CAR-T Cell Therapy: Revolutionizing Cancer Treatment and its Implications for Future Therapeutic Strategies: A Review

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ABSTRACT

Cancer is a multifactorial disease resulting from genetic alterations, environmental factors, and disrupted cellular pathways that drive uncontrolled cell growth. Traditional treatments, including chemotherapy and radiotherapy, often produce variable outcomes and are limited by toxicity and resistance. Chimeric antigen receptor (CAR) T-cell therapy has emerged as a transformative immunotherapy, particularly effective in relapsed or refractory hematological malignancies. This review synthesizes evidence from PubMed, Scopus, Web of Science, and regulatory sources (2015–2025) to assess FDA-approved CAR-T products and explore emerging CRISPR-based strategies. Approved CAR-T therapies targeting CD19 and BCMA show high overall and complete response rates, yet face challenges including cytokine release syndrome (CRS), immune effector cell–associated neurotoxicity syndrome (ICANS), long manufacturing times, and restricted efficacy in solid tumors. CRISPR/Cas9 gene editing offers potential enhancements such as multiplex gene knockouts, universal "off-the-shelf" CAR-T cells, and logic-gated designs, while highlighting the need for rigorous quality control and regulatory compliance. Integrating CAR-T therapy with CRISPR approaches may pave the way for next-generation personalized immunotherapies, contingent upon balancing efficacy, safety, accessibility, and cost.

Keywords: CAR-T cell therapy, CRISPR-Cas9 gene editing, CD19-targeted immunotherapy, BCMA-directed multiple myeloma, Cytokine release syndrome

Introduction

Cancer is a heterogeneous and multifactorial disease driven by genetic mutations, epigenetic alterations, and dysregulated cellular signaling pathways that promote uncontrolled proliferation, evasion of apoptosis, and metastatic spread.[1,2] Key molecular events include loss of tumor suppressor activity, activation of oncogenes, impaired DNA repair mechanisms, and epigenetic modifications such as DNA methylation, histone modifications, and hydroxymethylation, all of which contribute to tumor initiation and progression. Traditional therapies, including chemotherapy and radiotherapy, remain central to cancer treatment but are constrained by limited efficacy, systemic toxicities (e.g., cardiotoxicity, bone marrow suppression, organ damage), and the emergence of drug resistance.[3–5] These limitations have accelerated the development of novel therapeutic strategies aimed at achieving targeted and durable anti-cancer effects.

Immunotherapy, which harnesses the patient's immune system to combat malignancies, has emerged as a promising approach. Adoptive cell transfer (ACT), particularly chimeric antigen receptor (CAR) T-cell therapy, has demonstrated remarkable potential by genetically reprogramming patient-derived T cells to recognize and eliminate tumor cells independently of major histocompatibility complex (MHC) restrictions.[6] FDA-approved CAR-T therapies have achieved durable responses in hematologic cancers; however, their broader application is hindered by challenges such as cytokine release syndrome (CRS), neurotoxicity, complex and time-intensive manufacturing processes, and limited efficacy against solid tumors.[7]

To address these challenges, innovative strategies are being explored, including combinatorial regimens, next-generation CAR designs, and genome editing technologies. CRISPR/Cas9 has emerged as a transformative tool in this context, enabling precise gene modifications, disruption of inhibitory immune checkpoints, and the development of universal "off-the-shelf" CAR-T products.[8] These advancements hold the potential to enhance the safety, efficacy, and accessibility of CAR-T therapies, expanding their application to a wider spectrum of cancers and marking a significant step forward in personalized immunotherapy.[9]

Methods

This review was conducted to synthesize current evidence on FDA-approved CAR-T therapies and emerging CRISPR-based enhancements in cancer immunotherapy. A comprehensive literature search was performed across PubMed, Scopus, and Web of Science databases, as well as regulatory documents from the U.S. Food and Drug Administration (FDA), covering publications from 2015 to 2025. Search terms included "CAR-T cell therapy," "CRISPR/Cas9," "gene editing," "hematologic malignancies," "solid tumors," and "immunotherapy." Both preclinical and clinical studies, including regulatory reports, were considered to provide a comprehensive overview of therapeutic efficacy, safety, and translational developments.[10]

Inclusion criteria encompassed studies reporting clinical outcomes of FDA-approved CAR-T therapies, preclinical evaluations of CRISPR-modified CAR-T cells, and translational research describing gene editing strategies to improve efficacy, reduce toxicity, or enable universal CAR-T products. Articles not published in English, conference abstracts without full text, and studies lacking primary data were excluded. Data extraction focused on CAR-T target antigens, response rates, adverse effects, manufacturing characteristics, and CRISPR-based modifications. Comparisons among products and strategies were performed to highlight clinical relevance and potential future directions. While this review employed a structured

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approach inspired by PRISMA guidelines, it is presented as a narrative synthesis rather than a formal systematic review, to accommodate the heterogeneity of study types and reporting formats. Key outcomes and trends are summarized to provide a clear perspective on the evolving landscape of CAR-T and CRISPR-enhanced immunotherapies.[11]

