectory of the field suggests that CAR-T and related cellular therapies will not remain niche treatments but will become foundational components of modern immuno-oncology. The journey from proof-of-concept to global application reflects the challenge of harnessing living cells as medicines and the creativity driving this scientific revolution. Ultimately, the success of CAR-T therapy will depend on achieving a balance between efficacy, safety, accessibility, and durability, a balance that ongoing research and clinical innovation are actively pursuing.

Looking ahead, CAR-T cell therapy is poised to enter a new phase defined by engineering precision and biological insight. Allogeneic and in vivo CAR-T platforms aim to democratize access; synthetic circuits such as SynNotch, SNIPR, and logic-gated CARs promise spatially restricted activation and improved safety; and genome editing targeting the TRAC locus or other safe harbors ensures uniform expression with reduced tonic signaling. In parallel, AI-guided target discovery, surfaceome mapping, and conformation-specific proteomics are expanding the repertoire of tumor-restricted antigens, while ligand-based and nano-scaffold CARs enhance receptor stability and lower immunogenicity. Beyond T cells, CAR-modified NK cells, macrophages, and stem-cell–derived effectors are emerging as complementary platforms integrating innate and adaptive immunity. Ultimately, the convergence of synthetic biology, computational modeling, and translational science will transform CAR-T therapy from a curative option for select hematologic malignancies into a universally programmable cellular platform for cancer and immune-mediated diseases.

Financial support	None.
Conflict of interest	None.
Authorship role	Conception, design, acquisition, analysis, interpretation, writing, and critical review of the manuscript.

References

1. Zugasti I, Espinosa-Aroca, Lady, Fidyt K, Mulens-Arias V, Diaz-Beya M, Juan M, et al. CAR-T cell therapy for cancer: current challenges and future directions. Signal Transduct Target Ther [Internet]. 2025;10(1):210. Available from: https://doi.org/10.1038/s41392-025-02269-w

2. Petri K, D'Ippolito E, Künkele A, Köhl U, Busch DH, Einsele H, et al. Next-generation T cell immunotherapies engineered with CRISPR

base and prime editing: challenges and opportunities. Nat Rev Clin Oncol [Internet]. 2025;22(12):902–23. Available from: https://doi.org/10.1038/s41571-025-01072-4

3. Singh N, Maus M V. Synthetic manipulation of the cancer-immunity cycle: CAR-T cell therapy. Immunity [Internet]. 2023;56(10):2296–310. Available from: https://doi.org/10.1016/j.immuni.2023.09.010

4. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science (1979) [Internet]. 2018;359(6382):1361–5. Available from: https://doi.org/10.1126/science.aar6711

5. Mariuzza RA, Agnihotri P, Orban J. The structural basis of T-cell receptor (TCR) activation: An enduring enigma. Journal of Biological Chemistry [Internet]. 2020;295(4):914–25. Available from: https://doi.org/10.1016/s0021-9258(17)49904-2

6. Kong S, Zhang J, Wang L, Li W, Guo H, Weng Q, et al. Mechanisms of low MHC I expression and strategies for targeting MHC I with small molecules in cancer immunotherapy. Cancer Lett [Internet]. 2025;611:217432. Available from: https://doi.org/10.1016/j.canlet.2024.217432

7. Rodríguez-Nava C, Ortuño-Pineda C, Illades-Aguiar B, Flores-Alfaro E, Leyva-Vázquez MA, Parra-Rojas I, et al. Mechanisms of Action and Limitations of Monoclonal Antibodies and Single Chain Fragment Variable (scFv) in the Treatment of Cancer. Biomedicines [Internet]. 2023;11(6):1610. Available from: https://doi.org/10.3390/biomedicines11061610

8. Teoh PJ, Chng WJ. CAR T-cell therapy in multiple myeloma: more room for improvement. Blood Cancer J [Internet]. 2021;11(4):84. Available from: https://doi.org/10.1038/s41408-021-00469-5

9. Rafiq S, Hackett CS, Brentjens RJ. Engineering strategies to overcome the current roadblocks in CAR T cell therapy. Nat Rev Clin Oncol [Internet]. 2020;17(3):147–67. Available from: https://doi.org/10.1038/s41571-019-0297-y

10. van der Heide V, Humblin E, Vaidya A, Kamphorst AO. Advancing beyond the twists and turns of T cell exhaustion in cancer. Sci Transl Med [Internet]. 2022;14(670). Available from: https://doi.org/10.1126/scitranslmed.abo4997

11. Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, et al. Tisagenlecleucel in Adult Relapsed or Refractory Diffuse Large B-Cell Lymphoma. New England Journal of Medicine [Internet]. 2019;380(1):45–56. Available from: https://doi.org/10.1056/nejmoa1804980

