“In Vivo Delivery Systems For CAR T-Cell Therapy: Progress And Challenges”

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Abstract:

CAR T cell therapy, which genetically modifies T cells to target tumor antigens, has emerged as a viable immunotherapy strategy for the treatment of cancer. Notwithstanding its promising outcomes, CAR T cell treatment encounters some obstacles, including as toxicities like cytokine release syndrome (CRS) and "on-target, off-tumor" effects, logistical issues associated with production, and transport limitations in the in vivo setting. Many ways have been explored to overcome these obstacles, such as scaffold-based approaches, transfection using nanoparticles, safety switches, and the investigation of allogeneic "off-the-shelf" CAR T cells. Suicide genes and apoptosis-inducing pathways are examples of safety switches that may be used to reduce side effects without sacrificing the effectiveness of treatment. By facilitating the transfer of messenger RNA (mRNA) into T cells and stimulating the expression of CARs, nanoparticle-mediated transfection may be able to address long-term toxicity issues related to CAR T cell treatment. The goal of scaffold-based methods is to produce more CAR T cells outside of living things by creating an environment that is conducive to cell growth. Furthermore, the investigation of allogeneic "off-the-shelf" CAR T cells may be able to address the practical difficulties related to autologous CAR T cell therapy; nonetheless, issues with immune rejection and graft-versus-host disease (GvHD) still need to be addressed. In conclusion, even though CAR T cell therapy has a lot of potential to treat cancer, its widespread application depends on resolving issues with toxicity, production, and in vivo hurdles. To fully utilize CAR T cell therapy's potential to improve cancer

patient outcomes, more research and innovation in these fields are required.

Key words: CAR T-Cells, In-vivo delivery, Barriers, Polymers, Scaffold, Nanoparticles.

Introduction:

Traditional treatments such as surgery, chemotherapy, and radiation chemotherapy, in addition to more recent targeted medicines, have formed the foundation of cancer treatment throughout the course of history and throughout the course of several decades. Even though there have been significant breakthroughs in cancer treatment, the outlook for many cancers remains dismal. The introduction of targeted anticancer techniques, on the other hand, has made it possible to take a more individualized approach to treatment, which has led to increased rates of success. Because it makes use of the immune system of the body to fight cancer, immunotherapy has become a notable example of customized medicine. It has revolutionized views toward cancer treatment by utilizing the immune system of the body [1].

Monoclonal antibody therapy, tumor vaccines, immune checkpoint blockades, bispecific antibodies, tumor-infiltrating lymphocytes (TILs), and chimeric antigen receptor (CAR) T-cell therapies are some of the various ways that have been incorporated into immunotherapy. The scope of immunotherapy has substantially extended to include these various approaches. T-cells, which are an essential component of the adaptive immune system, are responsible for various functions, including the regulation of cytotoxic effects and the maintenance of cellular memory that is long-lasting of certain antigens. Despite the fact that tumor-specific TILs can be produced within the bodies of patients, their function is frequently impeded within the tumor microenvironment (TME), which causes them to be inactive [2].

The presence of tumor-associated or tumor-specific antigens (TAA and TSA) is necessary for the activation of CAR T-cells, in contrast to the endogenous activation of T-cells that occurs through the interaction between peptides that are presented by the major histocompatibility complex (MHC) and their T-cell

receptor (TCR). Incorporating a targeting domain, also known as a single chain variable fragment (scFv), onto the signaling domain of a T-cell results in the creation of CAR T-cells. This process effectively transforms the T-cell into a living therapeutic agent. The antigen specificity that is provided by the targeting domain is carried by CARs, which are made up of an antibody or ligand-derived targeting ectodomain, a hinge, a transmembrane domain, and intracellular T-cell signaling domains. CARs, in contrast to traditional T-cell receptors (TCRs), are able to recognize antigens independently of major histocompatibility complex (MHC) molecules. This feature enables CARs to target a wide variety of antigens, including non-protein targets such as carbohydrates, without the need for antigen processing and presentation. Because of this characteristic, as well as its therapeutic potential and its capacity to transcend human MHC restriction, CAR T-cell treatment is a method that shows promise in the field of adoptive T-cell therapy [3-5].

