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A review on CAR-T cell: Precise and personalized killer of tumor cells

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Abstract

Chimeric antigen receptor T-cell (CAR-T cell) therapy represents a transformative advancement in cancer immunotherapy, offering a highly precise and personalized approach to tumor eradication. By genetically engineering autologous T lymphocytes to express synthetic receptors that recognize tumor-associated antigens in a major histocompatibility complex-independent manner, CAR-T cells overcome key mechanisms of tumor immune evasion. Remarkable clinical success has been achieved in hematological malignancies, particularly B-cell acute lymphoblastic leukemia and diffuse large B-cell lymphoma, leading to multiple regulatory approvals and establishing CAR-T therapy as a novel therapeutic paradigm. Advances in CAR design, including the incorporation of co-stimulatory signaling domains and next-generation engineered constructs, have significantly improved T-cell persistence, expansion, and antitumor efficacy. Despite these achievements, challenges such as cytokine release syndrome, neurotoxicity, antigen escape, and limited efficacy in solid tumors continue to restrict broader clinical application. This review summarizes the historical evolution, structural design, and mechanism of action of CAR-T cells, highlights key clinical milestones, and discusses current limitations alongside emerging strategies aimed at enhancing safety, durability, and therapeutic scope. Collectively, CAR-T cell therapy exemplifies a powerful precision medicine approach with the potential to redefine cancer treatment.

Keywords: CAR-T cells, immunotherapy, adoptive cell therapy, t-cells

1. Introduction

Cancer immunotherapy has revolutionized modern oncology by exploiting the immune system's capacity to detect and eradicate malignant cells (June & Sadelain, 2018) ^[11]. Among the most innovative approaches is Chimeric Antigen Receptor T-cell (CAR-T cell) therapy, a form of adoptive cell transfer in which autologous T lymphocytes are genetically engineered to express synthetic receptors that recognize tumor-associated antigens with high specificity (Eshhar *et al.*, 1993; Gross *et al.*, 1989) ^[4, 7].

Unlike conventional T-cell receptor (TCR) signaling, which requires antigen presentation via the major histocompatibility complex (MHC), CAR-T cells recognize surface antigens in an MHC-independent manner, enabling effective targeting of tumor cells that evade immune surveillance (June & Sadelain, 2018) ^[11]. Structurally, CARs are composed of an extracellular antigen-recognition domain derived from a monoclonal antibody, a transmembrane region, and intracellular signaling domains responsible for T-cell activation and persistence (Kalos *et al.*, 2011) ^[15].

The incorporation of co-stimulatory domains such as CD28 and 4-1BB has markedly enhanced CAR-T cell proliferation, survival, and cytotoxicity, establishing CAR-T cells as precise and personalized killers of tumor cells (Imai *et al.*, 2004; Porter *et al.*, 2011) ^[8, 15]. These advancements have led to unprecedented clinical responses, particularly in hematological malignancies including B-cell acute lymphoblastic leukemia (B-ALL) and diffuse large B-cell lymphoma (DLBCL) (Kalos *et al.*, 2011) ^[15].

The clinical impact of CAR-T therapy was formally recognized with the approval of the first CAR-T cell products by the U.S. Food and Drug Administration in 2017, marking a milestone in gene and cell-based cancer therapies (Food and Drug Administration [FDA], 2017) ^[5].

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Despite these successes, challenges such as cytokine release syndrome, immune effector cell-associated neurotoxicity, antigen escape, and limited efficacy in solid tumors continue to drive innovation in CAR design and therapeutic strategies (June & Sadelain, 2018) ^[11]. Understanding the historical progression of CAR-T cell development provides a critical foundation for appreciating current advances and future directions in this rapidly evolving field.

1.1 Historical evolution of car-t cell therapy

The conceptual framework for CAR-T cell therapy originated in the late 1980s, when researchers demonstrated that antibody-derived antigen recognition domains could be fused with T-cell signaling components to create hybrid receptors capable of antigen-specific activation (Gross *et al.*, 1989) ^[7]. This foundational discovery paved the way for the development of first-generation CARs in the early 1990s.

First-generation CARs consisted of a single-chain variable fragment (scFv) linked to the CD3 ζ signaling domain. While these constructs enabled antigen-specific T-cell activation, they lacked adequate co-stimulatory signals, resulting in limited proliferation and poor *in vivo* persistence (Eshhar *et al.*, 1993; Brocker & Karjalainen, 1995) ^[7, 4].