12. Locke FL, Miklos DB, Jacobson CA, Perales MA, Kersten MJ, Oluwole OO, et al. Axicabtagene Ciloleucel as Second-Line Therapy for Large B-Cell Lymphoma. New England Journal of Medicine [Internet]. 2022;386(7):640–54. Available from: https://doi.org/10.1056/nejmoa2116133

13. 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. Journal of Clinical Oncology [Internet]. 2023;41(6):1265–74. Available from: https://doi.org/10.1200/JCO.22.00842

14. Hong DS, Van Tine BA, Biswas S, McAlpine C, Johnson ML, Olszanski AJ, et al. Autologous T cell therapy for MAGE-A4+ solid cancers in HLA-A*02+ patients: a phase 1 trial. Nat Med [Internet]. 2023;29(1):104–14. Available from: https://doi.org/10.1038/s41591-022-02128-z

15. Blache U, Popp G, Dünkel A, Koehl U, Fricke S. Potential solutions for manufacture of CAR T cells in cancer immunotherapy. Nat Commun [Internet]. 2022;13(1):5225. Available from: https://doi.org/10.1038/s41467-022-32866-0

16. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. New England Journal of Medicine [Internet]. 2018;378(5):439–48. Available from: https://doi.org/10.1056/nejmoa1709866

17. Locke FL, Ghobadi A, Jacobson CA, Miklos DB, Lekakis LJ, Oluwole OO, et al. Long-term safety and activity of axicabtagene ciloleucel in refractory large B-cell lymphoma (ZUMA-1): a single-arm, multicentre, phase 1–2 trial. Lancet Oncol [Internet]. 2019;20(1):31–42. Available from: https://doi.org/10.1016/S1470-2045(18)30864-7

18. 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. The Lancet [Internet]. 2020;396(10254):839–52. Available from: https://doi.org/10.1016/s0140-6736(20)31366-0

19. Wang M, Munoz J, Goy A, Locke FL, Jacobson CA, Hill BT, et al. KTE-X19 CAR T-Cell Therapy in Relapsed or Refractory Mantle-Cell Lymphoma. New England Journal of Medicine [Internet]. 2020;382(14):1331–42. Available from: https://doi.org/10.1056/nejmoa1914347

20. Munshi NC, Anderson LD, Shah N, Madduri D, Berdeja J, Lonial S, et al. Idecabtagene Vicleucel in Relapsed and Refractory Multiple Myeloma. New England Journal of Medicine [Internet]. 2021;384(8):705–16. Available from: https://doi.org/10.1056/nejmoa2024850

21. San-Miguel J, Dhakal B, Yong K, Spencer A, Anguille S, Mateos MV, et al. Cilta-cel or Standard Care in Lenalidomide-Refractory Multiple Myeloma. New England Journal of Medicine [Internet]. 2023;389(4):335–47. Available from: https://doi.org/10.1056/nejmoa2303379

22. 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. The Lancet [Internet]. 2021;398(10297):314–24. Available from: https://doi.org/10.1016/S0140-6736(21)00933-8

23. Peng L, Sferruzza G, Yang L, Zhou L, Chen S. CAR-T and CAR-NK as cellular cancer immunotherapy for solid tumors. Cell Mol Immunol [Internet]. 2024 Aug 12;21(10):1089–108. Available from: https://doi.org/10.1038/s41423-024-01207-0

24. Amini L, Silbert SK, Maude SL, Nastoupil LJ, Ramos CA, Brentjens RJ, et al. Preparing for CAR T cell therapy: patient selection, bridging therapies and lymphodepletion. Nat Rev Clin

Oncol [Internet]. 2022;19(5):342–55. Available from: https://doi.org/10.1038/s41571-022-00607-3

25. Hu B, Vaidya R, Ahmed F, Ehsan H, Moyo TK, Jacobs RW, et al. Real-World Analysis of Barriers to Timely Administration of Chimeric Antigen Receptor T Cell (CAR T) Therapy in Diffuse Large B-cell Lymphoma. Transplant Cell Ther [Internet]. 2024 Nov 1;30(11):1082.e1-1082.e10. Available from: https://doi.org/10.1016/j.jtct.2024.09.007

26. Lei T, Wang Y, Zhang Y, Yang Y, Cao J, Huang J, et al. Leveraging CRISPR gene editing technology to optimize the efficacy, safety and accessibility of CAR T-cell therapy. Leukemia [Internet]. 2024;38(12):2517–43. Available from: https://doi.org/10.1038/s41375-024-02444-y

