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REVIEW ARTICLE

In Cancer Immunotherapy Using CAR T Therapy and Emerging Advances in Stem Cell Therapy

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ABSTRACT: Chimeric antigen receptor (CAR) T-cell therapy has transformed cancer treatment by making precise antigen-specific tumor killing. However, its broader use remains limited by certain challenges such as T-cell exhaustion, short persistence, manufacturing complexity, high costs, and inconsistent patient-derived products. T-cell exhaustion is the progressive loss of function after chronic antigen exposure, while persistence describes the long-term survival of infused cells required for maintained tumor control. Recent innovations in stem cell reprogramming, or more specifically, induced pluripotent stem cells (iPSCs), allow for large-scale and easily gene-editable production of restored CAR T-cells with better longevity and stronger therapeutic function. This review goes over the advancements made in CAR receptor design from the first-generation models to the gene-edited armored CARs. It also includes workflows for generating iPSC-derived CAR T-cells and mentions strategies like cytokine engineering, receptor optimization, and gene editing to improve its effectiveness and safety. Clinical and preclinical findings are included too, adding extended persistence through co-stimulatory signaling, and promising early results in solid tumors such as gastric cancer and glioblastomas. Real-world impacts are also important, for example, the use of CAR T therapy in lower-income countries like India, and studies in China showed the survival benefits in patients with advanced cancers. These results show that the impact of CAR T therapy is already seen in clinics across different countries. Stem cell-based CAR T manufacturing is one of the biggest steps toward universal immunotherapies that can improve both access and patient outcomes in cancer treatment worldwide.

KEYWORDS: CAR T-cell Therapy, iPSC-derived CAR T-cells, Gene Editing, Cancer Immunotherapy, T-cell Exhaustion.

Introduction

Cancer immunotherapy uses the body's own immune system to find and kill harmful cells with the chimeric antigen receptor, with (CAR) T-cell therapy being one of the most important innovations in this field. In CAR T therapy, patient-derived T-cells or donor T-cells are genetically engineered to express synthetic receptors that identify specific tumor antigens, allowing MHC-free tumor cell killing.[1] This method has led to long-term recovery in some blood cancers, especially B-cell leukemia and lymphomas, frequently in patients who had no other remaining treatment alternatives.[2] A key factor in CAR T therapy is the balance of CD4+ helper cells, which support the immune response by producing cytokines and helping other cells, and CD8+ cytotoxic cells, which directly kill tumor cells. A balanced mix of these subsets has shown to improve persistence and overall outcomes.[3] However, this treatment still has many challenges. For example, autologous CAR T products are costly and time-consuming to produce; the engineered T-cells usually become exhausted, have limited persistence inside the body, and have reduced efficacy against solid tumors due to their immunosuppressive microenvironments. Manufacturing problems, donor cell limitations, and safety risks like cytokine release syndrome (CRS), neurotoxicity (ICANS), and graft versus host disease (GvHD) add even more problems when trying to make these therapies more widely available.[4] To find solutions to these problems, researchers are increasingly using induced pluripotent stem cells (iPSCs) as a renewable and easily engineerable source for CAR T manufacturing. iPSC-derived CAR T-cells can be produced at a large scale, gene edited to remove immunogenic features, add functional improvements, and be differentiated into balanced CD4+ helper and CD8+ cytotoxic subsets.[5] This review covers the biochemical advancements in CAR receptor design, the technical and clinical developments of iPSC-derived CAR T products, and the strategies being developed to move toward universal clinical cell-based immunotherapies.

Section 1: Evolution of CAR T Receptors Through Biochemical Innovation:

Over the last 2 decades, the chimeric antigen receptor (CAR) design has evolved from its basic prototype models into advanced therapeutic tools. The first-generation CARs were made of an antigen-binding single-chain variable fragment (scFv) linked to the CD247 signaling domain. This was enough to direct T-cell activation without the need for MHC, but they were very limited in their ability to expand, persist, and cause long-term tumor control.[6] Second-generation CARs added co-stimulatory domains such as CD28 or 4-1BB (CD137) improving T-cell activation and survival. The 4-1BB domain was shown to improve long-term persistence and boost mitochondrial fitness. CD28 provided faster expansion, but sometimes shorter activity and persistence.[7] These differences explain why some CAR T products persist for months or even years in patients, while others disappear faster. Third-generation CARs tried combining two co-stimulatory domains, CD28 and 4-1BB, together, but so far clinical outcomes have

