Int. J. Sci. R. Tech., 2025 2(9)
A Multidisciplinary peer-reviewed Journal
www.ijsrtjournal.com [ISSN: 2394-7063]

Original Article

CAR-T Cells in Cancer Therapy: From Structural Blueprint to Clinical Barriers

Shruti Shinde*, Vaibhavi Jadhav, Nilima Yadav, Sampada Waghmare

Matoshri College of Pharmacy, Eklahare, Nashik, Maharashtra, India

ABSTRACT

Chimeric Antigen Receptor T-cell (CAR-T) therapy is a transformative immunotherapy, particularly effective against hematological malignancies. By engineering a patient's T-cells to express synthetic receptors targeting tumor-associated antigens, CAR-T combines the specificity of monoclonal antibodies with T-cell cytotoxicity. Over successive generations, CAR designs have incorporated costimulatory domains, cytokine support, and advanced signaling to enhance persistence and efficacy. The therapeutic process involves autologous T-cell isolation, ex vivo genetic modification, and reinfusion to selectively destroy cancer cells. Despite remarkable success in B-cell leukemias, lymphomas, and multiple myeloma, challenges such as cytokine release syndrome, neurotoxicity, antigen escape, and poor efficacy in solid tumors persist. Strategies like dual-targeting CARs, armored CARs, suicide switches, and synthetic circuits are being developed to improve safety and control. Future directions include universal allogeneic CAR-T cells, CRISPR-based multiplex editing, off-the-shelf platforms, and integration with checkpoint inhibitors, targeted agents, nanotechnology, and synthetic biology to optimize delivery and tumor infiltration.

Keywords: CAR-T cell therapy, Adoptive Cell Transfer, Cancer Immunotherapy, Cytokine release syndrome (CSR)

INTRODUCTION

Cancer, commonly known as a malignant tumor, arises from neoplastic tissues and can typically be described as a collection of modified cells that exhibit irregular, uncontrolled growth and have the ability to invade adjacent tissues and/or spread to nearby lymph nodes and/or distant organs[1] .For many years, conventional treatments like surgery and radiotherapy have been employed to address cancers; however, these approaches come with certain restrictions, particularly regarding side effects that often negatively impact the patient's quality of life. The GLOBOCAN 2018 Cancer Incidence and Mortality Estimates, produced by the International Agency for Research on Cancer (IARC), reveal that lung cancer ranks as the most prevalent malignancy, making up 11.6% of all cancer cases, and is currently the leading cause of cancer-related deaths, followed by breast, prostate, colon, stomach, and liver cancers, among others [2]. In individuals with metastatic cancer, immunotherapy has demonstrated long-lasting anti-tumor effects. It has been demonstrated that patients with melanoma can fully recover with adoptive cell treatment (ACT). This is predicated on the idea that endogenous T cells may be genetically altered in vitro to target and kill tumor cells precisely. The tumor can then be eradicated by re-infusing the cells into the patient's body. Several cancer types are now being treated with this strategy. The effectiveness of ACT and other immunotherapies is significantly influenced by the immunotargeting of mutant "neoantigens" expressed on tumor cells. Additionally, it implies that analyzing the genomes of malignancies would reveal possible antigens on all tumors, posing new opportunities and problems for ACT [3]. Cancer immunotherapy has revolutionized the treatment of cancers, with Chimeric Antigen Receptor (CAR) T cell therapy being a remarkable advancement, particularly in blood cancers. CAR T cells are a type of T lymphocyte derived from the patient that are genetically modified to produce synthetic receptors, allowing them to specifically target tumors without relying on MHC presentation [4]. While achieving success against targets such as CD19 in leukemias and lymphomas derived from B-cells, applying this success to solid tumors has been more challenging due to variations in antigens, immune suppression present in the tumor microenvironment, and the potential for on-target/off-tumor toxicity. To overcome these

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

challenges, focus has turned to neoantigens—specific antigens unique to tumors that arise from somatic mutations and are exclusive to cancer cells [5]. In contrast to shared tumor-associated antigens (TAAs), neoantigens are exclusively found in cancerous tissues, which reduces the likelyhood of triggering autoimmune responses and improves the accuracy of T cell targeting. This attribute makes neoantigen-targeted CAR T cell therapy a highly promising approach for tailored cancer treatment. New platforms that combine next-generation sequencing (NGS) with bioinformatics prediction algorithms enable the swift identification of immunogenic neoantigens in specific tumors [6]. By utilizing scFvs or TCR-like constructs that identify neoepitopes, scientists have started creating CAR T cells specific to neoantigens that can precisely target distinct tumor mutations like KRAS^G12D or EGFR^Viii [7]. Without utilizing a major histocompatibility complex, this type of therapy's primary benefit is its immediate identification and destruction of the tumor antigen [8]. Since they established the groundwork for CAR T cell therapy for the first time, Gross and his associates were regarded as its pioneers. "Chimeric T cell receptors with antitumor specificity will enable testing the feasibility of this approach in combating human tumors," they said as they wrapped up their work, demonstrating the idea of genetically rerouting cytotoxic T lymphocytes to the tumor cells [9,10,11]

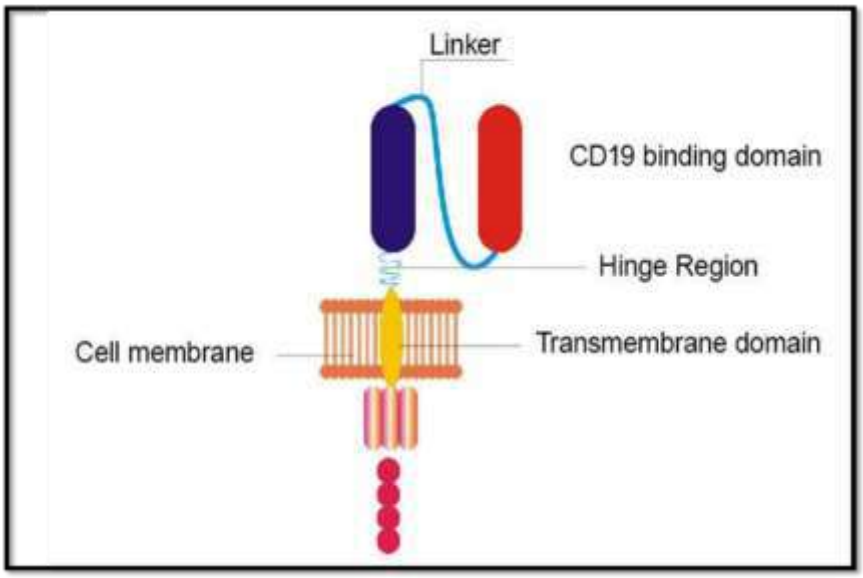

Figure 1: Structure of Car T Cell

CAR Structure:

Chimeric Antigen Receptor (CAR) T cells are synthetic T cells engineered to express a fusion receptor that enables them to recognize and kill cancer cells in an MHC-independent manner. The CAR molecule is composed of several modular domains, each contributing to antigen specificity, signal transduction, and T cell activation. The structure can be divided into 4 key components

1. Antigen Recognition Domain: -

The antigen recognition site recognizes antigens present in the Ectoderm of the receptor. This region is always exposed to the outside of the cell and interacts with the target antigen [12]. Single-chain variable fragment (scFv): Derived from the variable heavy (VH) and variable light (VL) chains of a monoclonal antibody. Responsible for recognizing and binding to the target antigen on tumor cells. These light and heavy chains are linked together with short linker peptides of Serine-glycine or glutamate –lysine [13].

2. Hinge/Spacer Region:

Usually located between the T cell's outer membrane and antigen recognition domain, the hinge region—also known as a spacer minuscule in comparison to the other receptor domain [14,15]. Provides flexibility and distance between the scFv and the cell membrane. Important for effective antigen binding, especially when the target epitope is close to the cell membrane. Derived from immunoglobulin (e.g., IgG1 Fc region), CD8α, or CD28 molecules. [16]

3. Transmembrane Domain: -

Anchors the CAR molecule to the T cell membrane. Commonly derived from CD3ζ, CD4, CD8, or CD28. Influences the stability and expression of the CAR on the T cell surface [17,18] It stabilizes the complete chimeric antigen receptor through a hydrophobic alpha-helix structure. The CD28 transmembrane domain is known to result in a highly expressed, stable receptor [19].

