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Overview of CAR-T Cell Therapy Application in Cancer

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Abstract

Genetically engineered T cells expressing chimeric antigen receptors (CARs) have shown notable therapeutic efficacy in persons with certain subtypes of B cell leukemia or lymphoma. Moreover, there is compelling evidence of their efficacy in individuals diagnosed with multiple myeloma. Nevertheless, several barriers impede the efficacy and widespread acceptance of CAR T cell treatment in these individuals, as well as in persons with other forms of cancer, particularly solid tumors. Significant challenges related to CAR T cells consist of severe toxicities, restricted capacity to travel to, infiltrate, and activate inside tumors, insufficient long-term persistence in the body, antigen evasion and variety, and issues in the manufacturing process. In order to expand the application of CAR T cells to a wider range of cancer types, it is crucial to enhance the designs of CARs beyond conventional structures. Investigators are using several engineering strategies to address the current challenges and improve the safety, efficacy, and user-friendliness of this therapy method. This paper presents an introduction to the CART cell, including its system of action, problems and limits, and its engineering.

Keywords: Solid Tumor, Chimeric antigen receptor, Immunotherapy, Adoptive-cell therapy.

An overview of CAR T cell treatments

Cancer has emerged as a prominent global cause of mortality, responsible for 21% of all fatalities. Annually, there are more than 16 million newly diagnosed cancer cases globally, resulting in over 9 million fatalities. Cancer immunotherapy is an innovative approach that is transforming the treatment of cancer. It utilizes your immune system to fight against cancer and leverages the potential of antibody-based treatment to achieve the same outcome (1). Despite the development of many treatments such as surgery, radiation, chemotherapy, and specific therapies, the outlook for patients with advanced and recurring cancers is still unsatisfactory. Cancer immunotherapy has provided optimism for individuals with cancer. Cancer immunotherapy offers a new and efficient approach to treating malignant tumors by stimulating the body's immune system. The primary objective of cancer immunotherapy is to stimulate the immune system and eradicate malignant cells. Hence, it offers enduring responses and the potential to develop enduring protection against relapses. Nevertheless, tumor cells have the ability to evade the immune system's reaction via many ways. The outcome is that the immune response of the body is unable to accurately identify and eliminate cancerous cells. In order to address this issue, it is necessary to

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intentionally alter the immune system (2, 3).

Over the last ten years, chimeric antigen receptor (CAR) T-cell treatment has accomplished extraordinary triumphs. While CAR T cell therapies show promising potential, they also pose hurdles due to the CAR structure, the physiology of antigen targeting, the risk of on-target off-tumor reaction, safety concerns, and the occurrence of severe side effects. Their future application focuses on selecting prospective inhibitors that may prevent the promotion of T cells in controlling brain tumors, managing toxicity that occur on-target but off-tumor, improving the safety of CARs, and studying the capacity of malignant cells to be depleted in CAR T cell therapy research models. This review presents an analysis of the efficacy of newly created chemicals in managing T cell therapy and investigates the structure of CAR T cells in relation to tumor management. Due to technological advancements, it is expected that conquering immunity and enhancing the efficacy of CAR T cell treatment would be achievable (4).

The United States Food and Drug Administration has granted approval for three CAR T cell treatments to treat certain forms of acute lymphoblastic leukemia and non-Hodgkin lymphomas. This signifies significant advancements in transforming the idea into practical medical therapy. CAR T cell therapies utilize redirected T cells to effectively kill tumor cells and their targeting of distinct tumor antigens and wide acceptance greatly enhance their versatile applications (5). Meanwhile, the maturation of cell technology makes progress in genetic modification for therapeutic purposes and decreases the obstacle in CAR optimization to enhance safety, primarily with attention on the cytokine release storm and on-target off-tumor toxicity observed in clinical practice. Furthermore, beyond the existing tumor-associated antigens, novel candidate targets are being exploited and explored to enlarge the CAR T cell therapeutic reagent spectrum. These advancements greatly broaden CAR T cell therapy application for hematological and non-hematological disorders (6). Since the approval of ipilimumab for use in the treatment of melanoma, more than 1,000 immuno-oncology drugs are currently undergoing evaluation, with high expectations for further expansion of the treatment field. At the same time, it has also led to a lack of scientific understanding and public confusion. Caring for patients with cancer requires an understanding of cancer immunotherapy and its use while trying to dissipate health literacy and ultimately impart a new and better understanding of the world of oncology and scientific research (6).