Because of their innate capacity to recognize and eradicate cancer cells, T cells are the cells of choice for adoptive cell therapies (ACTs). During this process, cytotoxic chemicals including perforin and granzyme, as well as cytokines like IL-2 and IFNγ, are released into the environment. To add insult to injury, T cells are responsible for inducing apoptosis in cancer cells through pathways that involve death ligands and death receptors. ACTs initially depended on the transfer of autologous tumor-infiltrating lymphocytes (TILs) derived from patients' tumors that had been surgically excised. After that, these TILs were extended outside of the body to levels that were enough for therapeutic reasons. It has been demonstrated that adoptive transfer of TILs is quite beneficial, particularly in patients who have metastatic melanoma. As a result, it is considered to be one of the most effective immune-based treatments that are currently accessible. The capacity of TILs to target neoantigens in melanoma has been brought to light by recent study, which further serves to reinforce the clinical benefits that have been shown for these cells [6-7].

Regrettably, not all cancers contain sufficient numbers of tumor-infiltrating lymphocytes (TILs), which restricts the more widespread applicability of this method to the treatment of other types of cancer. Adoptive cell therapy (ACT) has moved its attention to genetically modified T cells that are located in the circulation so that they express anti-tumor receptors in order to overcome this impediment. To begin, modified T cells were developed with the

intention of expressing cloned T cell receptors (TCRs) that were directed toward particular tumor antigens. Despite the fact that these modified T cells have the ability to target both surface and cytoplasmic tumor-associated proteins, which could possibly expand the range of antigens that could be targeted, TCRs need to be activated within the context of major histocompatibility complex (MHC) presentation. As a result of the fact that many cancers reduce the expression of MHC class I molecules in order to avoid being detected by the immune system, cancer cells become invisible to TCR-mediated recognition. Artificial chimeric antigen receptors, also known as CARs, have been developed as a solution to this problem. These receptors combine the high affinity of antibody-derived binding domains with the intracellular signaling regions of TCRs [8-9].

The aim of the present research study is to investigate the progress and challenges of in vivo delivery systems for CAR T-cell therapy. Through comprehensive analysis and evaluation, this study aims to provide insights into the current state of in vivo delivery methods for CAR T-cell therapy, including their efficacy, safety, and limitations. By examining existing data and exploring emerging trends, the research seeks to contribute to the advancement of CAR T-cell therapy by identifying areas for improvement and potential solutions to overcome existing challenges in in vivo delivery systems.

CAR T-Cells Therapy:

Reliable and effective gene transfer systems are necessary to increase the CAR T-cell treatment success rate. Leukapheresis is used to first isolate autologous T-cells, which are then genetically modified ex vivo using both viral and non-viral transfection techniques. Patients undergo lymphodepleting chemotherapy after quality control testing on the produced and expanded T-cells, and then they receive an infusion of CAR T-cells. Eshhar's group at the Weizmann Institute of Science in Israel invented the idea of chimeric receptors; the extracellular domain of CARs is made up of an antigen-binding moiety and a spacer. Three types of moieties can be identified: a human Fab fragment from phage display libraries, a scFv produced from antibodies, or naturally occurring ligands that bind to their corresponding receptor [10-13].