27. Kamali E, Rahbarizadeh F, Hojati Z, Frödin M. CRISPR/Cas9-mediated knockout of clinically relevant alloantigenes in human primary T cells. BMC Biotechnol [Internet]. 2021;21(1):9. Available from: https://doi.org/10.1186/s12896-020-00665-4

28. Georgiadis C, Preece R, Qasim W. Clinical development of allogeneic chimeric antigen receptor αβ-T cells. Molecular Therapy [Internet]. 2025;33(6):2426–40. Available from: https://doi.org/10.1016/j.ymthe.2025.03.040

29. Guo S, Lei W, Jin X, Liu H, Wang JQ, Deng W, et al. CD70-specific CAR NK cells expressing IL-15 for the treatment of CD19-negative B-cell malignancy. Blood Adv [Internet]. 2024;8(11):2635–45. Available from: https://doi.org/10.1182/bloodadvances.2023012202

30. Liu E, Marin D, Banerjee P, Macapinlac HA, Thompson P, Basar R, et al. Use of CAR-Transduced Natural Killer Cells in CD19-Positive Lymphoid Tumors. New England Journal of Medicine [Internet]. 2020;382(6):545–53. Available from: https://doi.org/10.1056/nejmoa1910607

31. Bot A, Scharenberg A, Friedman K, Guey L, Hofmeister R, Andorko JI, et al. In vivo chimeric antigen receptor (CAR)-T cell therapy. Nat Rev Drug Discov [Internet]. 2025. Available from: https://doi.org/10.1038/s41573-025-01291-5

32. Nyberg WA, Wang CH, Ark J, Liu C, Clouden S, Qualls A, et al. In vivo engineering of murine T cells using the evolved adeno-associated virus variant Ark313. Immunity [Internet]. 2025;58(2):499-512.e7. Available from: https://doi.org/10.1016/j.immuni.2025.01.009

33. Hamilton JR, Chen E, Perez BS, Sandoval Espinoza CR, Kang MH, Trinidad M, et al. In vivo human T cell engineering with enveloped delivery vehicles. Nat Biotechnol [Internet]. 2024;42(11):1684–92. Available from: https://doi.org/10.1038/s41587-023-02085-z

34. Xu J, Liu L, Parone P, Xie W, Sun C, Chen Z, et al. In-vivo B-cell maturation antigen CAR T-cell therapy for relapsed or refractory multiple myeloma. The Lancet [Internet]. 2025;406(10500):228–31. Available from: https://doi.org/10.1016/s0140-6736(25)01030-x

35. Long AH, Haso WM, Shern JF, Wanhainen KM, Murgai M, Ingaramo M, et al. 4-1BB costimulation ameliorates T cell exhaustion induced by tonic signaling of chimeric antigen receptors. Nat Med [Internet]. 2015;21(6):581–90. Available from: https://doi.org/10.1038/nm.3838

36. Lynn RC, Weber EW, Sotillo E, Gennert D, Xu P, Good Z, et al. c-Jun overexpression in CAR T cells induces exhaustion resistance. Nature [Internet]. 2019;576(7786):293–300. Available from: https://doi.org/10.1038/s41586-019-1805-z

37. Kumar B, Singh A, Basar R, Uprety N, Li Y, Fan H, et al. BATF is a major driver of NK cell epigenetic reprogramming and dysfunction in AML. Sci Transl Med [Internet]. 2024;16(764):4. Available from: https://doi.org/10.1126/scitranslmed.adp0004

38. Wherry EJ, Kurachi M. Molecular and cellular insights into T cell exhaustion. Nat Rev Immunol [Internet]. 2015;15(8):486–99. Available from: https://doi.org/10.1038/nri3862

39. Philipson BI, O'Connor RS, May MJ, June CH, Albelda SM, Milone MC. 4-1BB costimulation promotes CAR T cell survival through noncanonical NF-κB signaling. Sci Signal [Internet]. 2020;13(625):31. Available from: https://doi.org/10.1126/scisignal.aay8248

40. Roselli E, Boucher JC, Li G, Kotani H, Spitler K, Reid K, et al. 4-1BB and optimized CD28 co-stimulation enhances function of human mono-specific and bi-specific third-generation CAR T cells. J Immunother Cancer [Internet]. 2021;9(10):e003354. Available from: https://doi.org/10.1136/jitc-2021-003354

41. Roselli E, Boucher JC, Li G, Kotani H, Spitler K, Reid K, et al. 4-1BB and optimized CD28 co-stimulation enhances function of human mono-specific and bi-specific third-generation CAR T cells. J Immunother Cancer. 2021;9(10): 9:e003354. Available from: https://doi.org/10.1136/jitc-2021-003354