T-cell therapy is used to enhance the immune system's defense in order to treat some forms of cancer. More than six decades of research has been dedicated to studying the use of CAR T-cells, particularly in treatments aimed against non-solid organ cancers. Clinical studies that confirm the therapeutic efficacy of mast cells primarily target lymphomas, since these types of cancer are more vulnerable to cell-mediated destruction, as shown in TIL treatment for melanoma. For personalized treatment, the individual's own T-cells are collected and a chimeric antigen receptor is genetically modified into a lentivirus. Patients get high-dose chemotherapy to eliminate the residual bone marrow, enabling the modified T-cells to successfully establish themselves (7). Nevertheless, the significant toxic consequences, such as cytokine release syndrome, pulmonary edema, neurotoxicity, resistance, or impaired engraftment, persistently limit its effectiveness in patient treatment. To fully harness the potential of cell-based therapeutic strategy, it is necessary to acquire a more profound and enhanced comprehension of the methodologies and instruments used in CAR T-cell treatment (5, 7). Chimeric antigen receptor T cell therapy is a kind of cancer treatment that utilizes the immune system. Oncology clinics use this technique to identify and eliminate cancer cells by guiding a patient's genetically modified T-cells to specifically attack and eradicate the cancerous cells. The technique is significant due to its capacity to prevent the return of cancer, especially in individuals with tumors that are resistant to traditional treatment methods. Nevertheless, CAR T-cell treatment encounters a multitude of obstacles and restrictions that diminish its effectiveness in the field of clinical cancer (6). These issues include a variety of problems, including a lack of specificity in targeting tumors and the occurrence of harmful consequences, even in cells that are specifically targeted. This publication provides a comprehensive analysis of the tools, tactics, and expertise involved in the implementation of CAR T-cell therapy. It aims to provide a clearer understanding of the inherent constraints and difficulties in using CAR technology for cancer immunotherapy (8).

Immune cells play crucial roles in the body's ability to detect, control, and adapt to changes in the tumor, contributing in many ways to both the establishment and the restriction of cancer. The clinical research using this approach has led to unprecedented cures in patients with refractory and relapsed malignancies who failed traditional treatments and even allogeneic HSCT (hematopoietic stem cell transplant) rescue. The exciting action of the therapy is mainly based on antigens-specific targeting, activation, and continuous amplification, with genetic infections to the patient's T cells in vitro (9).

In recent times, because to the rapid progress in CAR T cell treatment, there has been a resurgence of interest in cancer immunosurveillance as a prominent field of study. The efficacy of cancer immunotherapy has significantly improved in the past few years, with

cellular immunotherapy seeing a rise with the advent of CAR T cells. CAR T cells, also known as gene-modified T cells, are a novel and very promising form of cancer immunotherapy. These cells are made up of an extracellular ligand binding domain, a hinge region, and a transcellular signal transduction domain (10). Once communicated, either the recipients or the allogenic HSCT donor T cells may be selectively activated against particular cell surface antigens. Ultimately, the activated T cells undergo rapid and uncontrolled division due to positive feedback immunological responses, specifically targeting the cancerous cells. Modified CAR T cells have been used in the treatment of both recipients and allogenic HSCT donors. Additionally, there have been documented cases of repeated infusions of MSCs-CAR T cells after allogenic HSCT. Refractory and relapsed cancers are successfully removed in over 80% of the treated patients, similar to the results shown in the clinical tests conducted on patients. They received authorization from the U.S. Food and Drug Administration (FDA) and later from the European Medicines Agency (EMA) for cancer immunotherapy.