CAR-T Cell Therapy: Therapeutic Mechanism and Generational Evolution

Chimeric Antigen Receptor T-cell (CAR-T) therapy is a breakthrough in personalized cancer immunotherapy, enabling targeted elimination of malignant cells using a patient's own genetically engineered T cells.[11] The process begins with the collection of peripheral blood from the patient, followed by isolation of T lymphocytes via leukapheresis.[12] These T cells are then genetically modified ex vivo using viral vectors to express a chimeric antigen receptor (CAR), a synthetic receptor composed of three key components: (i) an extracellular antigen-recognition domain, usually a single-chain variable fragment (scFv) derived from tumor-specific antibodies, which identifies tumor-associated antigens; (ii) a transmembrane domain that anchors the receptor to the T-cell membrane; and (iii) intracellular signaling domains, such as CD3ζ and costimulatory motifs, which initiate T-cell activation, proliferation, cytokine release, and cytotoxic effector functions. After expansion and quality control in vitro, these engineered CAR-T cells are reinfused into the patient, where they specifically recognize tumor-associated antigens, undergo clonal expansion, secrete cytotoxic cytokines, and mediate targeted lysis of malignant cells.[13,14]

CAR-T cells have undergone significant generational evolution to improve efficacy, persistence, and safety (Figure 1). First-generation CARs included only the CD3ζ signaling domain and provided limited T-cell activation and short-lived anti-tumor responses. Second-generation CARs added a single costimulatory domain, such as CD28 or 4-1BB, which enhanced T-cell proliferation, survival, and cytotoxicity, resulting in improved clinical outcomes in hematological malignancies. Third-generation CARs incorporated two costimulatory domains (e.g., CD28/4-1BB/CD3 or CD28/OX40/CD3), further increasing long-term persistence and tumor control, though efficacy against solid tumors remained limited due to the immunosuppressive tumor microenvironment and poor T-cell infiltration.[15,16]

Fourth-generation CARs, also called TRUCKs (T-cells Redirected for Universal Cytokine Killing), are engineered to secrete immune-stimulatory cytokines such as IL-12 or IL-18 upon antigen engagement.[17,18] These cytokines remodel the tumor microenvironment, recruit innate immune cells, and enhance anti-tumor activity, potentially overcoming some barriers posed by solid tumors. Traditional autologous CAR-T therapies are often time-consuming, expensive, and logistically challenging, particularly for multicenter applications.

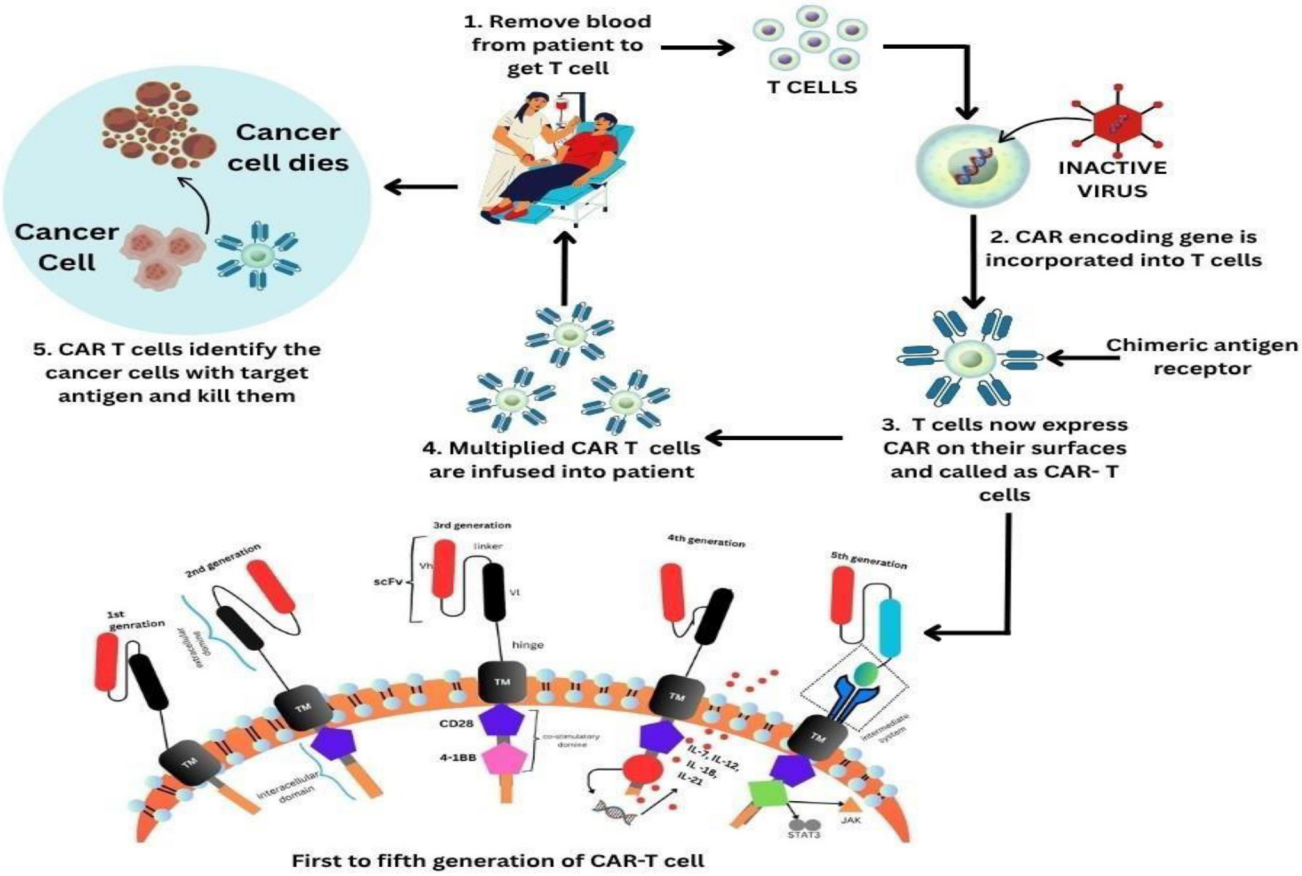

Fig 1 | CAR-T cell therapy: therapeutic mechanism and structural evolution from first to fifth generation receptors