42. Wijewarnasuriya D, Bebernitz C, Lopez A V., Rafiq S, Brentjens RJ. Excessive Costimulation Leads to Dysfunction of Adoptively Transferred T Cells. Cancer Immunol Res [Internet]. 2020;8(6):732–42. Available from: https://doi.org/10.1158/2326-6066.CIR-19-0908

43. Liu C, Qi T, Milner JJ, Lu Y, Cao Y. Speed and Location Both Matter: Antigen Stimulus Dynamics Controls CAR-T Cell Response. Front Immunol [Internet]. 2021;12. Available from: https://doi.org/10.3389/fimmu.2021.748768

44. 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 [Internet]. 2023;33(5):341–54. Available from: https://doi.org/10.1038/s41422-023-00789-0

45. Davenport AJ, Cross RS, Watson KA, Liao Y, Shi W, Prince HM, et al. Chimeric antigen receptor T cells form nonclassical and potent immune synapses driving rapid cytotoxicity. Proceedings of the National Academy of Sciences [Internet]. 2018 Feb 27;115(9):E2068–76. Available from: https://doi.org/10.1073/pnas.1716266115

46. Majzner RG, Rietberg SP, Sotillo E, Dong R, Vachharajani VT, Labanieh L, et al. Tuning the Antigen Density Requirement for CAR T-cell Activity. Cancer Discov [Internet]. 2020;10(5):702–23. Available from: https://doi.org/10.1158/2159-8290.CD-19-0945

47. Maher J, Brentjens RJ, Gunset G, Rivière I, Sadelain M. Human T-lymphocyte cytotoxicity and proliferation directed by a single chimeric TCRζ /CD28 receptor. Nat Biotechnol [Internet]. 2002;20(1):70–5.

Available from: https://doi.org/10.1038/nbt0102-70

48. Feucht J, Sun J, Eyquem J, Ho YJ, Zhao Z, Leibold J, et al. Calibration of CAR activation potential directs alternative T cell fates and therapeutic potency. Nat Med [Internet]. 2019;25(1):82–8. Available from: https://doi.org/10.1038/s41591-018-0290-5

49. Man K, Gabriel SS, Liao Y, Gloury R, Preston S, Henstridge DC, et al. Transcription Factor IRF4 Promotes CD8+ T Cell Exhaustion and Limits the Development of Memory-like T Cells during Chronic Infection. Immunity [Internet]. 2017;47(6):1129-1141.e5. Available from: https://doi.org/10.1016/j.immuni.2017.11.021

50. Li P, Spolski R, Liao W, Wang L, Murphy TL, Murphy KM, et al. BATF–JUN is critical for IRF4-mediated transcription in T cells. Nature [Internet]. 2012;490(7421):543–6. Available from: https://doi.org/10.1038/nature11530

51. Poorebrahim M, Melief J, Pico de Coaña Y, L. Wickström S, Cid-Arregui A, Kiessling R. Counteracting CAR T cell dysfunction. Oncogene [Internet]. 2021 Jan 14;40(2):421–35. Available from: https://doi.org/10.1038/s41388-020-01501-x

52. Seo H, Chen J, González-Avalos E, Samaniego-Castruita D, Das A, Wang YH, et al. TOX and TOX2 transcription factors cooperate with NR4A transcription factors to impose CD8 + T cell exhaustion. Proceedings of the National Academy of Sciences [Internet]. 2019;116(25):12410–5. Available from: https://doi.org/10.1073/pnas.1905675116

53. John LB, Devaud C, Duong CPM, Yong CS, Beavis PA, Haynes NM, et al. Anti-PD-1 Antibody Therapy Potently Enhances the Eradication of Established Tumors By Gene-Modified T Cells. Clinical Cancer Research [Internet]. 2013;19(20):5636–46. Available from: https://doi.org/10.1158/1078-0432.ccr-13-0458

54. Ouyang W, Jin SW, Xu N, Liu WY, Zhao H, Zhang L, et al. PD-1 downregulation enhances CAR-T cell antitumor efficiency by preserving a cell memory phenotype and reducing exhaustion. J Immunother Cancer [Internet]. 2024;12(4):e008429. Available from: https://doi.org/10.1136/jitc-2023-008429

55. Gumber D, Wang LD. Improving CAR-T immunotherapy: Overcoming the challenges of T cell exhaustion. EBioMedicine [Internet]. 2022;77:103941. Available from: https://doi.org/10.1016/j.ebiom.2022.103941

56. Gabriel SS, Tsui C, Chisanga D, Weber F, Llano-León M, Gubser PM, et al. Transforming growth factor-β-regulated mTOR activity preserves cellular metabolism to maintain long-term T cell responses in chronic infection. Immunity [Internet]. 2021;54(8):1698-1714.e5. Available from: https://doi.org/10.1016/j.immuni.2021.06.007

57. 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 [Internet]. 2019;11(499):5907. Available from: https://doi.org/10.1126/scitranslmed.aau5907

58. Morris EC, Neelapu SS, Giavridis T, Sadelain M. Cytokine release syndrome and associated neurotoxicity in cancer immunotherapy. Nat Rev Immunol [Internet].