Tumor-associated antigens (TAAs) on the cell surface of malignancies can be recognized and bound to by ScFv, a variable

monoclonal antibody fragment that is derived from mouse monoclonal antibodies (mAbs), humanized Abs, or fully human Abs. In contrast to typical TCRs, CARs are able to recognize carbohydrate glycolipid compounds produced on the surfaces of tumor cells as well as raw antigens, even in the absence of MHC-mediated antigen presentation. MHC class I and II barriers are crossed by CAR T-cells of both the CD8+ and CD4+ subsets, allowing for the target T-cell to be redirectedly recognized. Perforin and granzyme exocytosis are the main mechanisms of cytolysis used to eradicate CAR-mediated malignancies; death receptor signaling through Fas/Fas-ligand (Fas-L) or tumor necrosis factor (TNF)/TNF-receptor (TNFR) plays a small role in this process. The immunoglobulin (Ig)G1 hinge region usually acts as a spacer between the transmembrane domain and the antigen-binding domain [14-16].

The external antigen-binding domain and the intracellular signaling domain are connected by the transmembrane domain, which is typically derived from CD8 or CD28. The stability of the CD28 transmembrane receptor is well-known. The intracellular domain, which is mainly made up of CD3ζ, sends out the first signal that triggers T-cell activation and activity. Co-stimulatory signals generated in response to the second signal increase the production of cytokines like interleukin (IL)-2 and promote the T-cell's durability and in vivo proliferation. The intracellular signaling domain plays a major role in determining the functional behavior of CARs, hence careful preclinical and clinical evaluation is required [17].

Since 1989, four different generations of CAR T-cells have been distinguished from one another based on the structural makeup of the intracellular domain. The complex TCR/CD3 z chain (CD3z) is a characteristic unique to the first generation. The second generation, on the other hand, presents the idea of dual signaling for T-cell activation, in which contact with co-stimulatory molecules or detection of antigens causes activation. For instance, adding CD28/B7 as a co-stimulatory molecule can improve the production of IL-2, which in turn activates T-cells and inhibits their death. The inclusion of sequences from co-stimulatory signals, including OX40 (CD134), CD28, 4-1BB (CD137), CD27, and DNAX-activating protein 10 (DAP10) with CD3z, results in enhanced responses in the third generation of CARs. Especially in situations when antigen exposure is recurring, this combination of several co-stimulatory signals

improves CAR T-cell activity by encouraging cytokine production, T-cell proliferation, and antigen elimination. Notwithstanding these developments, there is still uncertainty regarding the extent to which the third generation of CARs improves patient outcomes when compared to the second generation, which calls for more research to validate the third generation's safety and effectiveness [18].

Known as CAR T-cells capable of universal cytokine-initiated killing (TRUCK), simplified CARs might provide an effective remedy. Cytokines such interferon-gamma (IFN-γ) and IL-12, which can elicit innate immune responses and lead to antigen-dependent tumor cell elimination, are produced transiently by TRUCK cells. While the second and fourth generations of CARs are similar, the fourth generation adds a protein, like IL-12, that is either constitutively or inducible produced upon CAR activation. Fourth-generation CARs are thought to include T-cells that have been steered for TRUCKs since their activation produces and secretes cytokines that improve tumor killing through a variety of synergistic methods. Currently, research is being done on a fifth generation of CARs, which are based on the second generation but have a binding site for the transcription factor signal transducers and activators of transcription 3 (STAT3) and a shortened cytoplasmic IL-2 receptor β-chain domain. Concurrent TCR, co-stimulatory, and cytokine signaling are produced when this receptor is activated, supplying the three complementary signals needed for thorough T-cell activation and proliferation [19].

Barriers and Challenges to CAR T-Cells:

The FDA approved five CAR-T cell medicines between 2017 and 2021. The first to be licensed for adult patients with specific forms of B-cell lymphoma is KYMRIAHTM (Tisagenlecleucel). For the treatment of B cell lymphoma, TECARTUSTM (Brexucabtagene autoleucel), BREYANZI® (Lisocabtagene maraleucel), and YESCARTATM (Axicabtagene ciloleucel) are also approved. Idecabtagene vicleucel, or ABECMA®, is used in the treatment of multiple myeloma. Although a sizable pipeline of CAR-T cells is being investigated in clinical trials, the general application of CAR-T therapy is hampered by a number of issues, such as related toxicity, immunosuppressive tumor microenvironments, and intricate manufacturing procedures [20].