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To address these challenges, allogeneic CAR-T cells derived from healthy donors are being developed. These T cells are genetically edited using technologies such as ZFN, TALEN, or CRISPR-Cas9 to disrupt the T-cell receptor (TCR) and HLA class I genes, reducing the risk of graft-versus-host disease, enhancing safety, and enabling off-the-shelf availability.[19,20]

Fifth-generation CARs represent the latest advancements, building on second-generation designs by incorporating cytokine receptor signaling domains, such as IL-2Rβ, which activate the JAK-STAT pathway. This enables precise regulation of T-cell activation and proliferation only in response to antigen engagement, enhancing anti-tumor efficacy while minimizing systemic toxicities. These CARs are designed to provide controlled immune responses, improve persistence, and reduce adverse events such as cytokine release syndrome (CRS) and neurotoxicity.[21,22]

Overall, the progressive evolution of CAR-T therapy—from first-generation to fifth-generation receptors—reflects continuous improvements in T-cell engineering, functionality, and clinical applicability. While current CAR-T therapies have shown remarkable success in hematological malignancies, challenges such as tumor antigen heterogeneity, immunosuppressive tumor microenvironments, high cost, complex manufacturing, and limited applicability in solid tumors remain. Ongoing innovations, including CRISPR-mediated gene editing and cytokine-modified CAR designs, aim to enhance safety, efficacy, and accessibility, moving the field closer to widely applicable, next-generation cancer immunotherapies.[23–25]

FDA-Approved CAR-T Cell Therapies

The U.S. Food and Drug Administration (FDA) has approved multiple CAR-T cell products for the treatment of hematological malignancies beginning in 2017. These therapies are engineered to recognize specific antigens, primarily CD19 or BCMA, and have demonstrated remarkable clinical outcomes.[26,27]

Tisagenlecleucel (Kymriah; Tisa-cel)

The first CAR T-cell product to gain regulatory approval was Tisagenlecleucel (Kymriah, formerly CTL019), licensed by the FDA in 2017 and representing a milestone in modern cancer immunotherapy. Developed through collaboration between Novartis and the Children's Hospital of Philadelphia, it is an autologous therapy, in which a patient's own T lymphocytes are collected, genetically engineered, and then reinfused. The genetic modification is carried out using a lentiviral vector that inserts a transgene encoding a chimeric antigen receptor (CAR) directed against the CD19 antigen, which is abundantly expressed on malignant B cells. The CAR construct integrates an extracellular single-chain variable fragment (scFv) for antigen recognition, linked to intracellular signaling domains comprising CD3ζ, the key activation signal, and the co-stimulatory domain 4-1BB (CD137), which enhances expansion, persistence, and durability of