The two adoptive cell therapies are CAR-T cell therapies-Chimeric Antigen Receptor T Cells (sometimes called designer T cells). The first two products are also the first two gene therapy products to gain market approval. When a T cell receives appropriate signals through the T-cell receptor (mainly from the peptide-MHC complex) and co-stimulatory receptors (mainly CD28 or 4-1BB), it triggers T-cell activation. The main purpose of CARs is to combine the ability of antibodies (binding to specific target proteins) with the signaling properties of T-cells (13). These CARs have three main parts: a domain which works as the scFv from an antibody binding to the antigens, and an extra-cellular hinge and stalk region (which joins the domains and charge molecules to help signal transduction). Some authors have reviewed the methods used to derive monoclonal antibodies binding to different TAAs (13).

The efficacy of CAR-engineered T cells in inducing durable remissions for individuals with recurrent or refractory B lymphoid tumors is now well recognized. Our primary objective is to analyze the specific attributes of CAR-T cells that continue to exist in patients who remain in remission for at least 6 months after injection (14). Considering the established long lifespan and efficacy of traditional immune-mediated therapies, such as T cells developing specificity to antigens showed by cancer cells during allogeneic stem cell transplant (alloSCT), we had high hopes that highly lasting CAR-T cell responses could be produced by increasing the abundance of CAR-T cells by a factor of 1000 through the elimination of inhibitory signaling in T cells. In this study, we show that functional CAR-T cells remain present in patients for up to 10 years of clinical follow-up, and even after illness resolution, they may be reactivated by exposure to antigens. Additionally, we observed that cellular transplant can extend the persistence of CAR-T cells by an additional year (14). CAR-engineered T cells have the ability to induce prolonged periods of disease absence in individuals with relapsed or refractory B cell malignancies. In light of the growing recognition of relapses that happen many months following cellular infusions, our objective was to determine if CAR-T cells may remain in patients who are still in remission more than 6 months after the infusion. Our study shows that CAR-T cells, which have been linked to long-lasting responses, remain present in the body for a minimum of 6 years after being infused, with a maximum follow-up period of 10 years. The efficacy of CAR-T cells is reduced comparing to the first period after infusion, however it may be restored with further exposure to antigens. CAR-T cells that react to the antigen may remain in the bloodstream for over ten years after the illness has been resolved (4, 15).

Summary of CAR-T Cell Therapy

CAR-T cell therapy is now recognized as a very attractive and innovative therapeutic approach for blood cancers. The method has seen significant success in achieving sustained remission rates in individuals who have extremely chemorefractory illness. The exceptional reactions are believed to be primarily attributed to the distinctive biology of the modified cells, characterized by the presence of distinct antigen receptors and their capacity to systematically eliminate cancerous cells. While the initial response rates of CAR-T cells may seem encouraging, the long-term effectiveness of these biologicals is generally limited by their intrinsic durability (16). The primary function of CAR-T cells is their capacity to undergo excessive proliferation upon binding to antigens, often resulting in cytokine release syndrome. Subsequently, more than 99% of the remaining cells are eliminated through a controlled and sequential process of T cell differentiation. The continued existence of CAR-T cells is contingent upon and seems to be directly related to the amount of antigens present. Therefore, in cases where the quantities of antigens are low, it is uncommon to see long-lasting responses, which is why a significant number of individuals have a recurrence following their first remission produced by CAR-T cell therapy (17).

There is a growing body of research suggesting that TAA-positive escape variants provide a major obstacle to the long-term survival and effectiveness of CAR-T cells. Following the first notable achievements

in using CAR-T cells in treating leukemia patients, other clinical studies are presently underway globally. Each research team employs distinct iterations of CAR-T cell treatment and evaluates diverse iterations of CAR-T cells for various blood malignancies and solid tumors. According to their findings, a considerable proportion of patients who were previously diagnosed with cancer had remission after receiving a simple infusion of reprogrammed T cells. Initial evidence suggests that CAR-T cell treatments have the potential to make a patient almost completely free of cancer for a prolonged period of time, namely up to 18 years. Nevertheless, a significant number of first recipients of CAR-T therapy have just recently undergone the treatment. CAR-T therapy, being a novel and groundbreaking approach, has the potential to save the lives of many individuals suffering from cancer. Consequently, our comprehension of the long-term consequences of CAR-T treatment remains incomplete. At now, CAR-T is the only category of immunotherapies that has been effectively reprogrammed, and there is a growing number of ongoing clinical studies for CAR-T treatment. We are still in the early phases of fully harnessing the promise of CAR-T cells for cancer treatment (19).