Subsequent incorporation of co-stimulatory domains such as CD28 or 4-1BB led to the development of second-generation CARs, which exhibited significantly improved expansion, survival, and antitumor efficacy (Imai *et al.*, 2004) ^[8]. These improvements culminated in landmark clinical trials demonstrating durable remissions in patients with refractory B-cell malignancies (Kalos *et al.*, 2011; Porter *et al.*, 2011) ^[15]. The approval of CAR-T therapies such as tisagenlecleucel and axicabtagene ciloleucel in 2017 marked a pivotal milestone in gene and cell-based cancer therapies (FDA,

2017). Current research efforts are focused on next-generation CAR designs, including armored CARs, multi-antigen targeting constructs, and allogeneic “off-the-shelf” CAR-T cells aimed at enhancing safety, scalability, and efficacy in solid tumors (Rafiq *et al.*, 2019) ^[16].

1.2 Tumor microenvironment and Immune evasion

Tumor progression is governed by a highly complex and dynamic tumor microenvironment (TME) composed of malignant cells, stromal elements, and diverse immune populations. Although cytotoxic CD8⁺ T cells and natural killer (NK) cells play central roles in antitumor immunity through the secretion of interferon- γ and cytolytic enzymes, tumors frequently establish immunosuppressive conditions that inhibit these effector functions (Mittal *et al.*, 2014) ^[14].

Immunosuppressive cell populations, including regulatory T cells, myeloid-derived suppressor cells, and M2-polarized macrophages, actively secrete inhibitory cytokines such as IL-10 and TGF- β , thereby dampening immune responses (Grivnikov *et al.*, 2010) ^[6]. Additionally, tumor cells downregulate MHC class I expression and activate immune checkpoint pathways involving PD-1 and CTLA-4, leading to T-cell exhaustion and immune tolerance (Intlekofer & Thompson, 2013) ^[9]. This hostile immunological environment presents a significant barrier to effective immunotherapy. CAR-T cells, while capable of bypassing MHC restrictions, still face challenges related to immunosuppressive signaling, antigen heterogeneity, and limited trafficking within solid tumors. Overcoming the TME remains a critical objective in advancing CAR-T therapy beyond hematological malignancies.

1.3 Car Structure

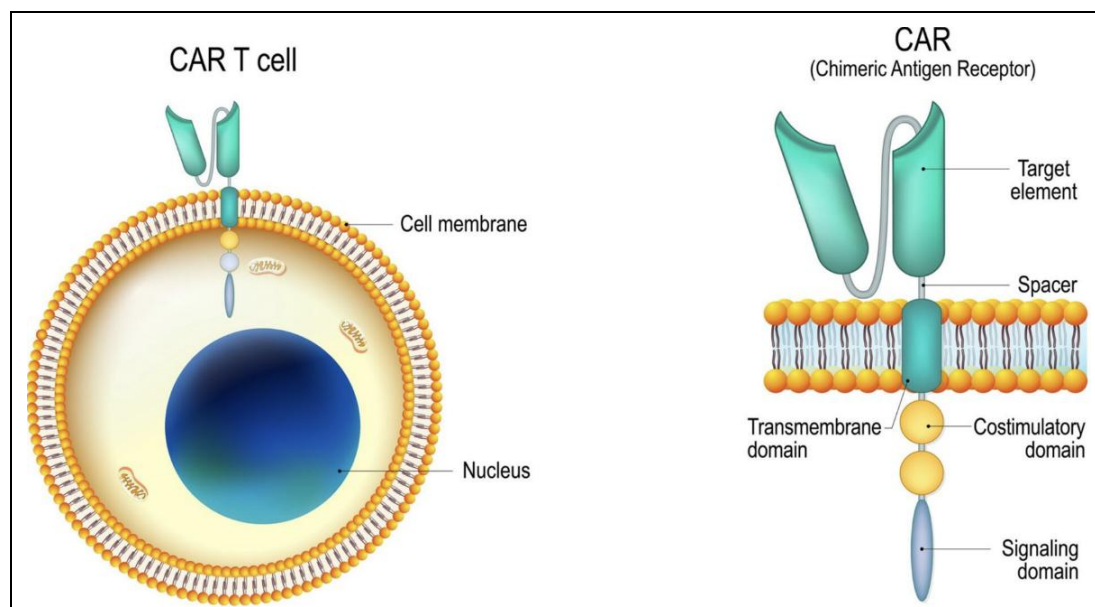


Fig 1: Different parts of CAR

Overview of Chimeric Antigen Receptor Architecture

Chimeric antigen receptors are synthetic, single-chain receptors engineered to integrate antigen recognition and T-cell activation into a unified molecular framework. Unlike native T-cell receptors (TCRs), which require peptide antigens to be presented by MHC molecules, CARs directly recognize intact surface antigens expressed on tumor cells (Gross *et al.*, 1989; June & Sadelain, 2018) ^[7]. This fundamental distinction allows CAR-T cells to overcome

tumor immune evasion strategies such as MHC down regulation.