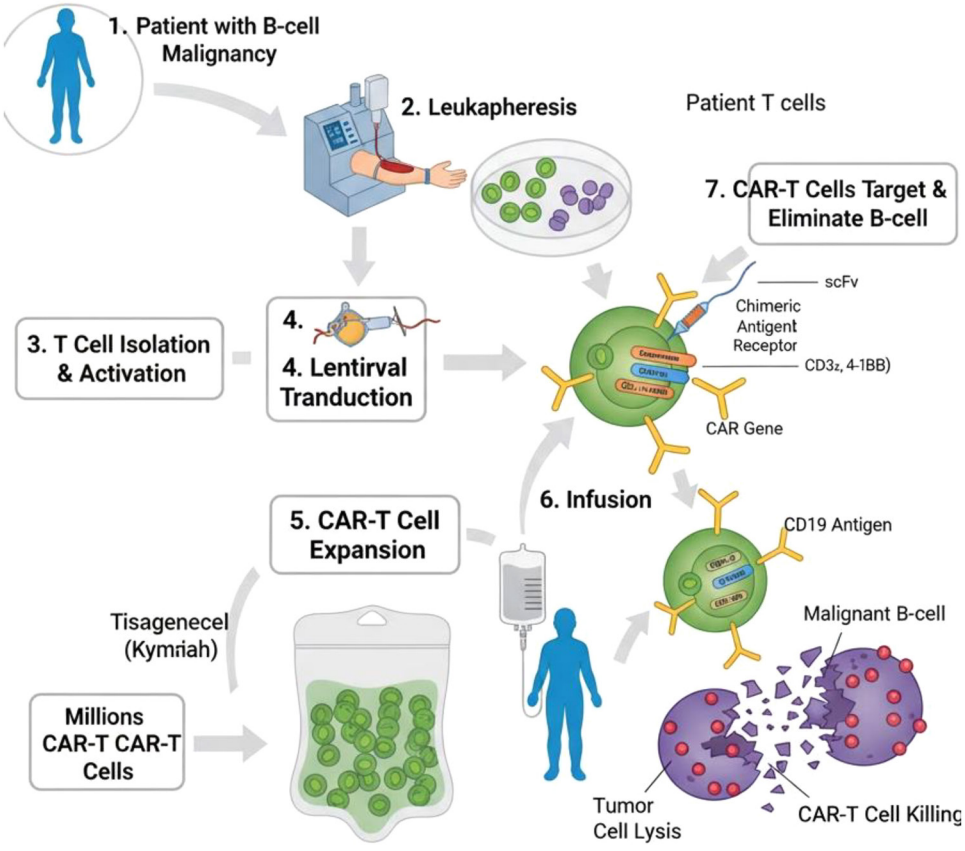

Fig 2 | Mechanism of action of tisagenleucel (kymriah) CAR-T therapy

anti-tumor responses (Figure 2).[28–30] Once administered, these engineered T cells are able to recognize and kill CD19-positive cells independently of major histocompatibility complex (MHC) presentation. The therapy was initially approved for pediatric and young adult patients (≤25 years) with relapsed or refractory B-cell acute lymphoblastic leukemia (ALL), later expanded to include adults with relapsed or refractory diffuse large B-cell lymphoma (DLBCL), high-grade B-cell lymphoma, and DLBCL arising from follicular lymphoma. Evidence from pivotal clinical trials highlights its transformative impact: in the ELIANA trial for ALL, tisagenlecleucel achieved an overall remission rate of 81% at three months, while in the JULIET trial for DLBCL, the overall response rate was 52%, including a 40% complete response rate, with some patients maintaining durable remissions.[31]

Despite its efficacy, tisagenlecleucel is associated with substantial toxicities. Cytokine release syndrome (CRS) occurs in most treated patients, with severe cases in approximately one-third; this is managed with the interleukin-6 receptor blocker tocilizumab and corticosteroids when required. Neurological complications, grouped under immune effector cell–associated neurotoxicity syndrome (ICANS), present as confusion, seizures, or encephalopathy and can be life-threatening, although they are usually reversible.[32,33] Additional risks include prolonged cytopenias (neutropenia, thrombocytopenia, anemia), opportunistic infections, and rare but severe conditions such as hemophagocytic lymphohistiocytosis (HLH). Key challenges with tisagenlecleucel include its manufacturing timeline of three to four weeks, which necessitates bridging chemotherapy in patients with aggressive disease, and its high cost, often exceeding USD 400,000 for a single infusion, creating barriers to access. Furthermore, disease relapse can occur due to antigen escape (loss of CD19 expression) or limited persistence of CAR-T cells. Thus, while tisagenlecleucel has revolutionized treatment for B-cell malignancies, ongoing research is focused on improving durability, expanding indications to solid tumors, and reducing toxicity and cost.[34,35]

Axicabtagene Ciloleucel (Yescarta; Axi-Cel)

Axicabtagene ciloleucel (Yescarta, Axi-cel), developed by Kite Pharma and approved by the FDA in 2017, was the second CAR T-cell therapy to enter clinical practice. It is indicated for adults with relapsed or refractory large B-cell lymphomas, including diffuse large B-cell lymphoma (DLBCL), primary mediastinal large B-cell lymphoma, high-grade B-cell lymphoma, and DLBCL not otherwise specified, after failure of at least two prior systemic therapies.[36] Axi-cel is an autologous therapy generated through leukapheresis, followed by retroviral transduction of patient T cells to express an anti-CD19 CAR. The CAR construct contains an extracellular scFv targeting CD19 linked to intracellular CD28 and CD3ζ domains, which provide potent activation signals leading to rapid T-cell proliferation, cytokine release, and tumor cell lysis. Clinical data from the ZUMA-1 trial demonstrated high efficacy, with an overall response rate (ORR) of 82% and a complete response (CR) rate of 54%, many of which were durable.[37] However, Axi-cel is associated with significant toxicities, primarily cytokine release syndrome (CRS) and immune effector cell–associated neurotoxicity syndrome (ICANS). CRS typically manifests as fever, hypotension, hypoxia, or organ dysfunction, while ICANS can present with confusion, seizures, and encephalopathy.[38] Most cases are manageable with tocilizumab and corticosteroids, but severe events can require intensive care support. While Axi-cel has transformed the treatment landscape for refractory B-cell lymphomas, key challenges remain, including its safety profile, prolonged manufacturing time, and high cost, alongside risks of relapse due to antigen escape or loss of CAR-T persistence. Nevertheless, its durable remissions in a subset of patients highlight its value as a pivotal advance in hematologic oncology.[39,40]

Brexucabtagene Autoleucel (Tecartus; Brexu-Cel)

Brexucabtagene autoleucel (Tecartus, brexu-cel), developed by Kite Pharma and approved by the FDA in July 2020, was the first CAR T-cell therapy authorized for relapsed or refractory mantle cell lymphoma (MCL), a highly aggressive B-cell malignancy with poor prognosis after conventional therapies.[41] It is also approved for adults with relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL). Tecartus is an autologous CD19-directed CAR-T product, manufactured by leukapheresis of patient T cells, followed by retroviral transduction to express a CAR containing an anti-CD19 scFv linked to CD28 and CD3ζ signaling domains, which drive robust T-cell activation and cytotoxicity.[42] The pivotal ZUMA-2 trial evaluated brexu-cel in heavily pretreated MCL patients and demonstrated an overall response rate of 87%, with 62% achieving complete remission, many of which were durable. These outcomes were unprecedented in this patient population and led to its accelerated approval. However, brexu-cel carries significant risks, particularly cytokine release syndrome (CRS) and neurotoxicity (ICANS), which occurred in more than 90% and 60% of patients, respectively, in ZUMA-2. While most adverse events were reversible, they often required interventions with tocilizumab, corticosteroids, and intensive care support. Despite these risks, brexu-cel represents a breakthrough for patients with otherwise refractory MCL and ALL. Ongoing challenges include its safety profile, limited applicability to solid tumors, manufacturing delays, and cost barriers. Research efforts are focusing on strategies to improve persistence, reduce toxicities, and expand the therapeutic reach of this potent CAR-T therapy.[43–45]