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not shown clear advantages compared to second-generation CARs and, therefore, have led to mixed results.[8] The newest fourth-generation "armoured" CARs, also called TRUCKs, included additional features like cytokine secretion (IL-12 and IL-15) to resist tumor suppression and increase cell longevity. IL-15 helps CAR T-cells live longer in the body, while IL-12 can help beat the solid tumor microenvironment that normally blocks T-cells.[9] Safety switches, like inducible caspase-9, were also included and allowed the doctors to destroy the treatment quickly in case there was a toxic reaction from the patient. Finally, modern CAR design now uses gene editing tools like CRISPR to remove inhibitory pathways like PD-1 or TGFB-RII that normally cause T-cell exhaustion to help increase the persistence.[10] The structural and functional progression from first-generation CARs to fourth-generation armored CAR designs is shown in Figure 1.

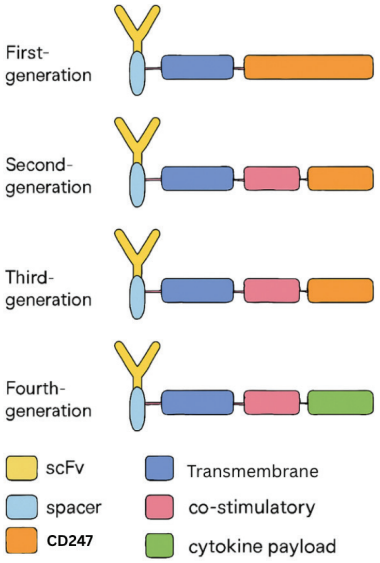

Figure 1: Structural evolution of CAR receptors from first to fourth generation designs. Figure created by Huseyn Ibrahimli. This progression shows how added co-stimulatory domains and cytokine engineering improved CAR T-cell persistence and effectiveness.

Section 2: Getting CAR T-Cells from Stem Cells:

The limits of autologous CAR T manufacturing, including the time, cost, donor variability, and complex procedures, have pushed a shift toward pluripotent stem cell products. iPSCs offer a limitless and easily engineered source for making CAR T-cells.[11] This scalability is especially important because iPSCs can be expanded much more easily while keeping the genetic stability, making them a better choice than donor-derived T-cells for regular production.[12] Research shows that mature T-cells can be reprogrammed into iPSCs and differentiated back into T-cells that keep their ability to target tumors.[13] The process of iPSC reprogramming, genome editing, and differentiation into functional CAR T-cells is shown in Figure 2. Clinical transition has focused on making the process safe and scalable, with GMP-compliant, Xeno-free, and feeder-free differentiation protocols.[14] Epigenetic tools like EZH1 inhibition have been used to improve cytokine secretion, cytotoxicity, and persistence *in vivo*.[15] In T-cell cancers, CD5-negative starting cells are used to avoid self-targeting.[16] At the same time, gene editing is used to delete TCR and HLA to reduce risks of GvHD and immune rejection, making "hypoimmunogenic" products that can be given to many patients without matching.[17] Several biotechnology companies like Fate Therapeutics are already testing these ideas, with iPSC-derived CAR T and NK therapies such as FT819 and FT576. Early results have shown good safety with no high-grade CRS or neurotoxicity.[18] Century Therapeutics is also developing iPSC-derived CAR NK products like Cnty 101, which has shown promising safety and activity in lymphoma.[19]

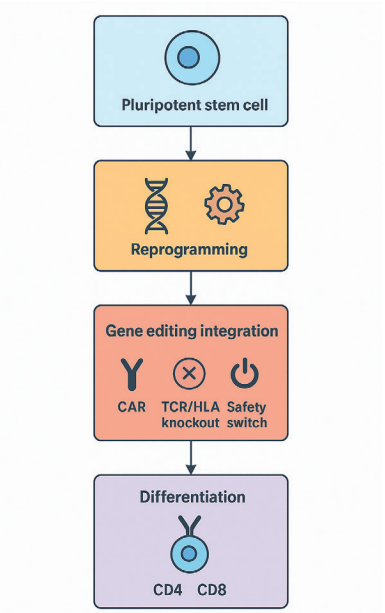

Figure 2: Workflow of iPSC reprogramming, genome editing, and differentiation into functional CAR T-cells. Figure created by Huseyn Ibrahimli. The workflow shows how stem cell engineering allows for scalable production of optimized CAR T-cells for broader clinical use.