2022;22(2):85–96. Available from: https://doi.org/10.1038/s41577-021-00547-6

59. Mulvey A, Trueb L, Coukos G, Arber C. Novel strategies to manage CAR-T cell toxicity. Nat Rev Drug Discov [Internet]. 2025;24(5):379–97. Available from: https://doi.org/10.1038/s41573-024-01100-5

60. Garcia J, Daniels J, Lee Y, Zhu I, Cheng K, Liu Q, et al. Naturally occurring T cell mutations enhance engineered T cell therapies. Nature [Internet]. 2024;626(7999):626–34. Available from: https://doi.org/10.1038/s41586-024-07018-7

61. Flugel CL, Majzner RG, Krenciute G, Dotti G, Riddell SR, Wagner DL, et al. Overcoming on-target, off-tumour toxicity of CAR T cell therapy for solid tumours. Nat Rev Clin Oncol [Internet]. 2023;20(1):49–62. Available from: https://doi.org/10.1038/s41571-022-00704-3

62. Monje M, Mahdi J, Majzner R, Yeom KW, Schultz LM, Richards RM, et al. Intravenous and intracranial GD2-CAR T cells for H3K27M+ diffuse midline gliomas. Nature [Internet]. 2025;637(8046):708–15. Available from: https://doi.org/10.1038/s41586-024-08171-9

63. Shabaneh TB, Stevens AR, Stull SM, Shimp KR, Seaton BW, Gad EA, et al. Systemically administered low-affinity HER2 CAR T cells mediate antitumor efficacy without toxicity. J Immunother Cancer [Internet]. 2024;12(2):e008566. Available from: https://doi.org/10.1136/jitc-2023-008566

64. Anderson J, Barone G, Zehner A. GD2 targeting CAR T cells for neuroblastoma. EJC Paediatric Oncology [Internet]. 2024 Dec 1;4:100179. Available from: https://doi.org/10.1016/j.ejcped.2024.100179

65. Zhu L, Liu J, Li J, Wang N, Zhao Y, Su H. Research progress on HER2-specific chimeric antigen receptor T cells for immunotherapy of solid tumors. Front Immunol [Internet]. 2025;16. Available from: https://doi.org/10.3389/fimmu.2025.1514994

66. Garfall AL, Cohen AD, Susanibar-Adaniya SP, Hwang WT, Vogl DT, Waxman AJ, et al. Anti-BCMA/CD19 CAR T Cells with Early Immunomodulatory Maintenance for Multiple Myeloma Responding to Initial or Later-Line Therapy. Blood Cancer Discov [Internet]. 2023;4(2):118–33. Available from: https://doi.org/10.1158/2643-3230.BCD-22-0074

67. 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 [Internet]. 2024;15(1):3371. Available from: https://doi.org/10.1038/s41467-024-47801-8

68. Cappell KM, Kochenderfer JN. Long-term outcomes following CAR T cell therapy: what we know so far. Vol. 20, Nature Reviews Clinical Oncology. Springer Nature; [Internet]. 2023. p. 359–71. Available from: https://doi.org/10.1038/s41571-023-00754-1

69. Wang Y, Li C, Xia J, Li P, Cao J, Pan B, et al. Humoral immune reconstitution after anti-BCMA CAR T-cell therapy in relapsed/refractory multiple myeloma. Blood Adv [Internet]. 2021;5(23):5290–9. Available from: https://doi.org/10.1182/bloodadvances.2021004603

70. Jiang Y li, Li Q, Yuan T, Jiang Y yu, Deng Q. <p>Case Report of Anti-CD123 Chimeric Antigen Receptor T-Cell Therapy Followed by Radiotherapy for a Recurrence of Blastic

Plasmacytoid Dendritic Cell Neoplasm After Allogeneic Hematopoietic Stem Cell Transplantation</p>. Onco Targets Ther [Internet]. 2020 Apr;Volume 13:3425–30. Available from: https://doi.org/10.2147/ott.s250016

71. Perna F, Parekh S, Diorio C, Smith M, Subklewe M, Mehta R, et al. CAR T-cell toxicities: from bedside to bench, how novel toxicities inform laboratory investigations. Blood Adv [Internet]. 2024;8(16):4348–58. Available from: https://doi.org/10.1182/bloodadvances.2024013044