CAR-T cell therapy, or chimeric antigen receptor T cell treatment, involves the modification of a patient's T cells, a specific kind of immune system cell, to specifically target and eliminate cancerous cells. The foundation for this emerging science was established some years ago, and only recently have we been able to advance and evaluate CAR-T cell therapy for those with cancer via clinical studies. CAR-T treatment involves the initial extraction of T cells from a blood sample in a cancer patient, followed by the introduction of a chimeric antigen receptor (CAR) into these T cells (20). After being reprogrammed, the T cells, which now possess the CAR protein, undergo rapid multiplication, resulting in a significant increase in the number of T cells capable of destroying cancer cells. Once these reprogrammed T cells are collected, they are reintroduced into the person's body. Although these reprogrammed T cells, which are designed to destroy cancer cells, are crucial in eliminating the tumor's malignant cells, their indefinite survival after the tumor has been eradicated is not attributed to the CAR component of the CAR-T cells (21).

CAR-T cells are T cells obtained from the individual's body via a blood collection technique known as leukapheresis. Subsequently, cells are sent to the manufacturing facility and genetically altered by introducing a gene that codes for a chimeric antigen receptor. A chimeric antigen receptor (CAR) is an engineered T cell receptor that combines the advantageous features of both antibodies and T cell receptors. Simultaneously, the duration of the CAR's lifetime is extensive. Once the CAR is inserted, the T cell has the capacity to selectively identify and attach to antigens. This leads to the stimulation of T cells, allowing the cell to multiply and produce significant quantities of cytokines. Additionally, the T cell develops cytotoxic function. Various CAR-T cells often recognize distinct antigens. These T cells are specifically engineered to identify and bind to tumor antigens, leading to the destruction of tumor tissue by the T cells. Hence, this technique signifies a paradigm-shifting advancement in the realm of cancer therapy. Simultaneously, the remarkable sensitivity and specificity of CAR-T cells render them highly responsive to the quantity of tumor antigens (22).

Types of Immunotherapy

CARs, also known as CAR-T cells, are highly potent immune cells that possess the ability to identify and target particular tumor antigens, therefore significantly bolstering the immune response. These super T cells exhibit both innate immune killing capabilities via NK ligand production and increase the immune system's response by binding to costimulatory signals (20, 21). Furthermore, the extracellular domain of fourth-generation CARs was modified to create sub-regions that functioned as a bispecific T-cell engager (BiTE). This modification not only increased the cytotoxic activity but also prevented any adverse impacts on the whole system. Following the first clinical report of CAR-T cell therapy in chronic lymphoblastic leukemia in 2011, the Food and Drug Administration (FDA) has granted approval for Kymriah in 2017 and Yescarta and Breyanzi (JCAR017) in 2018. These approvals allow for the use of CD19 CAR-T cell therapy in the clinical treatment of B-cell malignancy. Despite significant advancements in the treatment of blood cancers, the use of CAR-T cells for solid tumors still faces significant challenges in clinical practice (23).

Immunotherapy may be categorized into many groups, such as cytokines, adoptive cell transfer, and monoclonal antibodies. Adoptive cell transfer may be categorized into three subtypes: tumor-infiltrating lymphocytes, TCR-T cell treatment, and chimeric antigen receptor CAR-T therapy. These subtypes have shown encouraging clinical outcomes (24). The concepts of these forms of cell treatment rely on manipulating the in vitro culture by using non-specific (gamma chain cytokine and OKT3) or antigen-specific anti-CD3 monoclonal antibodies (mAbs) to activate T cells, prompting a cytotoxic response. Meanwhile, same T cells were grown along with γ-retrovirally or lentivirally transduced cells that had been modified to incorporate genes encoding TCR or CAR (24).