Structurally, CARs consist of four modular domains:

- Extracellular antigen-binding domain
- Hinge or spacer region
- Transmembrane domain
- Intracellular signaling domains

The modular nature of CARs enables systematic optimization of each domain to tailor receptor performance for specific tumor antigens, tissue contexts, and clinical applications. Importantly, CAR function emerges not from individual domains in isolation but from the integrated interaction of all components, underscoring the importance of holistic receptor design (Sadelain *et al.*, 2013) ^[17].

2. Extracellular Antigen-Binding Domain

2.1 Molecular Basis and Structural Organization

The extracellular antigen-binding domain determines the specificity of CAR-T cells toward tumor-associated antigens (TAAs). In most CAR constructs, this domain is derived from monoclonal antibodies and formatted as a single-chain variable fragment (scFv). The scFv consists of the variable heavy (VH) and variable light (VL) regions connected by a flexible linker, typically composed of glycine and serine residues to preserve conformational flexibility (Gross *et al.*, 1989) ^[7].

This antibody-derived configuration allows CAR-T cells to recognize antigens in their native conformations on the tumor cell surface, bypassing antigen processing and MHC presentation. As a result, CAR-T cells can target tumors that evade immune surveillance by altering antigen processing machinery (June & Sadelain, 2018) ^[11].

2.2 Antigen Density, Affinity, and Activation Thresholds

The interaction between scFv affinity and antigen density critically influences CAR-T cell activation. High-affinity scFvs can detect low antigen densities, enhancing tumor sensitivity; however, excessive affinity may promote tonic signaling, a phenomenon characterized by ligand-independent CAR clustering and chronic activation (Caruso *et al.*, 2015) ^[3]. Tonic signaling accelerates T-cell exhaustion, reduces proliferative capacity, and compromises long-term persistence.

Conversely, lower-affinity scFvs may improve selectivity for tumor cells over normal tissues, thereby reducing on-target, off-tumor toxicity. This balance between affinity and specificity is particularly important when targeting antigens that are not strictly tumor-exclusive.

2.3 Antigen Escape and Target Heterogeneity

Tumor antigen heterogeneity and antigen loss represent major challenges to CAR-T cell therapy. Selective pressure exerted by CAR-T cells can result in the outgrowth of antigen-negative tumor variants. Structural strategies to mitigate antigen escape include the development of dual-target CARs, tandem CARs, and logic-gated CAR systems, which rely on simultaneous recognition of multiple antigens (Rafiq *et al.*, 2019) ^[16].

2.4 Alternative Antigen-Binding Platforms

To overcome limitations associated with scFv-based CARs, alternative antigen recognition platforms have been developed. These include nano body-based CARs, which utilize single-domain antibodies with reduced immunogenicity, and TCR-like CARs, which recognize peptide-MHC complexes and expand the range of targetable intracellular antigens (Rafiq *et al.*, 2019) ^[16].

3. Hinge or Spacer Region

3.1 Structural and Biophysical Role

The hinge or spacer region connects the antigen-binding domain to the transmembrane domain and provides the

flexibility necessary for effective antigen engagement. This region determines the spatial orientation of the scFv relative to the tumor cell membrane and influences immunological synapse formation (Sadelain *et al.*, 2013) ^[17]. Common hinge sources include immunoglobulin Fc domains (IgG1 or IgG4) and sequences derived from CD8 α or CD28 molecules.

3.2 Spacer Length and Epitope Accessibility

Spacer length is a critical determinant of CAR function. Antigens located close to the tumor cell membrane require longer spacers to enable effective scFv engagement, whereas membrane-distal antigens are more effectively targeted with shorter spacers (Jensen & Riddell, 2015) ^[10]. Improper spacer length can impair synapse formation, reduce signal transduction, and limit cytotoxic efficacy.

3.3 Immunological Consequences and Safety

IgG-derived hinge regions may interact with Fc γ receptors expressed on innate immune cells, potentially triggering unintended immune activation or CAR-T cell clearance. To mitigate these risks, Fc regions are often modified to eliminate Fc receptor binding motifs, thereby enhancing CAR-T cell persistence and safety (Jensen & Riddell, 2015) ^[10].

4. Transmembrane Domain

4.1 Anchoring and Structural Stability

The transmembrane domain anchors the CAR molecule within the T-cell membrane and provides structural continuity between extracellular and intracellular domains. This hydrophobic α -helical segment is commonly derived from CD3 ζ , CD4, CD8 α , or CD28 (Sadelain *et al.*, 2013) ^[17].

4.2 Influence on CAR Expression and Assembly

The transmembrane domain affects receptor surface expression, stability, and assembly. CD3 ζ -derived transmembrane domains may associate with endogenous TCR complexes, potentially enhancing signaling strength but increasing the risk of receptor instability. In contrast, CD8 α and CD28-derived transmembrane domains generally confer improved stability and predictable signaling behavior (Brentjens *et al.*, 2003) ^[1].

