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REVIEW ARTICLE

Advances in CAR-T Cell Therapy, Clinical Breakthroughs, Challenges, and Future Directions: A Narrative Review

Zainab Khalid Khaleel [1], Zinah Makki Al-Yozbakee [2,*], Farqad M. Alhamdani [1], Ban M. Saeed [1]

[1]*Department of Microbiology, Al-Zahraa College of Medicine, University of Basrah, Basrah, Iraq*
[2]*Department of Microbiology, College of Medicine, University of Mosul, Mosul, Iraq*

[*]*Corresponding author email: zeenayouzbaki@uomosul.edu.iq*

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ABSTRACT

Chimeric antigen receptor T-cell (CAR-T) therapy is a landmark of oncologic immunotherapy, transforming care in selected hematologic malignancies and expanding toward solid tumors and autoimmune diseases. Durable remissions in leukemia and lymphoma are well established, whereas signals in solid tumors remain limited to early-phase studies and a few randomized evaluations. Key challenges persist, including therapy-related toxicities (notably cytokine release syndrome [CRS] and immune effector cell–associated neurotoxicity syndrome [ICANS]), antigen escape and other mechanisms of resistance within the immunosuppressive tumor microenvironment, and substantial logistical and cost barriers. This narrative review synthesizes clinical evidence from phase I–III trials and large real-world cohorts and summarizes advances in manufacturing, delivery, and toxicity mitigation. Literature was identified through PubMed, Embase, Scopus, Web of Science, and major publisher platforms (January 1, 2010–May 31, 2025) using terms such as "chimeric antigen receptor," "CAR-T," "bispecific CAR-T," "dual-targeting CAR-T," and "solid tumor CAR-T"; high-quality systematic reviews and meta-analyses informed context. Emerging innovations include in vivo approaches using lipid-nanoparticle–encapsulated mRNA to program T cells within the patient, allogeneic "universal" CAR-T candidates edited by CRISPR/Cas9 (e.g., TRAC and B2M) to reduce alloreactivity and enable off-the-shelf use, and computational/AI-aided receptor design to optimize efficacy and predict toxicity. Overall, CAR-T therapy continues to evolve with promising strategies to enhance outcomes and accessibility; however, broader confirmation in well-powered trials, biomarker-guided selection, scalable manufacturing, and equitable cost models remain essential for a widespread impact in refractory diseases.

Key words: CAR-T cell therapy; Hematologic malignancies; Toxicities; Cytokine release syndrome; Gene editing.

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INTRODUCTION

Chimeric antigen receptor (CAR) T-cell therapy redirects genetically modified T lymphocytes to recognize and eliminate malignant cells and has transformed the management of selected hematologic malignancies, with expanding exploration in solid tumors and autoimmune disease [1, 2].

CAR T cells recognize tumor-associated antigens independently of major histocompatibility complex (MHC) presentation through an engineered receptor composed of: (i) an extracellular, antibody-derived single-chain variable fragment (scFv) that confers antigen specificity; (ii) a hinge and transmembrane region providing structural support; and (iii) intracellular signaling domains that trigger T-cell activation and expansion [1].

Clinical success in B-cell malignancies has catalyzed rapid translational efforts across additional indications, while iterative receptor engineering—notably the incorporation of CD28 or 4-1BB costimulatory modules in second-generation constructs—has improved persistence, proliferation, and cytotoxic activity [3].

Given the pace of development and the growing breadth of applications, this narrative review synthesizes recent progress, emphasizes clinically meaningful advances, and highlights technological and translational issues likely to shape the field, including construct optimization, genome editing, and in vivo delivery strategies [4].

CAR Structure and Generational Evolution

CARs are synthetic receptors introduced into T cells to enable MHC-independent recognition of tumor antigens [4].

The structure of a CAR-T consists of three basic modules: an scFv specificity module, a transmembrane segment, and intracellular signaling domains that activate cytotoxic responses [5]. First-generation CARs bearing CD3ζ alone showed limited in vivo persistence; second-generation designs added a single costimulatory domain (e.g., CD28 or 4-1BB) with markedly enhanced activity [6]. Third-generation CARs combine costimulatory elements to further strengthen signaling [7]. Fourth-generation platforms (TRUCKs) include transgenes such as IL-12 to modulate the tumor microenvironment, and emerging fifth-generation designs incorporate additional signaling motifs to improve adaptability, safety, and antitumor potency [8].

Gene Editing Enhancements via CRISPR/Cas9

CRISPR/Cas9 editing enables targeted modifications that can augment CAR-T cell performance. Reported strategies include disruption of inhibitory receptors upregulated in the tumor microenvironment (e.g., PD-1, CTLA-4, LAG-3) to mitigate exhaustion, as well as edits in genes governing differentiation or apoptosis to improve persistence [9].