4. Intracellular Signaling Domains: -

CAR T cells are categorized into generations based on the number and type of intracellular signaling domains:

- First-generation CARs

Contain only the CD3ζ chain, which transmits the primary activation signal (Signal 1). Limited persistence and efficacy in vivo.

- Second-generation CARs

Include CD3ζ plus one co-stimulatory domain such as CD28 or 4-1BB (CD137). Provide both Signal 1 and Signal 2, improving T cell proliferation, survival, and antitumor activity.

- Third-generation CARs

Contain CD3ζ and two co-stimulatory domains, e.g., CD28 + 4-1BB.Designed to enhance T cell activation, but clinical benefits over second-generation CARs remain under investigation.

- Fourth-generation CARs (TRUCKs)

“T cells Redirected for Universal Cytokine Killing “Combine CAR structure with inducible cytokine genes (e.g., IL-12), enabling modulation of the tumor microenvironment. [20,21,22,23]

Clinical Preparation of CAR T Cell

In general, the customized clinical production of CAR-T cells involves a number of processes, all of which are followed by quality control testing. Leukapheresis is the first phase, in which leukocytes are removed from peripheral blood from the patient (autologous) or the donor (allogeneic). The remaining blood products are then put back into circulation. [24,25] Second, T cells are augmented, separated, and washed with leukapheresis buffer. [25] Third, certain antibody-coated bead conjugates or markers are used to segregate the T-cell subsets at the CD4/CD8 composition level. Purified allogeneic or autologous APCs or beads coated with anti-CD3 or anti-CD28 monoclonal antibodies (or both, in addition to feeder cells and interleukins) are then used to cultivate and activate the isolated cells. [26] Fourth, nucleic acid delivery to the obtained T cells has been made possible by a variety of methods. Generally, either viral or non-viral vectors can be used to deliver a foreign gene material (RNA or DNA) into human cells; viral vectors are preferred for basic and clinical research due to their diverse expression characteristics, short time to reach clinically desired numbers of cultured T cells, and high transfer competency.[24,27] CARs are encoded using viral vectors, which exploit their ability to reverse transcription to transform RNA into DNA that is permanently incorporated into the genome of the resulting T cells. Retroviruses, lentivirus, adenovirus, and adeno-associated virus are some of these viral vectors. Compared to gamma retroviral vectors, genetically modified retroviruses are the most often employed. Viral vectors are removed from the culture by medium exchange and dilution during the activation phase. [28] Viral vectors may pose a risk to public safety. The insertion mutation used to produce immune responses causes tumorigenesis and toxicity, and the viral vectors' limited carrier capacity and insufficiently high titers are among their drawbacks. [29] Therefore, non-viral vectors and mRNA transfection were employed in the creation of CAR-T cells in order to overcome the drawbacks of viral vectors. Transposon-based non-viral vectors were the most widely used, allowing for reliable and secure DNA transfer into CAR T cells. The latest alternative to viral-based vectors is the sleeping beauty (SB) transposon system. Both in vitro and in vivo, it has been utilized to produce CD19+ CAR T-cells with antitumor capabilities. [30,31] The fifth step is CAR-T cell expansion using bioreactors, which help cells divide and express CARs on the cell surface [32] Ultimately, the cells are reinfused into the patient as a therapeutic agent once they have reached the clinically necessary volume. 48–96 hours following

lymphodepletion chemotherapy, the infusion takes place to create space for the infused CAR-T cells. [33] During the first few days of infusion, the patient is then monitored for any potential side effects. The procedure takes around three weeks, with the most time-consuming part of treatment being cell preparation. [34]

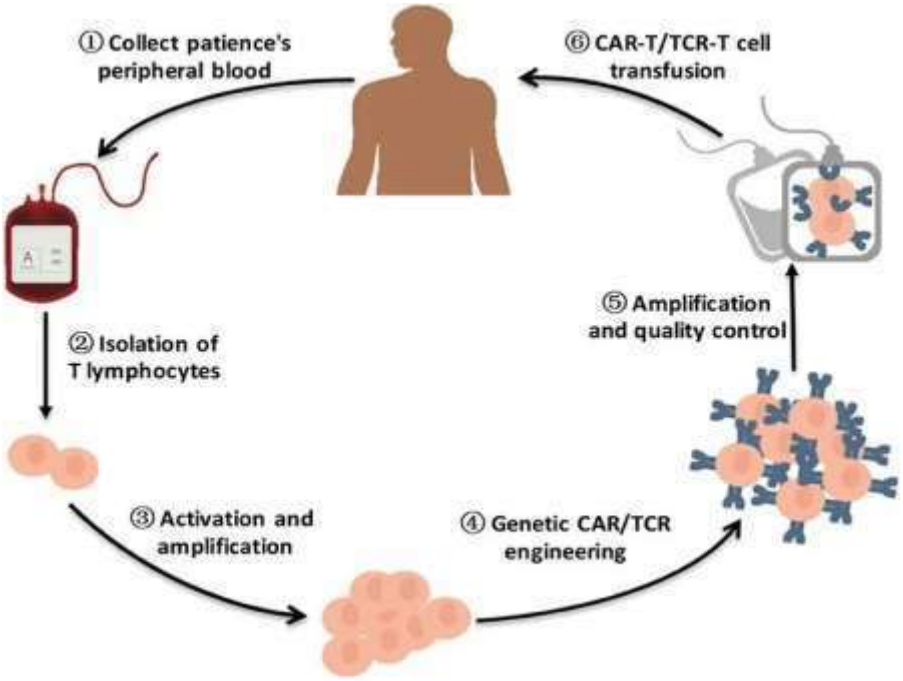

Figure 1:Preparation of CAR -T cell.

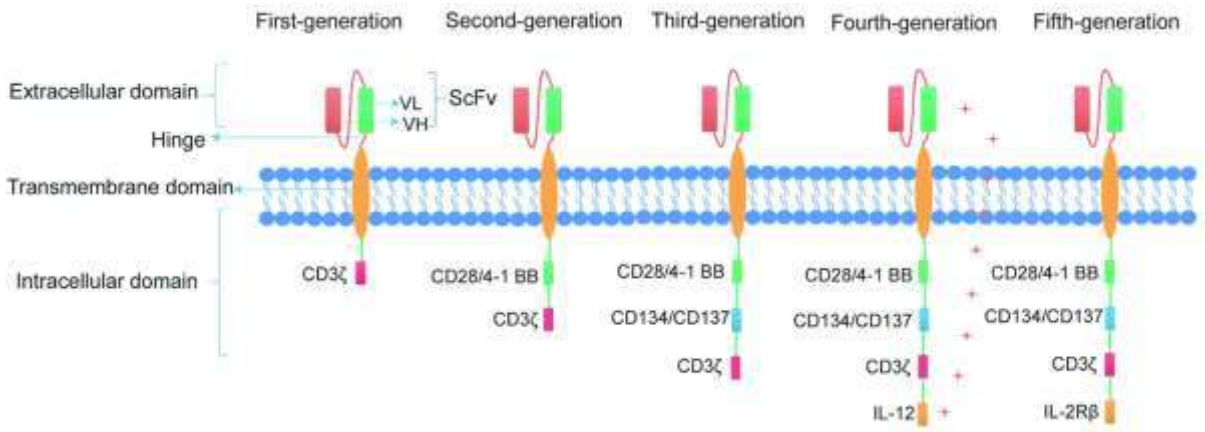

Figure 2:Five generations of CAR molecules

Mechanism of Action Of CAR-T Cell:

The basic mechanism by which CAR-T cells work is that their immune synapses attach to the antigenic targets on the surface of tumor cells, which then release chemicals like granzyme and perforin, creating holes in the target tumor cells' cell membranes. Granzyme then enters the target tumor cells through the holes, starting a cascade of cysteine-aspartate proteases that lysate the tumor cells. the CAR-T can autocrine release cytokines to promote its own activity and control the environment around the tumor. On the other hand, it can cause target cell death through the Fas-FasL pathway by binding specifically to TNF ligand (tumor necrosis factor) on CAR-T cells. [35]