Lisocabtagene Maraleucel (Breyanzi; Liso-Cel)

Lisocabtagene maraleucel (Breyanzi, liso-cel), developed by Juno Therapeutics/Bristol Myers Squibb, received its first FDA approval in February 2021 for adults with relapsed or refractory large B-cell lymphoma (LBCL) after at least two prior lines of therapy. Indications include diffuse large B-cell lymphoma

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(DLBCL), high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, and follicular lymphoma grade 3B.[46] Liso-cel is an autologous anti-CD19 CAR-T product, distinct from other approved CAR-T therapies because it is manufactured to deliver a defined composition of CD4+ and CD8+ T cells in a controlled ratio. Genetic modification is performed using a lentiviral vector that encodes a CAR with an anti-CD19 scFv, 4-1BB costimulatory domain, and CD3ζ signaling domain, providing potent activation with enhanced persistence and potentially lower toxicity compared to CD28-based constructs. The pivotal TRANSCEND NHL 001 trial demonstrated strong clinical activity, with an overall response rate (ORR) of ~73% and a complete response (CR) rate of ~54%. Importantly, liso-cel showed a more favorable safety profile than some earlier CD19 CAR-T products: any-grade cytokine release syndrome (CRS) occurred in ~42% of patients, and neurotoxicity (ICANS) in ~30%, with severe events being less frequent.[47,48] These features make Breyanzi an important therapeutic option for patients at higher risk of toxicity. Nevertheless, challenges remain, including manufacturing time, high cost, and disease relapse due to antigen escape or limited CAR-T persistence. Still, its favorable safety–efficacy balance positions liso-cel as a valuable advance in the treatment of refractory LBCL.[49,50]

Idecabtagene Vicleucel (Abecma; Ide-Cel)

Idecabtagene vicleucel (Abecma, ide-cel), developed by Bristol Myers Squibb and bluebird bio, became the first FDA-approved CAR T-cell therapy for multiple myeloma in March 2021. It is indicated for adult patients with relapsed or refractory multiple myeloma (RRMM) who have received at least four prior lines of therapy, including an immunomodulatory drug (IMiD), a proteasome inhibitor (PI), and an anti-CD38 monoclonal antibody. Ide-cel is an autologous CAR-T therapy targeting B-cell maturation antigen (BCMA), which is highly expressed on malignant plasma cells but minimally on normal tissues. The CAR construct incorporates an anti-BCMA scFv linked to a 4-1BB costimulatory domain and CD3ζ signaling domain, ensuring potent activation, expansion, and persistence of modified T cells. The pivotal KarMMa trial established its efficacy, reporting an overall response rate (ORR) of 72%, with 33% of patients achieving complete response (CR). The median progression-free survival (PFS) was approximately 8.8 months, with some patients achieving durable remissions despite heavy pretreatment. Toxicities are significant but generally manageable. Cytokine release syndrome (CRS) occurred in ~84% of patients (severe in ~6%), while neurotoxicity (ICANS) was observed in ~18%. Other common adverse effects include prolonged cytopenias, infections, and hypogammaglobulinemia due to on-target depletion of normal plasma cells. Despite these risks, ide-cel represents a major advance for patients with refractory myeloma who previously had limited treatment options. Ongoing trials are investigating its use in earlier lines of therapy, combinations with other agents, and strategies to overcome antigen escape and improve long-term persistence.[51,52]

Ciltacabtagene Autoleucel (Carvykti, Cilta-Cel)

Ciltacabtagene autoleucel (Carvykti, cilta-cel), developed by Janssen Biotech, is an autologous CAR T-cell therapy approved for the treatment of relapsed or refractory multiple myeloma. It targets B-cell maturation antigen (BCMA), a transmembrane glycoprotein highly expressed on malignant plasma cells but largely absent in normal tissues, making it a reliable therapeutic marker. The CAR construct includes an extracellular single-chain variable fragment (scFv) against BCMA, a hinge and spacer region, and intracellular signaling domains comprising CD3ζ for T-cell activation and 4-1BB for costimulatory signaling, which enhance proliferation, persistence, and anti-tumor activity. The therapy is manufactured by collecting patient-derived T cells via leukapheresis, genetically modifying and expanding them, purifying, and cryopreserving before reinfusion.[53,54] Upon administration, these engineered CAR T cells recognize BCMA-expressing tumor cells, rapidly expand, release cytokines, and mediate targeted cytotoxicity. Clinically, Carvykti has demonstrated high efficacy in heavily pretreated multiple myeloma patients, including those refractories to conventional therapies. However, it is associated with hematologic toxicities (neutropenia, leukopenia, anemia, thrombocytopenia), orthostatic hypotension, elevated liver enzymes, musculoskeletal pain, hypogammaglobulinemia, gastrointestinal and respiratory symptoms, as well as cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). Despite its potent anti-tumor activity, challenges such as high cost, complex manufacturing, risk of severe toxicities, and limitations in targeting solid tumors due to antigen heterogeneity and immunosuppressive tumor microenvironments must be considered for clinical application.[55,56]

Comparative Overview of FDA-Approved CAR-T Therapies and CRISPR-Cas9 Applications