Genome-wide CRISPR screens have uncovered tumor-intrinsic resistance mechanisms, including altered antigen presentation and immunosuppressive ligand expression—that nominate combinatorial targets [10]. Multiplex editing (e.g., TCR and MHC components) supports development of "off-the-shelf" allogeneic products; while off-target concerns remain, higher-fidelity nucleases and improved screening have increased safety margins [11].

Delivery Innovations: mRNA–LNP and In Vivo Reprogramming

In vivo reprogramming with lipid-nanoparticle (LNP)-encapsulated mRNA offers a manufacturing-sparing alternative to ex vivo cell processing, enabling transient expression of CARs within the patient and potentially reducing time, infrastructure, and cost [12]. Preclinical studies demonstrate robust CAR-T cell expansion, antitumor activity, and favorable tolerability in hematologic and autoimmune models [13]. Temporal control over expression may help mitigate long-term toxicities such as cytokine release syndrome (CRS) and neurotoxicity, while the modularity of mRNA facilitates rapid iteration for personalized applications [14].

METHODOLOGY

The study is a narrative review intended to integrate clinical experience and research progress in CAR-T cell therapy across hematologic malignancies, solid tumors, and emerging non-oncology indications. The aim is synthesis and interpretation rather than quantitative meta-analysis.

Information sources and search approach

Literature was identified in PubMed/MEDLINE, Embase, Scopus, Web of Science, and major publisher platforms (SpringerLink, ScienceDirect). Limited grey literature (regulatory communications and major conference abstracts) was consulted only when it clarified peer-reviewed findings. The search window spanned January 1, 2010, to May 31, 2025 (last search). Controlled vocabulary and free-text terms combined therapy, targets, indications, and development concepts, for example: "CAR-T cell*" OR "chimeric antigen receptor T" AND (CD19 OR BCMA OR CLDN18.2 OR HER2 OR GD2 OR MUC1 OR EGFR) AND (leukemia OR lymphoma OR myeloma OR "solid tumor*" OR gastric OR pancreatic OR sarcoma OR glioma OR autoimmune OR lupus) AND (trial OR phase OR toxicity OR CRS OR ICANS OR manufacturing OR cost OR access OR "in vivo" OR mRNA OR allogeneic). A database-by-database summary appears in Table 1.

Table 1. Search strategy for PubMed, Embase, Scopus, and publisher platforms.

Database	Date range	Search terms used (examples)	Search notes
PubMed/MEDLINE	Jan 1, 2010–May 31, 2025	"chimeric antigen receptor", "CAR-T cell", "bispecific CAR-T", "dual-targeting CAR-T", "allogeneic CAR-T", "solid tumor CAR-T"	MeSH + free text; limited to title/abstract/indexed terms.
Embase	Jan 1, 2010–May 31, 2025	Same terms as PubMed.	Emtree controlled vocabulary + keywords.
Scopus (Elsevier)	Jan 1, 2010–May 31, 2025	Title/Abstract/Keyword: as above; additional target terms (CD19, BCMA, CLDN18.2, HER2, GD2, MUC1, EGFR).	Subject filters (Medicine, Biochemistry, Immunology); English only.
SpringerLink; ScienceDirect	Jan 1, 2010–May 31, 2025	All fields: as above; "in vivo mRNA"; "LNP"; "allogeneic/off-the-shelf".	Refined by discipline (Oncology, Immunology, Cell Biology); article type: journal articles.

Abbreviations: LNP, lipid nanoparticle; MeSH, Medical Subject Headings.

Eligibility and selection

Priority was given to peer-reviewed clinical evidence (phase I–III trials, prospective/retrospective cohorts, and large real-world series). High-quality narrative/scoping reviews were used for context. Select preclinical or methodological reports were included when directly informative for mechanisms, manufacturing, or platform innovation. Single-patient anecdotes without broader relevance, non-English articles when an English equivalent existed, and sources lacking methodological clarity were excluded. Conference abstracts were generally excluded, with exceptions for first-in-human results or essential regulatory information not yet available as full papers.

Data extraction and synthesis

From eligible clinical studies, extracted items included indication; target/product; study design and phase; sample size; key efficacy outcomes (objective response rate, complete response, progression-free and overall survival); and safety signals, with emphasis on cytokine release syndrome (CRS) and immune effector cell–associated neurotoxicity syndrome (ICANS). Findings were synthesized narratively and organized thematically (hematologic malignancies, solid tumors, non-oncology indications, and manufacturing/access). Formal risk-of-bias scoring was *not* performed; instead, evidence was appraised qualitatively, noting common limitations (single-arm designs, small cohorts, short follow-up, and heterogeneity of products, dosing, and supportive care). Statements of efficacy or safety are linked to primary sources within the text.