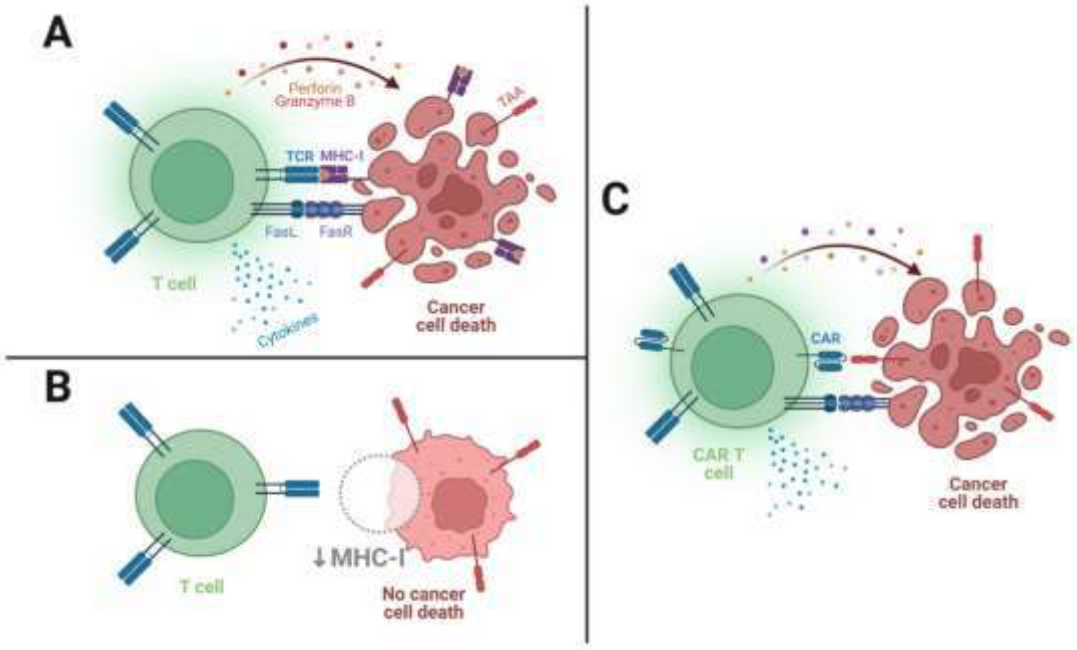

Figure 3:mechanism of action of CAR-T cell

Combined Application of Multiple Treatment:

Clinical trials involving patients with malignant tumors have demonstrated that those receiving a combination of CAR-T cell immunotherapy with either chemotherapy or radiotherapy experienced significantly longer overall survival compared to patients treated with chemotherapy or radiotherapy alone. This highlights the important role of CAR-T cell therapy when used alongside these conventional treatments in improving patient outcomes. In a mouse model of ROR1-positive non-small cell lung cancer (NSCLC), pre-treatment with the chemotherapy agents oxaliplatin and cyclophosphamide (Ox/Cy) improved the ability of CAR-T cells to infiltrate tumor tissues. This enhanced penetration led to an increase in chemokine secretion by tumor-associated macrophages. Moreover, the surface expression of PD-L1 on these macrophages was significantly upregulated after combined treatment with CAR-T cells and Ox/Cy. Chemotherapy with Ox/Cy prior to CAR-T cell administration boosted CAR-T cell migration into tumors. When this strategy was paired with anti-PD-L1 checkpoint inhibition, it further improved patient survival [36]. Radiotherapy studies employing an IFN-gamma-dependent mechanism showed that levels of adhesion molecules within the tumor microenvironment (TME) increased, thereby enhancing the ability of T cells to adhere to tumor tissue [37,38]. Researchers have also engineered a novel CAR-T cell by integrating the targeting capacity and blood-brain barrier permeability of CAR-T cells with the therapeutic advantages of SN-38, a chemotherapeutic agent known for deep vascular penetration and effectiveness against tumor heterogeneity and TME barriers. Using a thin-film-probe supershot method, they developed SN-38-loaded liposomes and incorporated them into a CAR-T construct targeting EGFRvIII, resulting in SN-38-L-EGFRvIII-CAR-T cells. These engineered cells not only retain immune-mediated tumor-killing ability but also act as carriers to deliver chemotherapy drugs directly to tumors, offering a promising dual-function treatment approach [39]. Additionally, because dendritic cells (DCs) play a critical role in presenting antigens and promoting T-cell infiltration, recent studies have explored combining DC vaccines with MSLN-targeted CAR-T cells. Immunofluorescence analysis showed that the DC vaccines significantly improved the proliferation, infiltration, and persistence of the MSLN-CAR-T cells [40]. Beyond chemotherapy, combining CAR-T therapy with radioimmunotherapy presents a new method to eliminate tumors that show resistance to CAR-T treatment. In one study, researchers used a NSG mouse model implanted with tumor cells. They administered ICAM-1-targeting CAR-T cells intravenously and used 177Lu-DOTATATE for radioimmunotherapy. With the help of SPECT-CT imaging, they monitored the distribution and therapeutic impact of the CAR-T cells. Results

indicated that this combination significantly enhanced treatment efficacy. The imaging confirmed that 177Lu-DOTATATE precisely targeted the tumor and measured the radiation dose absorbed. Although the findings suggest that this combination could be effective against tumors unresponsive to CAR-T therapy, the use of human xenograft tumor models limits broader applicability. Further research is needed to confirm the safety and effectiveness of this strategy [41]. Recent studies indicate that CAR-T cell therapy on its own shows limited success in treating solid tumors. To improve its effectiveness, various combination strategies are being explored.

• Combination with Chemotherapy

Research has shown that lymphodepletion enhances CAR-T therapy outcomes [42]. Several mechanisms have been proposed to explain the therapeutic benefits of lymphodepletion before administering adoptive T-cell immunotherapy [43]:

1. Nonmyeloablative chemotherapy creates temporary space for the incoming T cells.
2. The elimination of existing lymphocytes allows infused T-cells to better access vital cytokines like IL-2, IL-7, and IL-15 that support their survival and expansion.
3. Lymphodepletion can remove immunosuppressive cells, such as Tregs and MDSCs, while improving the function of antigen-presenting cells.
4. Some lymphodepleting drugs reduce immune suppression by targeting enzymes such as indoleamine 2,3-dioxygenase (IDO), which is involved in tryptophan metabolism.
5. Post-lymphodepletion, CAR-T cells show improved trafficking to tumor sites.

Common chemotherapy agents used for lymphodepletion in CAR-T therapy include cyclophosphamide and fludarabine [44]. Studies back the effectiveness of this combination. For instance, in patients with relapsed or treatment-resistant chronic lymphocytic leukemia (CLL) and other B-cell cancers, chemotherapy with cyclophosphamide before CD19 CAR-T therapy extended the persistence of CAR-T cells and improved efficacy [45]. Additionally, Srivastava et al. found that chemotherapy-induced lymphodepletion can trigger immunogenic cell death (ICD), releasing chemokines that attract CAR-T cells and increasing their infiltration into tumors [46]. Since the tumor microenvironment (TME) poses a major obstacle to CAR-T efficacy, chemotherapy can also help modify the TME to enhance therapy outcomes. Drugs like docetaxel [47] and gemcitabine [48,49] have been used as neoadjuvant therapies and shown to reduce MDSCs, thereby boosting the effects of GD2 CAR-T cells. Doxorubicin has demonstrated its ability to improve CAR-T cell activity by downregulating PD-L1 expression on osteosarcoma cells [50]. Oxaliplatin has been reported to alter the chemokine landscape in the TME, promoting recruitment of ROR1-CAR T cells. A Phase I clinical trial combining CAR-T therapy with paclitaxel and cyclophosphamide showed clinical benefits in 21 out of 28 patients who previously failed paclitaxel treatment [51].

• Combination with Radiotherapy

Solid tumors often develop an immunosuppressive and tolerant TME, transforming “hot” tumors into “cold” ones [52]. This suppresses CAR-T infiltration and promotes the recruitment of immune-suppressing cells, reducing the effectiveness of effector immune responses. However, recent evidence shows that radiotherapy (RT) can convert “cold” tumors into “hot” tumors by enhancing immune cell access to tumors and altering the suppressive TME [53]. As a result, combining RT with CAR-T therapy may yield stronger anti-tumor effects. In glioblastoma models, real-time imaging demonstrated that RT helped CAR-T cells rapidly infiltrate the tumor from blood vessels and expanded their presence in the TME, resulting in more durable immune responses [54]. Preclinical studies in mice with glioma also revealed that local tumor irradiation combined with NKG2D CAR-T cell therapy produced a synergistic effect—improving CAR-T cell tumor infiltration and increasing their cytotoxic function [55].