Cytokine release syndrome (CRS), immune effector cell-associated neurotoxicity syndrome (ICANS), and on-target/off-tumor toxicity are the main toxicities linked to the current CAR-T therapy. Following CAR-T cell therapy, inflammatory cytokines such as IL-6, IL-10, IL-2, and TNFa are released, which causes CRS and can result in organ failure, fever, hypotension, hypoxia, and potentially fatal side effects. In as many as 25% of patients, CRS can be severe or fatal. Usually within a week of receiving CAR-T cell therapy, ICANS manifests as neurological problems including toxic encephalopathy, aphasia, disorientation, and trouble pronouncing words. The expression of targeting proteins on both normal and malignant cells causes on-target/off-tumor toxicity. For instance, by eliminating CD19+ B cell progenitors, CD19 CAR-T cell therapy can result in B cell aplasia and hypogammaglobulinemia, which has anti-tumor effects [21].

Tumor microenvironment (MVT) immunosuppression delays T cell fatigue and prevents CAR-T cell activation. The immunosuppressive MVT's poor conditions are caused by a number of immunosuppressive cells, hypoxia, and the continuous production of coinhibitory receptors. The term "hypoxia" describes an oxygen shortage inside the MVT tumor. Tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and regulatory T cells (Tregs) are examples of the immunosuppressive cells present in the tumor microenvironment (MVT). Currently, the production of CAR-T cells is a multi-step, intricate procedure that includes T cell collection, genetic alteration, expansion, and reintroduction into patients. There are risks associated with these complex technologies and logistical procedures. Furthermore, creating standard operating procedures is quite difficult because manufacturing is a long-term, customized process. Many cancer patients who require this novel therapy are unable to obtain CAR-T cells due to their costly and technologically complex manufacturing processes [22].

In-vivo Delivery:

Gene-editing instruments and CAR genes are among the gene therapy products that have been administered using a range of delivery vehicles. Viral vectors are the most often used system among these since they have the highest transfection efficiency. They are linked to cellular toxicity and immunogenicity, though. Because they have less insertional mutagenesis than lentivirus and

adenovirus, adeno-associated viruses (AAV) are less hazardous than other viral vectors. However, compared to other viral vectors, AAV vectors have a smaller package size (~5.0 kb). Physical or chemical methods can be used to classify non-viral gene delivery systems. Physical treatments include procedures like gene guns, laser irradiation, needle injection, and electroporation. In example, electroporation is frequently used because it uses electric pulses to create pores on cell membranes and temporarily make genes permeable. Physical methods do not target interior organs, but they do show low immunogenicity [23].

Nano-delivery systems such as cationic lipids or polymer-based nanoparticles, silica nanoparticles, golden nanoparticles, quantum dots, carbon nanotubes, exosomes, ferritin, and cell membranes are mostly used in chemical procedures. As one of the most alluring non-viral vectors for gene transfer, lipid-based nanoparticles have multiple formulations licensed for clinical use. Notably, the CRISPR/Cas9 system for in vivo genome editing at therapeutically relevant levels and SARS-CoV-2 mRNA vaccines have both been effectively delivered via lipid nanoparticles. Since positively charged polymers can form stable polyplexes with genes, breaking cell membranes and enabling endosomal escape, polymer-based nanoparticles also hold potential for gene transport applications. Unfortunately, the toxicity and immunogenicity of polymer-based nanoparticles as a result of their interactions with negatively charged blood circulation proteins and cell membranes is one of their drawbacks [24].