Ethics

No human participants or identifiable data were involved; ethical approval and patient consent were not required.

RESULTS OF LITERATURE REVIEW AND DISCUSSION

A recent landscape analysis of registered CAR-T cell trials (n=1,580; April 2024) reported a strong predominance of hematologic indications (71.6%) versus solid tumors (24.6%) and autoimmune disease (2.8%), with the majority of studies in early phases, reflecting robust translational activity but limited late-stage validation [15]. Design trends show increasing use of dual-antigen targeting, armored constructs, and combinations with checkpoint modulation to enhance persistence and antitumor activity [5]. Streamlined and decentralized manufacturing concepts are emerging, including lipid–nanoparticle (LNP)–encapsulated mRNA for *in vivo* CAR expression; preclinical murine and primate data support dose-dependent activity, and first-in-human evaluations have begun in autoimmune indications [16].

Signals of activity in solid tumors remain mixed but noteworthy. A randomized phase II study in gastroesophageal junction/gastric cancer reported improvements in median overall survival (7.9 vs. 5.5 months) and progression-free survival (3.3 vs. 1.8 months) with CAR-T cell therapy compared with standard care, marking an important step toward controlled evidence in solid malignancies [17]. For central nervous system tumors, intrathecal delivery of dual-target CAR T cells (EGFR and IL-13Rα2) for recurrent glioblastoma produced early antitumor responses, although durability was limited, underscoring challenges posed by antigen heterogeneity and immune evasion in the CNS [18]. Beyond oncology, early case series in systemic lupus erythematosus demonstrate profound B-cell depletion and durable clinical remission in refractory disease, suggesting a potential new domain of application [19].

Table 2 summarizes representative advances spanning *in vivo* delivery, universal allogeneic platforms, CRISPR-enabled engineering, and exploratory work in autoimmune disease and solid tumors.

While CD19- and BCMA-directed products have reshaped outcomes in relapsed/refractory ALL, NHL, and multiple

Table 2. Recent advances in CAR-T cell therapy (2020–2025).

Advance	Key findings	Ref.
In vivo CAR T via mRNA–LNP	LNP-encapsulated mRNA enables direct, transient CAR expression in patients without ex vivo manufacturing; dose-dependent activity shown in animal/primate models; early clinical testing underway.	[20]
In vivo CAR T clinical entry	First-in-human studies targeting CD19 for autoimmune indications have initiated, representing a key translational milestone.	[21]
Universal off-the-shelf CAR T via CRISPR	CRISPR/Cas9-edited anti-CD19 CAR T cells (e.g., TRAC/B2M) exhibit long-memory phenotypes, strong *in vitro* activity, and reduced alloreactivity.	[22]
Checkpoint-knockout CAR T engineering	Disruption of PD-1/CTLA-4 enhances persistence and activity; potential to lower manufacturing cost in simplified platforms.	[23]
Bispecific CAR T in solid tumors	Bispecific designs (e.g., MUC1, CLDN6) demonstrate target specificity and early safety/feasibility in lung and ovarian settings.	[24]
CAR T in autoimmune disease	Autologous and allogeneic CD19 CAR T cells induce rapid B-cell aplasia and clinical remission in refractory SLE and related disorders.	[25]
Fibrosis-targeted CAR T (preclinical)	Fibroblast-specific CAR T cells reduce pathological fibrosis and improve metabolic profiles in cardiac models.	[26]
Non-viral CAR knock-in via CRISPR	HDR-based, non-viral integration achieves accurate, efficient CAR insertion in patient-derived T cells and can improve dose uniformity.	[27]
Solid-tumor engineering strategies	Reviews highlight logic-gated/armored, multiplexed, and scaffold-enabled approaches—including TRUCKs and bispecific CARs— to address TME barriers.	[28]

Abbreviations: LNP, lipid nanoparticle; HDR, homology-directed repair; TME, tumor microenvironment.

myeloma [29], claims of uniformly high complete remissions (e.g., 85–97%) require product- and cohort-specific context and careful attribution to pivotal trials [30, 31]. Solid-tumor progress remains constrained by antigen heterogeneity, hostile tumor microenvironments, and trafficking barriers; nevertheless, the randomized gastric/GEJ signal [32] and early studies in lung/ovarian cancer (MUC1/CLDN6) [33] justify continued, rigorously controlled development.