• Combination with Oncolytic Viruses

Oncolytic viruses (OVs) are gaining attention as cancer immunotherapy agents because of their ability to kill tumor cells and activate the immune system [56]. Despite this promise, OVs such as oncolytic adenoviruses (OAVs) face several hurdles like poor tumor penetration, off-target effects, resistance from

the host immune system, and a suppressive TME [57]. As monotherapy, their efficacy remains modest, but when combined with CAR-T therapy, OVs may help overcome these limitations.

Mechanisms by which OVs support CAR-T therapy include:

1. Direct lysis of tumor cells, releasing tumor-specific antigens.
2. Activation of innate immune responses and recruitment of immune cells.
3. Delivery of immune-modulating genes to shift the TME to a pro-inflammatory state [58].

These mechanisms have been confirmed by various in vitro and in vivo experiments. One study used a combination of Ad5-ZD55-CCL5-IL12, an OAV armed with CCL5 and IL-12, along with CAR-T cells targeting carbonic anhydrase 9 (CA9) in renal cancer. Results showed enhanced CAR-T cell infiltration, expansion, and activation [59]. In addition, single-cell sequencing and spatial transcriptomics in animal tumor models confirmed that OncoViron, a chimeric OAV, significantly altered gene expression in tumor cells and recruited large numbers of lymphocytes, NK cells, and macrophages to the TME. It also promoted macrophage polarization from M2 to M1 and increased the release of immune-stimulating cytokines, thereby enhancing the anti-tumor activity of CAR-T therapy [60]. Moreover, extensive research supports the promising results of combining CAR-T therapy with immune checkpoint inhibitors (ICIs), as discussed further in Sections "Challenges and coping strategies of immunosuppressive microenvironment" and "Challenges and coping strategies of limited persistence".

Major Challenges to Overcome for CAR T Cell Therapy:

Although CAR T cell therapy shows significant potential in the treatment of blood-related cancers, its broader application is hindered by the risk of severe, potentially life-threatening side effects. Two of the most commonly observed complications are cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS). CRS is caused by the excessive release of inflammatory cytokines such as IL-1 and IL-6, leading to symptoms like fever and hypotension. To manage these effects, the FDA approved tocilizumab in 2017—an antibody targeting the IL-6 receptor—for use during CAR T therapy. Other drugs under investigation include anakinra, an IL-1 receptor antagonist, and siltuximab, a chimeric antibody against IL-6 [61]. The pathophysiology of CRS and ICANS is complex. Animal studies have shown that monocytes and macrophages are the primary producers of IL-1 and IL-6 during CRS. Depleting these cells or inhibiting IL-6 signaling pathways has proven effective in preventing CRS, and anakinra has demonstrated efficacy in controlling both CRS and ICANS. Moreover, CD19—a common CAR T cell target in B cell malignancies—has also been detected in brain mural cells. This finding suggests a potential on-target, off-tumor mechanism for the neurotoxicity associated with CD19-CAR T cell therapy [62]. Several factors influence the severity of these adverse effects, including the patient's initial tumor burden, the intensity of the lymphodepletion regimen, and the administered CAR T cell dose. Studies have linked elevated cytokine levels during therapy with higher tumor burdens at the start of treatment. Although tumor burden does not appear to affect the peak expansion of CAR T cells, it may reduce the chances of complete remission and negatively impact overall survival [63]. Evidence supporting the impact of tumor burden on adverse effects also comes from CAR T cell trials in autoimmune diseases. In small-scale clinical studies involving patients with systemic lupus erythematosus (SLE), CAR T cell therapy resulted in substantial expansion of CAR T cells, rapid symptom relief, and minimal side effects. A similar response was observed in a patient with refractory antisynthetase syndrome (ASyS), who showed significant CAR T cell expansion with only mild adverse reactions [64]. In contrast, patients with hematological malignancies such as chronic lymphocytic leukemia (CLL) and B-cell acute lymphoblastic leukemia (B-ALL) often present with high circulating B cell levels, whereas patients with autoimmune conditions like SLE or ASyS usually have normal or decreased B cell counts [65]. The relatively low toxicity observed in autoimmune patients suggests that severe side effects in cancer patients are more likely a result of extensive tumor cell destruction rather than the direct effects of CAR T cells. This massive cell death may release

intracellular contents into the bloodstream, triggering tumor lysis syndrome (TLS), a potentially fatal complication [66]. These findings highlight the potential benefit of administering CAR T cell therapy during earlier stages of disease progression, when tumor burden is still low, to reduce the risk of severe toxicities.

• High Cost and Challenges in Autologous CAR T Cell Production

One of the primary obstacles in applying CAR T cell therapy broadly is the high expense involved in producing autologous CAR T cells. Treatment costs can soar up to $500,000, especially for patients suffering from severe cytokine release syndrome (CRS). Additionally, producing autologous CAR T cells takes around 21 to 35 days. This delay can be critical, as patients might need interim (bridging) therapies during this period, and in rapidly progressing cases, some may deteriorate before receiving the CAR T cells. Furthermore, T cells extracted from patients with advanced disease often exhibit functional exhaustion, reducing their therapeutic effectiveness compared to T cells from healthy individuals. To make CAR T therapy more accessible and cost-effective, alternative strategies are under investigation. These include the use of "off-the-shelf" allogeneic CAR T cells (allo-CAR T) and direct in vivo CAR T cell generation. Using allogeneic CAR T cells comes with immune-related risks such as graft-versus-host disease (GvHD) and potential rejection by the patient's immune system. To address this, gene-editing tools like CRISPR/Cas9 are employed to eliminate TCR genes from donor T cells, which helps reduce GvHD. Simultaneously, deleting the beta-2-microglobulin (B2M) gene prevents the formation of the HLA class I complex, thereby lowering the risk of immune rejection [67]. Despite these innovations, a significant limitation of allo-CAR T therapy is the short lifespan of these cells inside the patient's body, which may reduce their ability to fight cancer effectively. This reduced persistence might be due to natural killer (NK) cells targeting the HLA class I-deficient CAR T cells through a "missing-self" recognition process. Moreover, HLA class I molecules may be essential for T cell survival, as studies in B2M-deficient mice show a complete absence of CD8+ T cells. Another complication is the potential for CRISPR/Cas9 to introduce unintended genetic disruptions, such as large chromosomal deletions or karyotype abnormalities. To avoid these risks, base editing—which does not produce double-stranded DNA breaks—is being explored. This technique has been applied in creating base-edited allo-CAR T cells for patients with refractory T-cell acute lymphoblastic leukemia (T-ALL). Notably, in that study, the B2M gene remained intact, meaning the HLA class I structure was preserved. In addition, another genome-editing method, TALEN, has been utilized to disrupt both the TRAC and B2M genes, aiming to engineer "immune-evasive" universal CAR T cells that can be used across different patients without triggering rejection [68].

LIMITATIONS OF CAR-T CELL THERAPY:

Antigen escape

A significant challenge of CAR-T cell therapy is the emergence of tumor resistance to CAR constructs that target a single antigen. While CAR-T cells aimed at a specific antigen initially yield high response rates, many cancer cells in a considerable number of patients treated with these cells exhibit either a partial or complete loss of the targeted antigen expression. This occurrence is referred to as antigen escape. For instance, although 70–90% of patients with relapsed and/or refractory acute lymphoblastic leukemia (ALL) respond positively to CD19-targeted CAR-T cell therapy, recent follow-up findings indicate the development of a common resistance mechanism. This includes the downregulation or loss of CD19 antigen in 30–70% of patients who experience a recurrence of the disease after treatment. [69] Likewise, a decrease or absence of BCMA expression has been noted in multiple myeloma patients who are receiving treatment with BCMA-targeted CAR-T cells. [70,71] Comparable patterns of antigen escape resistance have been noted in solid tumors. For instance, a case report on CAR-T cell therapy aimed at IL13Ra2 in glioblastoma indicated that tumor recurrences showed reduced expression of IL13Ra2 In solid tumors, various tandem CARs have undergone testing in preclinical models, such as HER2 and IL13Ra2 in glioblastoma, as well as HER2 and MUC1 in breast cancer. In both instances, targeting two pathways led to better anti-tumor responses than therapies focused on a single target. In the glioblastoma research, CARs that target HER2

and IL13Ra2 demonstrated enhanced anti-tumor effectiveness and reduced antigen escape in comparison to two alternative dual-targeting treatments. [72] This research highlights the significance of enhancing the choice of target antigens that not only boost antitumor responses but also reduce the mechanisms of antigen escape to avert relapse.