72. Thompson JA, Schneider BJ, Brahmer J, Zaid MA, Achufusi A, Armand P, et al. NCCN Guidelines® Insights: Management of Immunotherapy-Related Toxicities, Version 2.2024. Journal of the National Comprehensive Cancer Network [Internet]. 2024;22(9):582–92. Available from: https://doi.org/10.6004/jnccn.2024.0057

73. Kumar AD, Atallah-Yunes SA, Rajeeve S, Abdelhak A, Hashmi H, Corraes A, et al. Delayed Neurotoxicity after CAR-T in Multiple Myeloma: Results from a Global IMWG Registry. Blood [Internet]. 2024;144(Supplement 1):4758–4758. Available from: https://doi.org/10.1182/blood-2024-201783

74. Parker KR, Migliorini D, Perkey E, Yost KE, Bhaduri A, Bagga P, et al. Single-Cell Analyses Identify Brain Mural Cells Expressing CD19 as Potential Off-Tumor Targets for CAR-T Immunotherapies. Cell [Internet]. 2020;183(1):126-142.e17. Available from: https://doi.org/10.1016/j.cell.2020.08.022

75. Ferguson ID, Patiño-Escobar B, Tuomivaara ST, Lin YHT, Nix MA, Leung KK, et al. The surfaceome of multiple myeloma cells suggests potential immunotherapeutic strategies and protein markers of drug resistance. Nat Commun [Internet]. 2022;13(1):4121. Available from: https://doi.org/10.1038/s41467-022-31810-6

76. Patiño-Escobar B, Perez-Lugo L, Wiita AP. *Technology Spotlight* Surface Target Discovery for Blood Cancer Immunotherapies. The Hematologist [Internet]. 2025;22(1). Available from: https://doi.org/10.1182/hem.v22.1.2025214

77. Mandal K, Wicaksono G, Yu C, Adams JJ, Hoopmann MR, Temple WC, et al. Structural surfaceomics reveals an AML-specific conformation of integrin β2 as a CAR T cellular therapy target. Nat Cancer [Internet]. 2023;4(11):1592–609. Available from: https://doi.org/10.1101/2022.10.10.511511

78. Kloss CC, Condomines M, Cartellieri M, Bachmann M, Sadelain M. Combinatorial antigen recognition with balanced signaling promotes selective tumor eradication by engineered T cells. Nat Biotechnol [Internet]. 2013;31(1):71–5. Available from: https://doi.org/10.1038/nbt.2459

79. Zhang W, He Q, Lopez B, Hu J, Kundu A, Andraza MC, et al. Abstract PO074: Logic-gating HER2 CAR-T to the tumor microenvironment mitigates on-target, off-tumor toxicity without compromising cytotoxicity against HER2-over-expressing tumors. Cancer Immunol Res [Internet]. 2021;9(2_Supplement):PO074–PO074. Available from: https://doi.org/10.1158/2326-6074.tumimm20-po074

80. Juillerat A, Marechal A, Filhol JM, Valogne Y, Valton J, Duclert A, et al. An oxygen sensitive self-decision making engineered CAR T-cell. Sci Rep [Internet]. 2017;7(1):39833. Available from: https://doi.org/10.1038/srep39833

81. Roybal KT, Williams JZ, Morsut L, Rupp LJ, Kolinko I, Choe JH, et al. Engineering T Cells with Customized Therapeutic Response Programs Using Synthetic Notch Receptors. Cell [Internet]. 2016;167(2):419-432.e16. Available from: https://doi.org/10.1016/j.cell.2016.09.011

82. Zhu I, Liu R, Garcia JM, Hyrenius-Wittsten A, Piraner DI, Alavi J, et al. Modular design of synthetic receptors for programmed gene regulation in cell therapies. Cell [Internet]. 2022;185(8):1431-1443.e16. Available from: https://doi.org/10.1016/j.cell.2022.03.023

83. Garcia JM, Burnett CE, Roybal KT. Toward the clinical development of synthetic immunity to cancer. Immunological Reviews. John Wiley and Sons Inc; [Internet]. 2023. Available from: https://doi.org/10.1111/imr.13245

84. Lin H, Yang X, Ye S, Huang L, Mu W. Antigen escape in CAR-T cell therapy: Mechanisms and overcoming strategies. Biomedicine & Pharmacotherapy [Internet]. 2024;178:117252. Available from: https://doi.org/10.1016/j.biopha.2024.117252

85. Lu Y, Zhao F. Strategies to overcome tumour relapse caused by antigen escape after CAR T therapy. Mol Cancer [Internet]. 2025;24(1):126. Available from: https://doi.org/10.1186/s12943-025-02334-6