4.3 Role in Signal Modulation

The transmembrane domain contributes to CAR clustering and oligomerization at the immunological synapse. These interactions influence activation thresholds, cytokine secretion profiles, and exhaustion kinetics, underscoring the importance of transmembrane domain selection in CAR design (Rafiq *et al.*, 2019) ^[16].

5. Intracellular Signaling Domains

5.1 Activation Domain: CD3 ζ Signaling

The intracellular signaling region initiates T-cell activation following antigen engagement. The CD3 ζ chain, which contains three immunoreceptor tyrosine-based activation motifs (ITAMs), is the most commonly used activation domain. Upon antigen binding, ITAM phosphorylation activates downstream signaling cascade involving ZAP-70, LAT, and transcription factors such as NF- κ B, NFAT, and AP-1 (June & Sadelain, 2018) ^[11]. First-generation CARs containing only CD3 ζ signaling demonstrated limited persistence and poor clinical efficacy, highlighting the necessity of co-stimulatory signaling (Brocker & Karjalainen, 1995) ^[2].

5.2 Co-Stimulatory Domains and T-Cell Fate

Co-stimulatory domains enhance CAR-T cell expansion, survival, metabolic fitness, and memory formation. Common co-stimulatory molecules include CD28, 4-1BB (CD137), ICOS, OX40, and CD27 (Imai *et al.*, 2004) ^[8]. CD28 signaling promotes rapid effector differentiation and glycolytic metabolism, whereas 4-1BB signaling supports mitochondrial biogenesis, oxidative metabolism, and long-term persistence. These differences have important implications for clinical outcomes and toxicity profiles (June & Sadelain, 2018) ^[11].

5.3 Advanced and Next-Generation Signaling Platforms

Fourth-generation CARs, also known as TRUCKs, incorporate inducible cytokine expression modules to reshape the tumor microenvironment. Fifth-generation CARs integrate cytokine receptor signaling domains, such as IL-2R β motifs, to further enhance proliferation and resistance to immune-suppressive signals (Rafiq *et al.*, 2019) ^[16].

6. Integrated Structure–Function Relationships

CAR-T cell behavior emerges from the coordinated interaction of all structural domains. Spacer length influences synapse formation, which affects the magnitude and duration of intracellular signaling. Co-stimulatory signaling modulates metabolic programming, differentiation, and exhaustion, while trans membrane domains influence receptor clustering and stability (Sadelain *et al.*, 2013) ^[17]. Rational CAR design therefore requires a holistic approach that considers how modifications to individual domains propagate through the entire receptor system.

6.1 Tumor Killing Mechanism

CAR-T cells mediate tumor destruction through multiple cytotoxic pathways. The primary mechanism involves the release of cytotoxic granules containing perforin and granzymes, which induce apoptotic cell death following immunological synapse formation (Stinchcombe *et al.*, 2006) ^[18]. In addition to granule-mediated killing, CAR-T cells can activate death receptor pathways such as the Fas/FasL axis, leading to caspase-dependent apoptosis (Martínez-Lostao *et al.*, 2015) ^[15]. Cytokine secretion by activated CAR-T cells further amplifies antitumor responses by modulating the tumor microenvironment, promoting macrophage polarization, and facilitating antigen-independent bystander killing (Textor *et al.*, 2014) ^[19].

6.2 FDA approved car-t cell therapy

To date, several CAR-T products targeting CD19 and B-cell maturation antigen (BCMA) have received regulatory approval for the treatment of relapsed or refractory hematological malignancies. These therapies have demonstrated high response rates in acute lymphoblastic leukemia, large B-cell lymphoma, mantle cell lymphoma, follicular lymphoma, and multiple myeloma, underscoring the transformative potential of CAR-T therapy (FDA, 2017-2022) ^[5].

7. Conclusion

CAR-T cell therapy represents one of the most significant breakthroughs in modern oncology, particularly for the treatment of hematological malignancies. The modular design of CARs enables extensive customization to optimize antigen recognition, signalling strength, and cellular persistence. However, challenges such as treatment-related toxicities,

tumor resistance mechanisms, limited efficacy in solid tumors, and high manufacturing costs continue to restrict broader clinical implementation. Comparative oncology models and ongoing technological innovations are expected to play a crucial role in overcoming these limitations.

8. Future Perspectives

Future advancements in CAR-T therapy will depend on large-scale clinical trials aimed at identifying predictors of efficacy and toxicity. The development of improved preclinical models is essential for anticipating adverse events such as cytokine release syndrome and neurotoxicity. Combinatorial strategies targeting multiple antigens and modulating the immunosuppressive tumor microenvironment are likely required to extend CAR-T therapy to solid tumors. Additionally, expanding the application of CAR-T cells to autoimmune and inflammatory diseases may open new therapeutic avenues.

Conflict of Interest

Not available

Financial Support

Not available

Reference

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