On-target effects that occur outside of the tumor.

Identifying solid tumor antigens is difficult since they frequently appear in normal tissues as well, which raises the risk of "on-target off-tumor" toxicity. Consequently, meticulous antigen selection is vital for the design of CARs. An effective approach to enhance specificity involves targeting tumor-specific post-translational modifications, such as the overexpressed truncated O-glycans like Tn and sialyl-Tn.While initial generation CAR-T cells aimed at TAG72 in colorectal cancer did not generate an anti-tumor effect, newer models of second generation TAG72-CAR-T cells along with various tumor-specific post-translational modifications are now under exploration.[73]Continued advancement of creative approaches to diminish antigen escape and identify antigens that can trigger adequate antitumor effectiveness, while reducing toxicity issues, will be essential to broaden the clinical application of CAR-T cell therapies for both hematological cancers and solid tumors.

Movement of CAR-T cells and their penetration into tumors

One approach to address these limitations is by employing delivery methods beyond systemic administration, as localized treatment (1) reduces the requirement for CAR-T cells to travel to the sites of disease and (2) decreases on-target off-tumor toxicities because the CAR-T cells' activity is specifically directed at tumor cells, minimizing engagement with healthy tissues. Preclinical studies have shown enhanced therapeutic effectiveness of intraventricular administration of CAR-T cells targeting HER2[ref.14] and IL13Ra2 in brain metastases of breast cancer and glioblastoma. These findings have prompted three current clinical trials examining the intraventricular delivery of CAR-T cells for glioblastoma (NCT02208362, NCT03389230) and for recurrent brain or leptomeningeal metastases (NCT03696030). In a similar vein, preclinical research indicated that intrapleural injection of CAR-T cells leads to improved treatment outcomes for malignant pleural mesothelioma, resulting in an ongoing phase 1 clinical trial (NCT02414269). [74] While localized injection seems to demonstrate greater effectiveness, this method is theoretically constrained to individual tumor lesions or oligometastatic conditions. A newly developd approach that seems to greatly enhance CAR-T cell movement entails the expression of chemokine receptors on CAR-T cells that align with and react to chemokines produced by tumors. Physical obstacles like the tumor stroma hinder CAR-T cell therapy since these barriers prevent the effective penetration of tumor cells. The stroma mainly consists of an extracellular matrix, with heparan sulfate proteoglycan (HSPG) being the key element that CAR-T cells need to break down to infiltrate the tumor. CAR-T cells modified to produce heparanase, an enzyme that breaks down heparan sulfate proteoglycans (HSPG), exhibit improved infiltration into tumors and increased antitumor effectiveness. Likewise, CAR-T cells targeting fibroblast activation protein (FAP) show enhanced cytotoxic abilities by diminishing tumor-associated fibroblasts in animal studies. [75]

Future Developments and Latest Prospects In CAR-T Cell Therapy:

1) Structural and Functional Modification of CAR-T Cells

There are two approaches for designing CAR-T cells that target multiple antigens. The first involves introducing CAR molecules with different scFvs into the same T cell to create a parallel configuration of dual/multi-specific CAR-T cells, while the second involves incorporating two or more scFvs into a single CAR molecule that is then transferred into one T cell to establish a tandem configuration of dual/multi-specific CAR-T cells.[76] Multi-targeted CAR-T cells can effectively mitigate the issue of antigen loss that can occur during therapy and also decrease the likelihood of CRS. The innovative cell therapy product STAR-T incorporates a TCRab-based dual-chain receptor that links immunoglobulin heavy and light chain variable regions (VH and VL) with TCR-

Ca and TCR-Cb, respectively, differing from AbTCR, which utilizes TCRgd, and the single-chain scFv-derived TAC and TRuC receptors. The structure of STAR closely resembles that of a natural TCR. This receptor has both TCR and antigen recognition capabilities, and its sensitivity to antigens surpasses that of CAR-T cells, which significantly lowers the chances of tumor recurrence caused by antigen loss in various solid tumor models. [77] Allo-715 is designed to eliminate GvHD and reduce the rejection of CAR-T cells. The excessive expression of RUNX3, a key modulator of T-cell immune response, was discovered to effectively decrease the depletion of CAR-T cells when activated by antigens. [78]

2) Boolean Logic Gate Designs

Employing Boolean logic gates has become a promising approach that can control the function of CAR-T cells under particular circumstances by programming them to function as switches, thus enhancing their specificity. (LINK) CAR is a Boolean logic and gated CAR-T cell platform that operates quickly and reversibly, demonstrating superior performance compared to other systems. AdCAR-T comprises a dual-component signaling system utilizing a split recognition/activation mechanism, where the labeled adapter molecules (AM) convey the antigen-recognition signal to stimulate T cells through the anti-labeled CAR, enhancing specificity and effector control. [79]

3) Armored CAR-T Cells

The idea of "armored" CAR-T cells holds significant promise in the field of immunotherapy. This approach has been proposed to address challenges related to tumor evasion and unintended toxicity, and it involves enhancing cells with additional protective features alongside second- or third-generation CAR-T cells. This includes the simultaneous expression of essential cytokines, chemokines, or co-stimulatory ligands to boost their immunomodulatory capabilities and anti-tumor effectiveness. For instance, mice given “armored” CAR-T cells demonstrated a much greater reduction in tumor size compared to those receiving standard CAR-T cell treatment. [80] The release of chemokines or cytokines by “armored” CAR-T cells can improve the cells’ ability to move towards the tumor location and mitigate immunosuppressive signals.

4) Research on Novel Targets

CD317 is a novel target antigen for glioblastoma, which is among the most aggressive solid tumors. Normal neurons and microglial cells do not express CD317, but it promotes the proliferation of various malignant cells, such as those from liver and breast cancers, as well as glioblastoma. A recent investigation described the development of CAR-T cells through lentiviral transduction of CD317. In a mouse model of glioma, these CAR-T cells demonstrated significant anti-tumor efficacy. B7-H3 CAR-T cell therapy demonstrated enhanced targeted suppression of tumor cell proliferation and cytokine release without inducing significant toxic side effects, as evidenced by experiments conducted on mice. The modified MUC1-CAR-T cells exhibited notable anti-tumor activity against esophageal cancer cells. [81]

5) CAR-NK Cell Therapy

CAR-NK cells exhibit considerable potential. Similar to the structure and overall design of CAR-T cells, CAR-NK cells are composed of extracellular signaling domains, transmembrane segments, and intracellular domains. Clinical studies have shown that the safety profile of CAR-NK cells is better than that of CAR-T cells, as the cytokines they produce are primarily GM-CSF and IFN-g, which are not associated with the cytokines that trigger CRS, resulting in a significantly lower risk of CRS. By employing bifunctional lipid nanoparticles (DLNPs) to stimulate and effectively transport mRNA that encodes CAR. The research also investigated how DLNPs activate and regulate NK cells, in addition to optimizing the concentration of the cationic lipid 1,2-dioleoyl-3-trimethylammonium propane (DOTAP) within lipid nanoparticles to enhance gene delivery effectiveness and minimize cytotoxicity. CAR-NK cells that target Glypcan-3 showed notable therapeutic effectiveness. [82]

6) Combination Therapies

Individuals who underwent treatment with a combination of CAR-T cell immunotherapy and chemotherapy or radiotherapy experienced

considerably improved overall survival compared to those who received chemotherapy or radiotherapy alone.SN-38-L-EGFRvIII-CAR-T cells possess the ability not only to produce their immunological effects but also to serve as a vehicle for the precise delivery of chemotherapy agents. DC vaccines greatly improved the growth, infiltration, and longevity of the MSLN-CAR-T cells. Combining radioimmunotherapy with CAR-T cell therapy may significantly enhance treatment efficacy. [83]