Exosomes are naturally occurring nanoscale extracellular vesicles that are being studied extensively as potential vectors for gene delivery because of their low immunological clearance and intrinsic biocompatibility. Nevertheless, further work is required to solve issues with isolation, purification, and production. For the purpose of delivering genes, biomimetic vectors made from cell membranes—such as those found in red blood cells and platelets—offer inherent biocompatibility and targeting capabilities. However, they still need to improve their transfection efficiency. The distinct features of each of the other chemical nano-vectors affect how effective they are at delivering genes. Although some have the potential to treat a variety of illnesses, the best delivery methods for clinical application have not yet been established [25].

In-Vivo Delivery Techniques:

The creation of autologous CAR T cell products is necessary for conventional CAR T cell treatment. Unfortunately, this procedure is labor-intensive and time-consuming, which drives up the expense of therapy. Furthermore, patients may not receive therapy in a timely manner for diseases that proceed quickly. The production of allogeneic "off-the-shelf" or universal CAR T cells has been suggested as a solution to these problems. However, their application is limited by the rejection of allogeneic T cells and the potential for graft-versus-host disease (GvHD) [26].

Different delivery systems have been developed to address these problems. In vivo generation of CAR T cells has been achieved through the use of genome editing techniques. CAR T cell production in situ has been achieved with the use of synthetic DNA nanocarriers. Biodegradable polymer nanoparticles containing plasmid DNA encoding CARs are surface-coupled with ligands that target T cells. The engagement of circulating T lymphocytes is improved by these ligands. Moreover, peptide-containing nanoparticles help the microtubule transport mechanism carry genetic material into the cell nucleus. Transgene integration is made possible by the co-delivery of a transposase. This method successfully transduces T cells, producing transfected lymphocytes that proliferate vigorously. According to studies, mice given DNA nanocarrier treatment had survival rates that are similar to those of mice given traditional CAR T cell therapy. All things considered, this delivery method has the potential to shorten the time and expense needed to generate CAR T cells [27].

A number of scaffolds have been developed to improve CAR T cell ex vivo generation. One widely utilized technique is the commercially available therapeutic cell expansion product Dynabeads, polystyrene beads coated with antigen molecules. As a substitute strategy, Cheung et al. presented APC-mimetic scaffolds (APC-ms), which are intended to both stimulate CAR T cells and encourage the growth of primary T cells. These APC-ms were made up of additional biomolecules and lipid bilayer-coated silica microrods. These scaffolds were used to cultivate T cells and supplied pro-survival cytokines and stimulation. It was shown that the APC-ms, which replicated the interaction between T cells and natural antigen-presenting cells (APCs), were more successful than Dynabeads. In particular, functionalized lipid bilayers comprising T cell activating chemicals like IL-2 and anti-CD3/CD28 antibodies were coated onto the mesoporous silica micro-rods. Comparing

APC-ms to Dynabeads, CAR T cell growth was dramatically increased, with a five-fold increase. Crucially, these scaffolds continued to be incorporated into the finished cell product and were biodegradable. Scaffolds based on biomaterials were also created for T cell transduction. T cell movement was aided by calcium-crosslinked alginate scaffolds with macroporous architectures. Retroviral particles were placed into these scaffolds to effectively transduce T cells while preserving their complete functionality [28].

A viable method for introducing messenger RNA (mRNA) into T cells and promoting the production of chimeric antigen receptors (CARs) is through transfection with nanoparticles. This technique has the potential to be non-viral transfected, enabling temporary CAR expression to reduce the possibility of long-term damage related to CAR T cells. Ionizable lipid nanoparticles (LNPs) were used by Margaret et al. to transfer mRNA to T cells. The quantity of CAR expression and the amount of mRNA delivered were shown to be correlated, indicating that mRNA concentration regulation may lessen the possibility of uncontrollably high levels of toxicity. Because of their ionizable lipid cores, which shielded them from environmental pH fluctuations, LNPs provided effective nucleic acid delivery. However, the anticancer efficacy of CAR T cells created with LNPs was only moderate. Gene delivery via polymeric nanoparticles has also been studied. Several ligands were grafted onto the surface of these nanoparticles to engage with particular cell surface receptors in order to improve the precision of targeted delivery. The goal of this strategy is to increase the efficiency and specificity of gene delivery to target cells [29].