86. Samur MK, Fulciniti M, Aktas Samur A, Bazarbachi AH, Tai YT, Prabhala R, et al. Biallelic loss of BCMA as a resistance mechanism to CAR T cell therapy in a patient with multiple myeloma. Nat Commun [Internet]. 2021;12(1):868. Available from: https://doi.org/10.1182/bloodadvances.2023010025

87. Samur MK, Aktas Samur A, Corre J, Lannes R, Shah P, Anderson K, et al. Monoallelic deletion of *BCMA* is a frequent feature in multiple myeloma. Blood Adv [Internet]. 2023;7(21):6599–603. Available from: https://doi.org/10.1182/bloodadvances.2023010025

88. 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 [Internet]. 2021;27(10):1797–805. Available from: https://doi.org/10.1038/s41591-021-01497-1

89. 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 [Internet]. 2021;27(8):1419–31. Available from: https://doi.org/10.1038/s41591-021-01436-0

90. Simon S, Riddell SR. Dual Targeting with CAR T Cells to Limit Antigen Escape in Multiple Myeloma. Blood Cancer Discov [Internet]. 2020;1(2):130–3. Available from: https://doi.org/10.1158/2643-3230.bcd-20-0122

91. Zhou D, Sun Q, Xia J, Gu W, Qian J, Zhuang W, et al. Anti-BCMA/GPRC5D bispecific CAR T cells in patients with relapsed or refractory multiple myeloma: a single-arm, single-centre, phase 1 trial. Lancet Haematol [Internet]. 2024;11(10):e751–60. Available from: https://doi.org/10.1016/s2352-3026(24)00176-5

92. Mailankody S, Devlin SM, Landa J, Nath K, Diamonte C, Carstens EJ, et al. GPRC5D-Targeted CAR T Cells for Myeloma. New England Journal of Medicine [Internet]. 2022;387(13):1196–206. Available from: https://doi.org/10.1056/nejmoa2209900

93. Tong C, Zhang Y, Liu Y, Ji X, Zhang W, Guo Y, et al. Optimized tandem CD19/CD20 CAR-engineered T cells in refractory/relapsed B-cell lymphoma [Internet]. 2020. Available from: https://doi.org/10.1182/blood.2022017502

94. Hegde M, Mukherjee M, Grada Z, Pignata A, Landi D, Navai SA, et al. Tandem CAR T cells targeting HER2 and IL13Rα2 mitigate tumor antigen escape. Journal of Clinical Investigation [Internet]. 2016;126(8):3036–52. Available from: https://doi.org/10.1172/jci83416

95. 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 [Internet]. 2013;5(215). Available from: https://doi.org/10.1126/scitranslmed.3006597

96. FDA Requires Boxed Warning for T cell Malignancies Following Treatment with BCMA-Directed or CD19-Directed Autologous Chimeric Antigen Receptor (CAR) T cell Immunotherapies [Internet]. FDA. 2024. Available from: https://www.fda.gov/vaccines-blood-biologics/safety-availability-biologics/fda-requires-boxed-warning-t-cell-malignancies-following-treatment-bcma-directed-or-cd19-directed

97. Prasad V. T-Cell Lymphoma From CAR T-Cell Therapy—A New Safety Notice. JAMA [Internet]. 2024;331(5):389. Available from: https://doi.org/10.1001/jama.2023.27885

98. Hamilton MP, Sugio T, Noordenbos T, Shi S, Bulterys PL, Liu CL, et al. Risk of Second Tumors and T-Cell Lymphoma after CAR T-Cell Therapy. New England Journal of Medicine [Internet]. 2024;390(22):2047–60. Available from: https://doi.org/10.1056/nejmoa2401361

99. Perica K, Jain N, Scordo M, Patel R, Eren OC, Patel U, et al. CD4+ T-Cell Lymphoma Harboring a Chimeric Antigen Receptor Integration in *TP53*. New England Journal of Medicine [Internet]. 2025;392(6):577–83. Available from: https://doi.org/10.1056/nejmoa2411507

100. Aleman A, Van Oekelen O, Melnekoff DT, Grossman L, Mouhieddine TH, Kurowski A, et al. Targeted Therapy of CAR+ T-Cell Lymphoma after Anti-BCMA CAR T-Cell Therapy. New England Journal of Medicine [Internet]. 2025 Aug 21;393(8):823–5. Available from: https://doi.org/10.1056/NEJMc2504588

101. Gehrke L, Gonçalves VDR, Andrae D, Rasko T, Ho P, Einsele H, et al. Current Non-Viral-Based Strategies to Manufacture CAR-T Cells. Int J Mol Sci [Internet]. 2024;25(24):13685. Available from: https://doi.org/10.3390/ijms252413685