7) Advanced Manufacturing and Delivery Technologies

The study utilized an LNP (antigenic lipid-like nanoparticle), which incorporates CD3 and CD28 antibody fragments, to activate and expand T cells. Initially, the lipid-like nanoparticles were conjugated with CD3 and CD28 antibody fragments. Subsequently, CAR mRNA was encapsulated within aLNP to promote effective transcription in T cells. Lastly, the aLNP was co-cultured with T cells to facilitate the uptake of CAR mRNA by the T cells. The results of the experiment revealed that aLNP effectively delivered CAR mRNA to T cells, leading them to express CAR. [84]

8) Dual-Targeting CAR Platforms

AbTCR-CSR was effective in eliminating AML cells. Analysis through flow cytometry revealed that AbTCR-CSR demonstrated a notable capacity for recognizing and killing AML cells, while showing no cytotoxic effects on normal peripheral blood mononuclear cells (PBMCs). [85]

CONCLUSION:

CAR-T cell therapy is a paradigm shift in cancer treatment, providing long-lasting remissions in otherwise treatment-refractory hematological malignancies. The development of CARs from the first to the next generation has shown how important it is to integrate costimulatory domains, cytokine support, and safety switches to improve efficacy and control. However, there are still major obstacles to overcome, such as severe toxicities like cytokine release syndrome and neurotoxicity, antigen escape, limited trafficking to solid tumors, and the immunosuppressive tumor microenvironment. To overcome these obstacles, a multifaceted approach is needed, ranging from dual-targeting and armored CAR designs to synthetic circuits and gene-edited universal cell products. The field's future lies in combining CAR-T therapy with checkpoint inhibitors, targeted drugs, and advanced delivery systems, while utilizing nanotechnology and synthetic biology to improve precision, persistence, and monitoring.

REFERENCE

1. Um, P. Cancer, Definition. In Encyclopedia of Metagenomics; Highlander, S.K., Rodriguez-Valera, F., White, B.A., Eds.; Springer:Boston, MA, USA, 2015; p. 65. ISBN 978-1-4899-7475-4
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries (vol 68, pg 394, 2018). Ca-a Cancer J Clin. (2020) 70:313.
3. Yang JC, Rosenberg SA. Adoptive T-cell therapy for cancer. Tumor Immunol Adv Immunol. (2016) 1302016:279–94
4. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science. 2018 Mar 23;359(6382):1361–5.
5. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. Nature. 2017 Jul;547(7662):217–21
6. Zhang M, Yang J, Hua Y, Deng M, Kong Y, Zhao H, et al. Monitoring neoantigen-specific T-cell immunity to identify immunogenic mutations and predict patient prognosis. Oncoimmunology.
7. Zhou Y, Wen W, Yao Y, Jiang R, Li P, Zhu Y, et al. Neoantigen-reactive CD8+ T cells correlate with clinical outcomes in long-term survivors of pancreatic cancer. Nature Cancer. 2021 May;2(5):473–86.
8. Z. Zhao, Y. Chen, N.M. Francisco, Y. Zhang, M. Wu, The application of CAR-T cellTherapy in hematological malignancies: advantages and challenges, Acta Pharm.Sin. B 8 (4) (2018) 539–551.
9. G. Gross, Z. Eshhar, Therapeutic potential of T cell chimeric antigen receptors (CARs) in cancer

treatment: counteracting off-tumor toxicities for safe CAR T cell Therapy, Annu. Rev. Pharmacol. Toxicol. 56 (2016) 59–83.

10. P. Vormittag, R. Gunn, S. Ghorashian, F.S. Veraitch, A guide to manufacturing CAR T cell therapies, Curr. Opin. Biotechnol. 53 (2018) 164–181.
11. C. Graham, A. Jozwik, A. Pepper, R. Benjamin, Allogeneic CAR-T cells: more than Ease of access? Cells 7 (10) (2018) 155.
12. S. Rafiq, C.S. Hackett, R.J. Brentjens, Engineering strategies to overcome the Current roadblocks in CAR T cell therapy, Nat. Rev. Clin. Oncol. 17 (3) (2020) 147–167.
13. M. Nakajima, Y. Sakoda, K. Adachi, H. Nagano, K. Tamada, Improved survival of Chimeric antigen receptor-engineered T (CAR-T) and tumor-specific T cells caused By anti-programmed cell death protein 1 single-chain variable fragment-producing CAR-T cells, Cancer Sci. 110 (10) (2019) 3079
14. L. Alabanza, M. Pegues, C. Geldres, V. Shi, J.J. Wiltzius, S.A. Sievers, S. Yang,J.N. Kochenderfer, Function of novel anti-CD19 chimeric antigen receptors with Human variable regions is affected by hinge and transmembrane domains, Mol.Ther. 25 (2017) 2452–2465.
15. A.Künkele, A.J. Johnson, L.S. Rolczynski, C.A. Chang, V. Hoglund, K.S. Kelly-Spratt, M.C. Jensen, Functional tuning of CARs reveals signaling threshold above Which CD8þ CTL antitumor potency is attenuated due to cell Fas–FasL-dependent AICD, Cancer Immunol. Res. 3 (4) (2015) 368–379.
16. S. Lankipalli, U.A. Ramagopal, A Structural Perspective on the Design of Decoy Immune Modulators, Pharmacological Research, 2021, p. 105735.
17. L. Morales, J.M. Paramio, Cell therapies in bladder cancer management, Int. J. Mol.Sci. 22 (6) (2021) 2818.
18. J. Julamanee, S. Terakura, K. Umemura, Y. Adachi, K. Miyao, S. Okuno, E. Takagi,T. Sakai, D. Koyama, T. Goto, R. Hanajiri, Composite CD79A/CD40 co-stimulatory Endodomain enhances CD19CAR-T cell proliferation and survival, Mol. Ther.(2021).
19. Y.D. Muller, D.P. Nguyen, L.M. Ferreira, P. Ho, C. Raffin, R.V.B. Valencia, Z. Congrave-Wilson, T.L. Roth, J. Eyquem, F. Van Gool, A. Marson, The CD28-Transmembrane domain mediates chimeric antigen receptor heterodimerization With CD28, Front. Immunol. 12 (2021) 500.
20. E. Lanitis, G. Coukos, M. Irving, All systems go: converging synthetic biology and Combinatorial treatment for CAR-T cell therapy, Curr. Opin. Biotechnol. 65 (2020) 75–87.
21. C.A. Janeway Jr., P. Travers, M. Walport, M.J. Shlomchik, Antigen receptor Structure and signaling pathways, in: Immunobiology: the Immune System in Health and Disease, fifth ed., Garland Science, 2001.
22. A. Loskog, V. Giandomenico, C. Rossig, M. Pule, G. Dotti, M.K. Brenner, Addition of The CD28 signaling domain to chimeric T-cell receptors enhances chimeric T-cell Resistance to T regulatory cells, Leukemia 20 (10) (2006) 1819–1828.
23. R. Weinkove, P. George, N. Dasyam, A.D. McLellan, Selecting co-stimulatory Domains for chimeric antigen receptors: functional and clinical considerations, Clin.Transl. Immunol. 8 (5) (2019) e1049.
24. Brown C. E., Adusumilli P. S. (2016). Next Frontiers in CAR T-Cell Therapy. Mol. Ther. - Oncolytics 3, 16028. 10.1038/mto.2016.28
25. Zhang C., Liu J., Zhong J. F., Zhang X. (2017). Engineering CAR-T Cells. Biomark. Res. 5, 22. 10.1186/s40364-017-0102-y
26. Guedan S., Calderon H., Posey A. D., Maus M. V. (2019). Engineering and Design of Chimeric Antigen Receptors. Mol. Ther. - Methods & Clin. Dev. 12, 145–156. 10.1016/j.omtm.2018.12.009
27. Gomes-Silva D., Ramos C. A. (2018). Cancer Immunotherapy Using CAR-T Cells: From the Research Bench to the Assembly Line. Biotechnol. J. 13, 1700097. 10.1002/biot.201700097
28. McGarrity G. J., Hoyah G., Winemiller A., Andre K., Stein D., Blick G., et al. (2013). Patient Monitoring and Follow-Up in Lentiviral Clinical Trials. J. Gene Med. 15, 78–82. 10.1002/jgm.2691
29. Wang G. P., Garrigue A., Ciuffi A., Ronen K., Leipzig J., Berry C., et al. (2008). DNA Bar Coding and Pyrosequencing to Analyze Adverse Events in Therapeutic Gene Transfer. Nucleic Acids Res. 36, e49. 10.1093/nar/gkn125