Chimeric Antigen Receptor T-cell (CAR-T) therapies have revolutionized the management of hematological malignancies by genetically reprogramming autologous T cells to selectively target and eliminate malignant cells.[57,58] The U.S. Food and Drug Administration (FDA) has approved multiple CAR-T products since 2017 for B-cell malignancies, including acute lymphoblastic leukemia (ALL), large B-cell lymphoma (LBCL), mantle cell lymphoma (MCL), follicular lymphoma (FL), and relapsed or refractory multiple myeloma (MM). Representative examples include Kymriah (tisagenlecleucel, 2017) for B-ALL and DLBCL, Yescarta (axicabtagene ciloleucel, 2017; expanded 2021) for LBCL and FL, Tecartus (brexucabtagene autoleucel, 2020) for MCL, Breyanzi (lisocabtagene maraleucel, 2021) for LBCL, Abecma (idecabtagene vicleucel, 2021) for MM, and Carvykti (ciltacabtagene autoleucel, 2022) for MM. These therapies differ in their design and application, with variations in target antigens

such as CD19 or BCMA, costimulatory domains including 4-1BB or CD28, and vector platforms such as lentiviral or retroviral systems.[59,60] Clinical outcomes also vary, with differences in overall and complete response rates, durability of remission, and safety profiles, particularly regarding cytokine release syndrome (CRS) and neurotoxicity. While FDA-approved CAR-T products have achieved unprecedented efficacy in otherwise refractory cancers, limitations such as CRS, neurotoxicity, antigen escape, limited persistence, and high treatment costs remain significant challenges. In this context, CRISPR-Cas9 genome-editing technology has emerged as a promising approach to refine CAR-T therapies by enhancing target specificity, minimizing off-target effects, improving persistence, and overcoming tumor resistance mechanisms. The integration of CRISPR with CAR-T development represents a major step toward next-generation engineered cell therapies with broader clinical applicability, including potential use against solid tumors.[61]

CAR-T Cell Clinical Trials: An Overview

Clinical trials are essential for establishing the safety, efficacy, and long-term outcomes of CAR-T cell therapies across diverse hematological malignancies.[62] These trials are conducted in sequential phases, starting with phase I studies that primarily assess safety, tolerability, and optimal dosing, followed by phase II and III trials that evaluate therapeutic efficacy, durability of response, and management of adverse events. Globally, ongoing and completed CAR-T trials are investigating a variety of antigen targets, including CD19, CD7, CD30, CD37, FLT3, BCMA, and SLAM7, reflecting the expansion of CAR-T therapy beyond B-cell malignancies to conditions such as multiple myeloma, acute myeloid leukemia, and Hodgkin lymphoma. Trials vary in design, including participant demographics, inclusion/exclusion criteria, disease stage, and geographic location. The accompanying table summarizes major CAR-T cell clinical trials, highlighting trial phase, start and completion dates, disease focus, targeted antigens, patient populations, and trial sites. This overview demonstrates the rapid translation of CAR-T research from laboratory discoveries to clinical applications, emphasizing the evolving landscape of personalized immunotherapy.[63,64]

CRISPR-Cas9 Technology: An Emerging Tool

CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats – CRISPR associated protein 9) is a revolutionary genome-editing technology that has transformed modern molecular biology and therapeutic research.[65,66] Initially discovered as a natural adaptive immune system in bacteria and archaea, CRISPR-Cas9 protects microbial cells by targeting and cleaving the DNA of invading viruses and plasmids. The technology was adapted for laboratory use in 2012, allowing scientists to precisely edit genes in a wide variety of organisms, including humans, plants, and animals. Its simplicity, high efficiency, and versatility make it superior to older gene-editing tools like zinc-finger nucleases (ZFNs) and TALENs (transcription activator-like effector nucleases). CRISPR-Cas9 enables not only gene knockout and correction but also gene regulation, epigenetic modification, and advanced genome engineering strategies.[67]

Mechanism of CRISPR-Cas9

The CRISPR-Cas9 system functions as a programmable genome-editing tool that allows precise targeting and modification of specific DNA sequences. It relies on a single-guide RNA (sgRNA), which is engineered to match the target DNA sequence, guiding the Cas9 endonuclease to the desired genomic locus. Upon recognition of a complementary DNA sequence adjacent to a Protospacer Adjacent Motif (PAM), Cas9 binds and introduces a double-strand break (DSB) at the target site. The cellular DNA repair machinery then responds to this break through two primary pathways. Non-homologous end joining (NHEJ) is an error-prone repair mechanism that can introduce small insertions or deletions, often resulting in gene disruption or knockout. In contrast, homology-directed repair (HDR) is a precise mechanism that utilizes a donor DNA template to accurately correct mutations or insert new genetic material. This RNA-guided DNA cleavage and repair process provides CRISPR-Cas9 with exceptional specificity, efficiency, and versatility, making it a powerful tool for genetic engineering, functional genomics, and therapeutic applications such as enhancing the efficacy of CAR-T cell therapies (Table 1).[68,69]

Advantages of CRISPR-Cas9 in Genetic Engineering

CRISPR-Cas9 offers several distinct advantages over earlier genome-editing technologies. Its high precision and specificity allow the single-guide RNA (sgRNA) to be designed to target almost any DNA sequence within the genome, minimizing off-target effects when properly optimized. This level of accuracy makes CRISPR-Cas9 highly reliable for both research and therapeutic applications. Additionally, the system provides remarkable efficiency and speed, enabling simultaneous editing of multiple genes through multiplexing. Compared to older technologies such as zinc finger nucleases (ZFNs) or transcription activator-like effector nucleases (TALENs), CRISPR-Cas9 is faster, simpler, and reduces experimental time significantly.