102. Eyquem J, Mansilla-Soto J, Giavridis T, van der Stegen SJC, Hamieh M, Cunanan KM, et al. Targeting a CAR to the TRAC locus with CRISPR/Cas9 enhances tumour rejection. Nature [Internet]. 2017;543(7643):113–7. Available from: https://doi.org/10.1038/nature21405

103. Mansilla-Soto J, Eyquem J, Haubner S, Hamieh M, Feucht J, Paillon N, et al. HLA-independent T cell receptors for targeting tumors with low antigen density. Nat Med [Internet]. 2022;28(2):345–52. Available from: https://doi.org/10.1038/s41591-021-01621-1

104. Moghanloo E, Mollanoori H, Talebi M,

Pashangzadeh S, Faraji F, Hadjilooei F, et al. Remote controlling of CAR-T cells and toxicity management: Molecular switches and next generation CARs. Transl Oncol [Internet]. 2021;14(6):101070. Available from: https://doi.org/10.1016/j.tranon.2021.101070

105. D'Aloia MM, Zizzari IG, Sacchetti B, Pierelli L, Alimandi M. CAR-T cells: the long and winding road to solid tumors. Cell Death Dis [Internet]. 2018;9(3):282. Available from: https://doi.org/10.1038/s41419-018-0278-6

106. Gattinoni L, Speiser DE, Lichterfeld M, Bonini C. T memory stem cells in health and disease. Nat Med [Internet]. 2017;23(1):18–27. Available from: https://doi.org/10.1038/nm.4241

107. 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. [Internet]. 2014;123(24):3750–9. Available from: https://doi.org/10.1182/blood-2014-01-552174

108. Torres Chavez AG, McKenna MK, Gupta A, Daga N, Vera J, Leen AM, et al. IL-7 armed binary CAR T cell strategy to augment potency against solid tumors. Front Immunol [Internet]. 2025;16. Available from: https://doi.org/10.3389/fimmu.2025.1618404

109. Shen Z, Sang Z, Shi Y. Nanobodies as a powerful platform for biomedicine. Trends Mol Med [Internet]. 2022 Nov 1;28(11):1006–7. Available from: https://doi.org/10.1016/j.molmed.2022.08.007

110. Nix MA, Mandal K, Geng H, Paranjape N, Lin YHT, Rivera JM, et al. Surface Proteomics Reveals CD72 as a Target for In Vitro – Evolved Nanobody-Based CAR-T Cells in KMT2A/MLL1 -Rearranged B-ALL. Cancer Discov [Internet]. 2021;11(8):2032–49. Available from: https://doi.org/10.1158/2159-8290.CD-20-0242

111. Schmidts A, Ormhøj M, Choi BD, Taylor AO, Bouffard AA, Scarfò I, et al. Rational design of a trimeric APRIL-based CAR-binding domain enables efficient targeting of multiple myeloma. Blood Adv [Internet]. 2019;3(21):3248–60. Available from: https://doi.org/10.1182/bloodadvances.2019000703

112. Kasap C, Izgutdina A, Patiño-Escobar B, Kang AS, Chilakapati N, Akagi N, et al. Targeting high-risk multiple myeloma genotypes with optimized anti-CD70 CAR T cells. Blood [Internet]. 2025;146(7):819–33. Available from: https://doi.org/10.1182/blood.2024025536

113. 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 [Internet]. 2024;12(5):e008417. Available from: https://doi.org/10.1136/jitc-2023-008417

114. Patino-Escobar B, Kasap C, Ferguson I, Hale M, Wiita A. P-103: Profiling the myeloma cell surface proteome reveals CCR10 as a potential immunotherapeutic target. Clin Lymphoma Myeloma Leuk [Internet]. 2021;21:S94–5. Available from: https://doi.org/10.1016/s2152-2650(21)02237-0

115. Chilakapati N, Patiño-Escobar B, Chen EY, Dalal R, de Montagnac J, Johnson H, et al. Structure-guided engineering of CCL27 enhances natural ligand CAR T-cells against CCR10 for multiple myeloma [Internet]. 2025. Available from: https://doi.org/10.1101/2025.08.19.670119

116. Chen AXY, Yap KM, Kim JS, Sek K, Huang YK, Dunbar PA, et al. Rewiring endogenous genes in CAR T cells for tumour-restricted payload delivery. Nature [Internet]. 2025;644(8075):241–51. Available from: https://doi.org/10.1038/s41586-025-09212-7

117. Edelstein HI, Cosio A, Ezekiel ML, Corcoran WK, Morris AH, Leonard JN. Conversion of natural cytokine receptors into orthogonal synthetic biosensors. Nat Chem Biol [Internet]. 2025;21(11):1719–30. Available from: https://doi.org/10.1101/2024.03.23.586421