30. Singh H., Moyes J. S. E., Huls M. H., Cooper L. J. N. (2015). Manufacture of T Cells Using the Sleeping Beauty System to Enforce Expression of a CD19-specific Chimeric Antigen Receptor. Cancer Gene Ther. 22, 95–100. 10.1038/cgt.2014.69
31. Chicaybam L., Abdo L., Carneiro M., Peixoto B., Viegas M., De Sousa P., et al. (2019). CAR T Cells Generated UsingSleeping BeautyTransposon Vectors and Expanded with an EBV-Transformed Lymphoblastoid Cell Line Display Antitumor ActivityIn VitroandIn Vivo. Hum. Gene Ther. 30, 511–522. 10.1089/hum.2018.218
32. Harrison R. P., Zylberberg E., Ellison S., Levine B. L. (2019). Chimeric Antigen Receptor-T Cell Therapy Manufacturing: Modelling the Effect of Offshore Production on Aggregate Cost of Goods. Cytotherapy 21, 224–233. 10.1016/j.jcyt.2019.01.003
33. Turtle C. J., Hanafi L.-A., Berger C., Gooley T. A., Cherian S., Hudecek M., et al. (2016). CD19 CAR-T Cells of Defined CD4+:CD8+ Composition in Adult B Cell ALL Patients. J. Clin. Invest. 126, 2123–2138. 10.1172/JCI85309
34. Zhao L., Cao Y. J. (2019). Engineered T Cell Therapy for Cancer in the Clinic. Front. Immunol. 10, 2250. 10.3389/fimmu.2019.02250
35. Sadelain M, RiviSre I, Riddell S. Therapeutic T cell engineering. Nature. (2017) 545:423–31. doi: 10.1038/nature22395
36. Srivastava S, Furlan SN, Jaeger-Ruckstuhl CA, Sarvothama M, Berger C, Smythe KS, et al. Immunogenic chemotherapy enhances recruitment of CAR-T cells to lung tumors and improves antitumor efficacy when combined with checkpoint blockade. Cancer Cell. (2021) 39:193. doi: 10.1016/j.ccell.2020.11.005
37. Hassan R, Williams-Gould J, Steinberg SM, Liewehr DJ, Yokokawa J, Tsang KY, et al. Tumor-directed radiation and the immunotoxin SS1P in the treatment of mesothelin-expressing tumor xenografts. Clin Cancer Res. (2006) 12:4983–8. doi: 10.1158/1078-0432.CCR-06-0441
38. Heckmann M, Douwes K, Peter R, Degitz K. Vascular activation of adhesion molecule mRNA and cell surface expression by ionizing radiation. Exp Cell Res. (1998) 238:148–54. Doi: 10.1006/excr.1997.3826
39. Xing H. Feasibility of anti EGFRvIII-CAR-T cells as drug carriers in the treatment of glioma. Huazhong University of Science and Technology (2023). PhD thesis.
40. Zhang MM, Wang YY, Chen XZ, Zhang F, Chen JN, Zhu HQ, et al. DC vaccine enhances CAR-T cell antitumor activity by overcoming T cell exhaustion and promoting T cell infiltration in solid tumors. Clin Trans Oncol. (2023). Doi: 10.1007/ s12094-023-03161-1
41. Yang YP, Vedvyas Y, Alcaina Y, Son JY, Min IM, Jin MM. Low-dose targeted radionuclide therapy synergizes with CAR T cells and enhances tumor response. Front Immunol. (2024) 15. Doi: 10.3389/fimmu.2024.1355388
42. Muranski P, Boni A, Wrzesinski C, Citrin DE, Rosenberg SA, Childs R, Restifo NP. Increased intensity lymphodepletion and adoptive immunotherapy–how far can we go? Nat Clin Pract Oncol. 2006;3(12):668–81.
43. Bechman N, Maher J. Lymphodepletion strategies to potentiate adoptive T-cell immunotherapy – what are we doing; where are we going? Expert Opin Biol Ther. 2021;21(5):627–37.
44. Sesques P, Ferrant E, Safar V, Wallet F, Tordo J, Dhomps A, Karlin L, Brisou G, Vercasson M, Hospital-Gustem C, et al. Commercial anti-CD19 CAR T cell therapy for patients with relapsed/refractory aggressive B cell lymphoma in a European center. Am J Hematol. 2020;95(11):1324–33.
45. Brentjens RJ, Rivière I, Park JH, Davila ML, Wang X, Stefanski J, Taylor C, Yeh R, Bartido S, Borquez-Ojeda O, et al. Safety and persistence of adoptively transferred autologous CD19-targeted T cells in patients with relapsed or chemotherapy refractory B-cell leukemias. Blood. 2011;118(18):4817–28.
46. Srivastava S, Furlan SN, Jaeger-Ruckstuhl CA, Sarvothama M, Berger C, Smythe KS, Garrison SM, Specht JM, Lee SM, Amezquita RA, et al. Immunogenic Chemotherapy Enhances Recruitment of CAR-T Cells to lung tumors and improves Antitumor Efficacy when combined with checkpoint blockade. Cancer Cell. 2021;39(2):193–e208110.

47. Zhang X, Sun S, Miao Y, Yuan Y, Zhao W, Li H, Wei X, Huang C, Hu X, Wang B, et al. Docetaxel enhances the therapeutic efficacy of PSMA-specific CAR-T cells against prostate cancer models by suppressing MDSCs. J Cancer Res Clin Oncol. 2022;148(12):3511–20.
48. Enblad G, Karlsson H, Gammelgård G, Wenthe J, Lövgren T, Amini RM, Wikstrom KI, Essand M, Savoldo B, Hallböök H, et al. A phase I/IIa trial using CD19-Targeted third-generation CAR T cells for Lymphoma and Leukemia. Clin Cancer Res. 2018;24(24):6185–94.
49. Eriksson E, Wenthe J, Irenaeus S, Loskog A, Ullenhag G. Gemcitabine reduces MDSCs, tregs and TGFβ-1 while restoring the teff/treg ratio in patients with pancreatic cancer. J Transl Med. 2016;14(1):282.
50. Chulanetra M, Morchang A, Sayour E, Eldjerou L, Milner R, Lagmay J, Cascio M, Stover B, Slayton W, Chaicumpa W, et al. GD2 chimeric antigen receptor modified T cells in synergy with sub-toxic level of doxorubicin targeting Osteosarcomas. Am J Cancer Res. 2020;10(2):674–87.
51. Qi C, Gong J, Li J, Liu D, Qin Y, Ge S, Zhang M, Peng Z, Zhou J, Cao Y, et al. Claudin18.2-specific CAR T cells in gastrointestinal cancers: phase 1 trial interim results. Nat Med. 2022;28(6):1189–98.
52. Bindea G, Mlecnik B, Tosolini M, Kirilovsky A, Waldner M, Obenauf AC, Angell H, Fredriksen T, Lafontaine L, Berger A, et al. Spatiotemporal dynamics of intratumoral immune cells reveal the immune landscape in human cancer. Immunity. 2013;39(4):782–95.
53. Formenti SC, Demaria S. Systemic effects of local radiotherapy. Lancet Oncol. 2009;10(7):718–26.
54. Murty S, Haile ST, Beinat C, Aalipour A, Alam IS, Murty T, Shaffer TM, Patel CB, Graves EE, Mackall CL, et al. Intravital imaging reveals synergistic effect of CAR T-cells and radiation therapy in a preclinical immunocompetent glioblastoma model. Oncoimmunology. 2020;9(1):1757360.
55. Weiss T, Weller M, Guckenberger M, Sentman CL, Roth P. NKG2D-Based CAR T-cells and Radiotherapy Exert Synergistic Efficacy in Glioblastoma. Cancer Res. 2018;78(4):1031–43.
56. Feist M, Zhu Z, Dai E, Ma C, Liu Z, Giehl E, Ravindranathan R, Kowalsky SJ, Obermajer N, Kammula US, et al. Oncolytic virus promotes tumor-reactive infiltrating lymphocytes for adoptive cell therapy. Cancer Gene Ther. 2021;28(1–2):98–111.
57. Yang M, Olaoba OT, Zhang C, Kimchi ET, Staveley-O'Carroll KF, Li G. Cancer Immunotherapy and Delivery System: an update. Pharmaceutics 2022, 14(8).
58. Sonzogni O, Zak DE, Sasso MS, Lear R, Muntzer A, Zonca M, West K, Champion BR, Rottman JB. T-SIGn tumor reengineering therapy and CAR T cells synergize in combination therapy to clear human lung tumor xenografts and lung metastases in NSG mice. Oncoimmunology. 2022;11(1):2029070.
59. Fang L, Tian W, Zhang C, Wang X, Li W, Zhang Q, Zhang Y, Zheng J. Oncolytic adenovirus-mediated expression of CCL5 and IL12 facilitates CA9-targeting CAR-T therapy against renal cell carcinoma. Pharmacol Res. 2023; 189:106701.
60. Su Y, Li J, Ji W, Wang G, Fang L, Zhang Q, Ang L, Zhao M, Sen Y, Chen L et al. Triple-serotype chimeric oncolytic adenovirus exerts multiple synergistic mechanisms against solid tumors. J Immunother Cancer 2022, 10(5)
61. Giavridis T, van der Stegen SJC, Eyquem J, Hamieh M, Piersigilli A, Sadelain M. CAR T cell-induced cytokine release syndrome is mediated by macrophages and abated by IL-1 blockade. Nat Med (2018) 24:731–8. Doi: 10.1038/s41591-018-0041-7
62. Parker KR, Migliorini D, Perkey E, Yost KE, Bhaduri A, Bagga P, et al. Single-cell analyses identify brain mural cells expressing CD19 as potential off-tumor targets for CAR-T immunotherapies. Cell (2020) 183:126–142.e117. doi: 10.1016/ j. cell.2020.08.022
63. Li M, Xue SL, Tang X, Xu J, Chen S, Han Y, et al. The differential effects of tumor burdens on predicting the net benefits of ssCART-19 cell treatment on r/r b-ALL patients. Sci Rep (2022) 12:378. Doi: 10.1038/s41598-021-04296-3
64. Hallek M. Chronic lymphocytic leukemia: 2020 update on diagnosis, risk stratification and treatment. Am J Hematol (2019) 94:1266–87. Doi: 10.1002/ajh.25595