Cost-effectiveness is another major advantage, as sgRNAs are easy to design and synthesize, and the method requires minimal specialized equipment compared to traditional genome-editing approaches. Furthermore, CRISPR-Cas9 demonstrates exceptional versatility. It can be used for gene knockouts, precise gene knock-ins, base editing, prime editing, and even modulation of gene expression through CRISPR activation (CRISPRa) or inhibition (CRISPRi). The system also allows modification of epigenetic marks, broadening its applications in functional genomics and biotechnology.

The broad applicability of CRISPR-Cas9 is evident in its functionality across a wide range of organisms and cell types, making it a powerful tool for both basic research and therapeutic development. It holds

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Table 1 | FDA-approved CAR-T cell therapies and CRISPR-Cas9 applications: a comparative overview[60,6]

Medication (Brand Name)	Abecma (Idecabtagene Vicleucel)	Breyanzi (Lisocabtagene Maraleucel)	Kymriah (Tisagenlecleucel)	Tecartus (Brexucabtagene Autoleucel)	Yescarta (Axicabtagene Ciloleucel)
FDA Approval (Indication)	Multiple myeloma (2021)	Large B-cell lymphoma (2021)	B-cell ALL & DLBCL (2017)	Mantle cell lymphoma (2020)	Large B-cell lymphoma (2017), Follicular lymphoma (2021)
CAR Construct	BCMAscFv, 4-1BB, CD3ζ	CD19scFv, 4-1BB, CD3ζ	CD19scFv, 4-1BB, CD3ζ	CD19scFv, CD28, CD3ζ	CD19scFv, CD28, CD3ζ
Vector	Lentiviral vector	Lentiviral vector	Lentiviral vector	Retroviral vector	Retroviral vector
Target Antigen	BCMA	CD19	CD19	CD19	CD19
Bridging Chemotherapy	Yes	Yes	Yes	Yes	No
CAR-T Dose	450 × 10^6 CAR-T cells	50–110 × 10^6 CAR-T cells	0.2–5 × 10^6 CAR-T cells/kg	2 × 10^6 CAR-T cells/kg	2 × 10^6 CAR-T cells/kg
Efficacy (Overall / Complete Response)	72% / 33%	73% / 54%	52% / 40%	87% / 62%	82% / 54%
Safety (CRS / Neurotoxicity)	84% / 18%	42% / 30%	58% / 21%	91% / 63%	93% / 64%
Common Side Effects	Cytokine release syndrome	Cytokine release syndrome	B-cell aplasia, CRS	Cytokine release syndrome	Cytokine release syndrome

significant potential for treating genetic disorders, cancers, viral infections, and for engineering immune cells, including CAR-T cells. By integrating CRISPR-Cas9 into CAR-T therapy, next-generation immunotherapies can be developed with improved specificity, reduced off-target effects, enhanced persistence, and greater efficacy. This approach can also overcome tumor resistance mechanisms, address T-cell exhaustion, and improve the safety and durability of engineered T cells. Specific applications include knocking out PD-1 or endogenous T-cell receptors to reduce exhaustion and alloreactivity, precise insertion of CAR constructs at controlled genomic loci, and multiplexed editing to target multiple immune checkpoints simultaneously. Together, these advantages position CRISPR-Cas9 as a transformative tool in genetic engineering and immunotherapy development.[70]

Combination Therapy: CRISPR-Cas9 and CAR-T Cell Therapy

CAR-T cell therapy has emerged as a highly effective immunotherapy for B-cell malignancies, yet several limitations restrict its broader application. Challenges include limited CAR-T cell persistence, T-cell exhaustion, cytokine release syndrome (CRS), neurotoxicity, antigen escape, and high costs associated with autologous cell production. These obstacles reduce therapeutic durability and hinder applicability in solid tumors. CRISPR-Cas9-based genome editing offers a powerful solution by enabling precise and programmable modifications in CAR-T cells, enhancing their anti-tumor activity, safety, and persistence while potentially lowering production costs.[71,72]

The CRISPR-Cas9 system is an RNA-guided genome editing tool that allows targeted modifications at specific loci. It involves three key steps: (i) spacer acquisition, where short sequences derived from foreign DNA are incorporated into the CRISPR array; (ii) DNA interference, in which the Cas9 nuclease is guided to complementary target DNA sequences; and (iii) CRISPR and Cas expression, producing CRISPR RNA (crRNA) that directs Cas9 to the target locus. Using CRISPR, negative regulators of T-cell activity can be disrupted, or CAR constructs can be precisely integrated into safe genomic loci, such as the T-cell receptor α constant (TRAC) locus, ensuring uniform CAR expression and reducing the risk of mispairing with endogenous TCRs.[73]

Several Delivery Strategies for CRISPR-Cas9 Editing in CAR-T Cells Exist[73]

- Plasmid DNA delivery, encoding both Cas9 and single-guide RNA (sgRNA) in a single vector.
- mRNA delivery, combining Cas9 mRNA with sgRNA for transient expression.
- Ribonucleoprotein (RNP) complexes, consisting of Cas9 protein bound to sgRNA, which is considered the most advantageous due to minimal off-target effects and no permanent DNA integration, as the complex is rapidly degraded after editing.