Section 3: Enhancing CAR T Receptor Efficiency and Differentiation Strategies:

Enhancing receptor efficiency and cellular function is the main goal. Persistence is very important as long-lived CAR T-cells are strongly linked with durable remission. T-cell exhaustion happens when chronic antigen exposure decreases effector function, limiting its benefit. Strategies to improve persistence include co-stimulatory domains like 4-1BB.[20] Inserting cytokines like IL-15 under activation promoters can also help T-cells stay active longer without external support.[21] Deleting inhibitory receptors like PD-1 with CRISPR protects cells from exhaustion.[22] The balance of CD4 and CD8 subsets is another important factor since studies show that an even amount of CD4 to CD8 (about a 1:1 ratio) results in better persistence and antitumor activity.[23] Achieving a balanced CD4+ and CD8+ phenotype remains challenging for iPSC-derived products. Three-dimensional thymic organoid systems, including ligand 4 (DLL4), have been developed to restore physiological thymopoiesis.[24] Gene editing has also improved safety and performance. Deleting cytokine toxicity mediators like GM-CSF helps reduce CRS and neuroinflammation while keeping good antitumor activity.[25] Epigenetic modifications are being explored to make T-cells last longer

and resist exhaustion.[26] The receptor engineering approaches and iPSC differentiation strategies used to enhance CAR T-cell persistence and function are shown in Figure 3.

Category	Strategy	Tool	Benefits
receptor engineering	CRISPR editing	TGF βRII, GM CSF, IL 6, IL 15	IL 6, IL 15 Boost function, reduce toxicity
	Epigenetic tuning	TET2, PRDM1	Memory like phenotype, persistence
iPSC Differentiation	CD4+/CD8+ balance	Address CD8+ Bias	Improve helper/killer synergy
	Optimized iPSC source	CD62L+ naive/memory T cells	Longer lasting CAR-T cells
	3D organoid culture	MS5 DLL4 system	Better T cell maturation
	Epigenetic acceleration	EZH1 inhibition	Faster, feeder free T cell output

Figure 3: Summary of receptor engineering and iPSC differentiation strategies used to enhance CAR T-cell performance. Figure created by Huseyn Ibrahimli. These strategies improve CAR T-cell persistence, safety, and functionality in cancer treatment.

Clinical Developments and Real-World Impacts:

Clinical studies show the progress of CAR T in both blood cancers and solid tumors. In B-cell leukemias and lymphomas, remission rates of 70–90% have been reported.[27] Solid tumor progression is slower. A gastric cancer trial in China showed improved survival from 5.5 to 7.9 months.[28] Glioblastoma dual-target EGFR/IL13Ra2 CARs given into the cerebrospinal fluid caused tumor shrinkage in 62% of patients. Toxicities like CRS and ICANS remain challenges but are manageable.[27] Global access is also improving, with lower-cost CAR T therapy in India and the development of iPSC-derived products by industry.[29]

■ Conclusion

This review covered the evolution from early CAR receptor design to stem cell-based CAR T manufacturing and clinical use. CAR designs have evolved from first-generation models into advanced, gene-edited, multifunctional versions. iPSC-derived platforms allow large-scale universal products. Challenges like immune rejection, CD4/CD8 balance, neurological side effects, and solid tumor barriers remain. Advances in receptor design, differentiation methods, gene editing, and real-world clinical applications suggest a future with safer, more effective, globally accessible CAR T therapies.[1–29]

Declaration by Authors

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Authors

Huseyn Ibrahimli has an academic interest in cancer immunotherapy, specifically CAR T-cell therapy and gene editing strategies for cancer treatment. His work focuses on reviewing advances that improve the effectiveness, persistence, and accessibility of the next generation of immunotherapies.