65. Arce E, Jackson DG, Gill MA, Bennett LB, Banchereau J, Pascual V. Increased frequency of pre-germinal center b cells and plasma cell precursors in the blood of children with systemic lupus erythematosus. J Immunol (2001) 167:2361–9. Doi: 10.4049/jimmunol.167.4.2361
66. Rahmani B, Patel S, Seyam O, Gandhi J, Reid I, Smith N, et al. Current understanding of tumor lysis syndrome. Hematol Oncol (2019) 37:537–47. Doi: 10.1002/hon.2668
67. . Mailankody S, Matous JV, Chhabra S, Liedtke M, Sidana S, Oluwole OO, et al. Allogeneic BCMA-targeting CAR T cells in relapsed/refractory multiple myeloma: Phase 1 UNIVERSAL trial interim results. Nat Med (2023) 29(2):422–9. Doi: 10.1038/S41591-023-02306-7
68. Kuhn NF, Purdon TJ, Van Leeuwen DG, Lopez AV, Curran KJ, Daniyan AF,et al. CD40 ligand-modified chimeric antigen receptor T cells enhance antitumor function by eliciting an endogenous antitumor response. Cancer Cell (2019) 35:473–488.e476. doi: 10.1016/j.ccell.2019.02.006
69. Majzner, R. G. & Mackall, C. L. Tumor antigen escape from CAR T-. cell therapy. Cancer Discov. 8, 1219–1226 (2018).
70. Green, D. J. et al. Fully human bcma targeted chimeric antigen receptor T cells administered in a defined composition demonstrate potency at low Doses in advanced stage high risk multiple myeloma. Blood. 132, 1011–1011 (2018).
71. Brudno, J. N. et al. T cells genetically modified to express an anti-B-cell Maturation antigen chimeric antigen receptor cause remission of poor-Prognosis relapsed multiple myeloma. J. Clin. Oncol. 36, 2267–2280 (2018).
72. Hegde, M. et al. Tandem CAR T cells targeting HER2 and IL13Rα2 mitigate Tumor antigen escape. J. Clin. Invest. 126, 3036–3052 (2016).
73. Murad, J. P. et al. Effective targeting of TAG72+ peritoneal ovarian tumors via regional delivery of CAR-engineered T cells. Front. Immunol. 9, 2268 (2018).
74. Adusumilli, P. S. et al. Regional delivery of mesothelin-targeted CAR T cell Therapy generates potent and long-lasting CD4-dependent tumor immunity.Sci. Transl. Med. 6, 261ra151 (2014).
75. Wang, L.-C. S. et al. Targeting fibroblast activation protein in tumor stroma with chimeric antigen receptor t cells can inhibit tumor growth and augment host immunity without severe toxicity. Cancer Immunol. Res. 2,154–166 (2014).
76. Kozani PS, Kozani PS, O'Connor RS. In like a lamb; out like a lion: marching CAR T cells toward enhanced efficacy in B-ALL. Mol Cancer Ther. (2021) 20:1223–33. Doi: 10.1158/1535-7163.MCT-20-1089
77. Liu Y, Liu GN, Wang JS, Zheng ZY, Jia LM, Rui W, et al. Chimeric STAR Receptors using TCR machinery mediate robust responses against solid tumors. Sci Trans Med. (2021) 13. Doi: 10.1126/scitranslmed. abb5191
78. Zhu XX, Li WL, Gao JD, Shen JJ, Xu YM, Zhang CC, et al. RUNX3 improves CAR-T cell phenotype and reduces cytokine release while maintaining CAR-T function. Med Oncol. (2023) 40. doi: 10.1007/s12032-022-01913-7
79. Seitz CM, Mittelstaet J, Atar D, Hau J, Reiter S, Illi C, et al. Novel adapter CAR-T cell technology for precisely controllable multiplex cancer targeting. Oncoimmunology. (2021) 10. doi: 10.1080/2162402X.2021.2003532
80. Hoyos V, Savoldo B, Quintarelli C, Mahendravada A, Zhang M, Vera J, et al. Engineering CD19-specific T lymphocytes with interleukin-15 and a suicide gene to Enhance their anti-lymphoma/leukemia effects and safety. Leukemia. (2010) 24:1160–70. Doi: 10.1038/leu.2010.75
81. Shin H, Han JH, Park JD, Park M, Han JU, Kang MH, et al. Enhancing CAR-NK cells against solid tumors through chemical and genetic fortification with DOTAP-functionalized lipid nanoparticles. Advanced Funct Materials. (2024). doi: 10.1002/adfm.202315721
82. Xing H. Feasibility of anti EGFRvIII-CAR-T cells as drug carriers in the treatment Of glioma. Huazhong University of Science and Technology (2023). PhD thesis.
83. Yang YP, Vedvyas Y, Alcaina Y, Son JY, Min IM, Jin MM. Low-dose targeted Radionuclide therapy synergizes with CAR T cells and enhances tumor response. Front Immunol. (2024) 15. Doi: 10.3389/fimmu.2024.1355388

84. Metzloff AE, Padilla MS, Gong NQ, Billingsley MM, Han XX, Merolle M, et al. Antigen presenting cell mimetic lipid nanoparticles for rapid mRNA CAR T cell cancer immunotherapy. Advanced Materials. (2024). doi:10.1002/adma.202313226
85. Dao T, Xiong GY, Mun SS, Meyerberg J, Korontsvit T, Xiang JY, et al. A dual-Receptor T-cell platform with Ab-TCR and costimulatory receptor achieves specificity and potency against AML. Blood. (2024) 143:507–21. Doi: 10.1182/blood.2023021054.

HOW TO CITE: Shruti Shinde*, Vaibhavi Jadhav, Nilima Yadav, Sampada Waghmare, CAR-T Cells in Cancer Therapy: From Structural Blueprint to Clinical Barriers, Int. J. Sci. R. Tech., 2025, 2 (9), 22-37. https://doi.org/10.5281/zenodo.17066739