Early proof-of-concept studies, such as those by Eyquem et al., demonstrated that inserting a CD19-specific CAR into the TRAC locus enhanced CAR-T cell proliferation, persistence, and anti-tumor efficacy. CRISPR-mediated gene editing also enables the generation of allogeneic or "universal" CAR-T cells by knocking out TCR and HLA class I genes, reducing the risk of graft-versus-host disease and making off-the-shelf CAR-T therapies feasible. Furthermore, CRISPR technology allows the introduction of cytokine-secreting modules or checkpoint blockade genes to improve tumor infiltration and overcome immunosuppressive microenvironments, particularly in solid tumors. The combination of CRISPR-Cas9 and CAR-T therapies addresses several key clinical challenges: enhancing the endurance and persistence of CAR-T cells, increasing efficacy in solid tumors, reducing cytokine-mediated toxicities, and lowering the cost and complexity of CAR-T production. Preclinical and early-phase clinical studies indicate that CRISPR-edited CAR-T cells can achieve more uniform CAR expression, improved tumor targeting, and controlled immune activation. This integration of genome editing into CAR-T therapy has the potential to create next-generation, safer, and more

effective immunotherapies, paving the way for broader application across multiple cancer types and addressing unmet clinical needs. Given the global demand for innovative cancer treatments, CRISPR/Cas9-based CAR-T strategies represent a promising avenue to improve patient outcomes while optimizing manufacturing efficiency and therapeutic safety.

Potential Benefits of CRISPR-Cas9 in CAR-T Cell Therapy[74]

CRISPR-Cas9 genome editing offers several potential advantages for enhancing CAR-T cell therapy, addressing current limitations in efficacy, persistence, safety, and scalability.

- **Improved CAR-T Cell Function:** CRISPR can selectively disrupt genes in T cells that negatively regulate their activity, such as immune checkpoint molecules (e.g., PD-1). This enhances CAR-T cell persistence, proliferation, and tumor-homing capabilities, allowing them to maintain long-term anti-tumor activity. Additionally, CRISPR enables the design of multi-antigen targeting CARs, which can recognize multiple tumor-associated antigens simultaneously, reducing the likelihood of tumor escape and improving overall therapeutic effectiveness.
- **Reduced Toxicity:** Cytokine release syndrome (CRS) and other immune-related toxicities are major concerns in CAR-T therapy. By modifying genes that influence T-cell activation or cytokine production, CRISPR allows fine-tuning of CAR-T responses, enhancing anti-tumor activity while minimizing systemic toxicities.
- **Gene Correction and Targeting:** CRISPR technology offers the potential to correct genetic mutations that contribute to cancer progression or impair immune function. Integrating gene correction with CAR-T therapy not only eliminates tumor cells but may also restore normal immune functions in patients with underlying genetic aberrations.
- **Off-the-Shelf (Allogeneic) CAR-T Therapies:** CRISPR enables the generation of "universal" CAR-T cells from healthy donors by knocking out genes such as T-cell receptor (TCR) and human leukocyte antigen (HLA), reducing the risk of graft-versus-host disease and immunological rejection. These allogeneic CAR-T cells can be produced in bulk and used in multiple patients, significantly improving accessibility, reducing manufacturing time, and lowering costs.
- Overall, CRISPR-Cas9 integration into CAR-T therapy has the potential to enhance efficacy, reduce adverse events, and expand clinical applicability, paving the way for next-generation, safer, and more widely available cancer immunotherapies.

Limitations and Future Perspectives

This review has several limitations. The included studies are heterogeneous, comprising preclinical experiments, early-phase clinical trials, and regulatory documents, which limits direct comparability of outcomes. Long-term safety and efficacy data for both FDA-approved CAR-T therapies and CRISPR-enhanced strategies remain limited, particularly for solid tumors. Publication bias may have influenced the available evidence, as studies with positive results are more likely to be published. Additionally, this review is a narrative synthesis rather than a full systematic review, and some relevant studies might have been inadvertently excluded. Rapid advancements in gene-editing technologies and CAR-T designs mean that some emerging innovations may not yet be reflected in the literature.

Looking forward, several avenues hold promise for improving CAR-T and CRISPR-based therapies. Future research should focus on enhancing efficacy and safety in solid tumors, reducing treatment-related toxicities such as cytokine release syndrome (CRS) and neurotoxicity, and developing universal "off-the-shelf" CAR-T products. Integration of CRISPR/Cas9 for precise gene editing, logic-gated designs, and multiplex modifications may further optimize therapeutic outcomes. Additionally, long-term follow-up studies and real-world clinical data are essential to validate these approaches and inform regulatory and clinical decision-making. Together, these strategies could expand the applicability, accessibility, and personalization of next-generation cancer immunotherapies.

Conclusion

CAR-T cell therapy has transformed the landscape of cancer treatment, offering a powerful, patient-specific immunotherapy for hematological malignancies such as lymphomas and leukemias. With six FDA-approved therapies including Kymriah, Yescarta, Tecartus, Breyanzi, Abecma, and Carvykti patients with limited treatment options now have access to highly targeted approaches that harness their own immune system to recognize and eliminate malignant cells. These therapies have demonstrated remarkable efficacy, durability, and specificity, establishing CAR-T cells as a cornerstone of modern oncology. The integration of CRISPR-Cas9 gene-editing technology represents a transformative advancement in this field. By precisely modifying T cells, CRISPR can enhance CAR-T cell persistence, tumor targeting, and anti-cancer potency, while mitigating adverse effects such as cytokine release syndrome (CRS). CRISPR-edited CAR-T cells allow for immune checkpoint disruption, multi-antigen targeting, and uniform CAR expression, addressing many limitations of conventional CAR-T therapy and expanding its potential to solid tumors and other challenging malignancies. Together, the convergence of CAR-T cell therapy and CRISPR-Cas9 genome editing promises the development of next-generation immunotherapies that are safer, more effective, and broadly accessible. These innovations not only highlight the rapid progress in personalized cancer treatment but also offer a roadmap for overcoming current clinical barriers, ultimately paving the way for more precise, durable, and widely applicable cancer therapies.

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