Toxicities remain central barriers to broader adoption. Cytokine release syndrome and ICANS demand standardized grading and prompt intervention (e.g., tocilizumab for CRS and judicious corticosteroids), recognizing potential impacts on efficacy [34–36]. On-target/off-tumor effects—including B-cell aplasia after CD19 targeting—and antigen overlap driving mucositis or cytopenias necessitate continued pursuit of truly tumor-restricted targets and smart control circuits. Manufacturing and access challenges persist: current autologous processes require weeks, bridging therapy, and stringent quality controls [37, 38]; costs for approved products exceed $350,000 per infusion in many systems, exclusive of hospitalization and supportive care [39, 40]. Point-of-care manufacturing, universal allogeneic platforms, and *in vivo* generation may mitigate these constraints but face regulatory, safety, and scale-up hurdles [40, 41]. Table 3 outlines key challenges and mitigation concepts (42–48). Looking ahead, logic-gated and switchable receptors, multiplex antigen strategies, and AI-enabled design and biomarker selection may improve precision and durability, while CRISPR-based allogeneic programs promise broader access if safety is maintained [42].

Table 3. Recent challenges and limitations in CAR-T cell therapy and candidate mitigation strategies.

Challenge	Key insights from recent publications	Proposed mitigation strategies	Ref.
Tumor microenvironment (TME)	Immunosuppressive myeloid cells (TAMs), Tregs, hypoxia, metabolic competition, and dense ECM limit CAR-T cell function in solid tumors.	Local cytokine delivery (*armored* CARs), anti-VEGF/chemokine targeting, ECM remodeling, and scaffold-supported delivery.	[43, 44]
CRS & ICANS toxicity	Recognition and management have improved; neurotoxicity rates can approach ~30–65% in selected cohorts, varying by product and grading.	Early grading; tocilizumab for CRS; corticosteroids when indicated; prophylactic pathways and close neurologic monitoring.	[45]
On-target/off-tumor toxicity	Antigen overlap with healthy tissues produces autoimmune-like effects (e.g., B-cell aplasia with CD19).	Logic-gated/affinity-tuned CARs; suicide genes or drug-inducible off-switches; combinatorial targeting to increase specificity.	[46]
Manufacturing complexity & cost	Lengthy autologous processes, variable batch quality, and viral-vector dependency hinder scale and equity.	Non-viral gene delivery; *in vivo* CAR T generation; point-of-care production; allogeneic (off-the-shelf) platforms.	[47]
Antigen heterogeneity & escape	Antigen loss/downregulation (e.g., 30–50% CD19 loss in B-ALL) and spatial heterogeneity limit durability, especially in solid tumors.	Multispecific/tandem CARs; oncolytic/conditioning combinations; modeling-informed precision design and adaptive targeting.	[48]
Checkpoint-mediated exhaustion	PD-1 and other checkpoints induced by the TME reduce persistence and effector function.	Combination with checkpoint blockade; armored CARs expressing checkpoint antagonists; metabolic rewiring and combination immunotherapy.	[49]

Abbreviations: TAMs, tumor-associated macrophages; ECM, extracellular matrix; CRS, cytokine release syndrome; ICANS, immune effector cell–associated neurotoxicity syndrome; TME, tumor microenvironment.

CONCLUSION

CAR-T cell therapy has transformed outcomes in selected hematologic malignancies and is being explored in solid tumors and autoimmune disease. Despite compelling remissions in B-cell cancers, broader impact is limited by acute toxicities (CRS, ICANS), antigen escape, hostile tumor microenvironments, and the cost and complexity of individualized manufacturing. Emerging solutions—including logic-gated and multispecific receptors, armored designs, non-viral and CRISPR-enabled engineering for allogeneic "off-the-shelf" products, and *in vivo* mRNA–LNP programming—may improve efficacy, safety, and access. Real progress now depends on well-powered randomized trials in solid tumors and non-oncology indications, validated biomarkers for patient selection and response monitoring, scalable and equitable manufacturing models, and standardized toxicity prevention and outpatient pathways. As synthetic biology, computational design, and systems immunology converge, CAR T therapy is poised to extend beyond oncology into immune-mediated disorders; realizing that promise will require parallel advances in scientific innovation, implementation, and affordability.

ETHICAL DECLARATIONS

Ethics Approval and Consent to Participate

Not required

Consent for Publication

None.

Availability of Data and Material

None.

Competing Interests

The authors declare that there is no conflict of interest.

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Use of Generative Artificial Intelligence

The authors declare that no generative AI tools were used in the preparation, writing, or editing of this manuscript.

Authors' Contributions

All authors contributed to the literature review, study design, data collection, and manuscript preparation. All authors have read and approved the final version of the manuscript.

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