Two major toxicities that have been linked to CAR T cell infusion into patients are "on-target, off-tumor" toxicity and cytokine release syndrome (CRS). CRS is a serious adverse event brought on by CAR T cells' overproduction of pro-inflammatory cytokines. Conversely, "on-target, off-tumor" toxicity happens when CAR T cells identify and target normal cells and tissues that also express the same antigen that the T cells are targeting, in addition to tumor cells. Tissue-associated antigens (TAAs) that are either not expressed at all or only slightly expressed by normal tissues make it difficult to find appropriate antigens for CAR targeting in solid tumors. Preclinical research has investigated a number of approaches to overcome these safety problems [30].

Suicide genes are one type of safety switch that can be added to CAR T cells in order to enable their removal and regulate negative effects. For example, ganciclovir can be used to selectively kill transduced cells by introducing the herpes simplex virus thymidine kinase (HSV-TK) gene into T cells. Empirical data has indicated that this approach is efficacious in mitigating graft-versus-host disease (GvHD) subsequent to allogeneic transplantation. However, HSV-TK's strong immunogenicity limits its applicability in clinical settings. A different technique uses dimerization agents and joins proteins to cause apoptosis. By combining human caspase-9 with the human FK506-binding protein, an inducible caspase-9 system (iC9) was created that allows apoptosis to be triggered by a chemical inducer of dimerization [31].

In vivo, this approach efficiently eradicates more than 85% of T cells transduced with iC9. Interestingly, low concentrations of the dimerization agent can reduce inflammatory cytokine production without totally destroying CAR T cells, which nevertheless maintain control over tumor recurrence. This means that iC9 can also manage the function of CAR T cells. As an alternative, transplanted T cells can be eliminated via antibody-dependent depletion mechanisms. For example, in the event of toxicity, the anti-CD20 antibody rituximab can be used to target CAR T cells that express human CD20 and cause depletion. When cetuximab is used as a target, co-expression of an inactive, truncated human EGFR molecule on the cell surface may also aid in the antibody-mediated depletion of CAR T cells [32].

Conclusion:

In conclusion, by utilizing the immune system's ability to identify and eradicate tumors, the field of CAR T cell therapy has enormous potential to transform the treatment of cancer. However, there are a number of obstacles standing in the way of the broad application of CAR T cell treatment, including logistical difficulties associated with production and delivery, as well as toxicities such cytokine release syndrome (CRS) and "on-target, off-tumor" consequences. Numerous tactics have been developed in an attempt to improve the efficacy and safety of CAR T cell treatment. Suicide genes and apoptosis-inducing pathways are examples of safety switches that may be used to reduce side effects without sacrificing the effectiveness of treatment. Furthermore, improvements in scaffold-based methods and nanoparticle-mediated transfection

offer potential for enhancing CAR T cell generation and delivery. Additionally, the investigation of allogeneic "off-the-shelf" CAR T cells offers a chance to get around some of the logistical issues that arise with autologous CAR T cell therapy. Nonetheless, more research on immune evasion and tolerance tactics is required because of worries about graft-versus-host disease (GvHD) and immunological rejection.

Moreover, since "on-target, off-tumor" toxicity presents a serious safety risk, identifying appropriate antigens for CAR targeting in solid tumors continues to be an important research focus. Enhancing the specificity and safety of CAR T cell therapy requires preclinical research focused on antigen selection and CAR design optimization. Overall, even though CAR T cell therapy has advanced significantly, further research is required to solve unresolved issues and realize the full promise of this ground-breaking method of treating cancer. Through continuous innovation and interdisciplinary cooperation, CAR T cell therapy has the potential to revolutionize the field of cancer treatment and enhance patient outcomes globally.

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