The research and development of CAR T treatment

has been at the forefront for over three decades. The first iteration of CAR T cells may alone identify tumor-associated antigens (TAAs) in a way that is not reliant on major histocompatibility complex (MHC), thereby failing to produce the intended anti-tumor impact. The second generation of CAR T cells has an enhanced architecture that incorporates individual or multiple insertions of costimulatory molecules such as CD28, 4-1BB, OX40, ICOS, CD27, and CD40, among others. The second generation of CAR T cells has been extensively and effectively used in several clinical research, thanks to their ongoing improvement in functionality. Tisagenlecleucel, Axicabtagene ciloleucel, and Lisocabtagene maraleucel are commercially available treatments that have been used to treat CD19+ B lymphoblastic leukemia and lymphoma (25). The third generation of CAR T-cells typically incorporates two costimulatory domains, with many of the second costimulatory molecules containing a beneficial cytokine or signal target. This enhances their toxicity reactions to the receptor and reduces the rate at which targeted cells can evade the immune response. The third generation of CAR T-cells has the most potential to be the most effective option for CAR T therapy. Moreover, the fourth-generation CAR T-cells are often known as armored CAR T-cells. These cells include transgenic products that encode small interfering RNA (siRNA), chimeric cytokine receptors (CCRs), or cytokines that improve the function of T cells and/or antigen-presenting cells (APC) (26). This enhancement primarily operates by increasing the specificity, synthesis of cytokine levels, and promoting the altered homing potential. It also supports various cytocidal activities of polyfunctional CAR T-cell populations that are highly AMP. Additionally, it extends the proliferation, persistence, and overall quality of CAR T-cells. Only a limited number of clinical experiments have used the fourth CAR T lineage in medicinal applications. Furthermore, it implies that this therapy has the potential to provide improved safety and decrease the immunosuppressive environment caused by the tumor (26).

Mechanism of Action of CAR T Cells

The best-documented evidence of a CAR derived from T cell receptor (TCR)-α and -β chains is the CD8 molecule, which recognizes the class I MHC/antigen peptide complex (pMHC). CARs are synthesized from single-chain variable fragments (scFvs), made up of paired variable regions of the heavy and light chains (VH and VL, respectively) of a monoclonal antibody (27) connected with a short linker peptide (21). The extracellular ectodomain is typically synthesized by combining VH and VL by a flexible peptide linker of 10-20 amino acids; the transmembrane domain links the extracellular portion to the endodomain and is both required for correct positioning within the membrane lipid bilayer and signal transduction. The endodomain is the key molecular component where the signaling cascades are triggered following the engagement of the CAR with its antigen. In most cases (but not all), it contains variants of well-known intracellular signaling domains, such as the CD3-ζ subunit of the T cell receptor and one or more additional co-stimulating molecules, such as 4-1BB or other elements from the tumor necrosis factor receptor (TNFR) superfamily, such as CD28 (28). Collectively, the composition of the various functional antigen recognition units indicates that CAR function ultimately draws upon the signaling machinery of the TCR, which is required to also produce polyclonal activation in unresponsive T cells, while at the same time not being able to exhaust the cells. In this sense, the CAR is representative of active receptor engineering. The challenges in translating a substantial amount of progress from decades spent studying TCR signaling cascades, science and human therapeutics, into CAR T cells are newly brought to the fore. With CARs in numerous pipelines to treat cancer, immune and inflammatory disorders, integrating novel insights into their activity versus endogenous TCR could offer more focused and selective therapeutic strategies (29).

Recent advancements in the field of T cell engineering have successfully transformed alternative T cell immunotherapy into a viable treatment option for patients. The integration of TCR and gene-editing technologies has facilitated the fast progression of CAR T cells from theoretical validation to practical use in a few of years. Undoubtedly, the discovery of very precise tumor antigens, together with the use of T-cells produced from the patient's own body, makes CAR-based treatment a perfect subject for personalized medicine. Patients in dire need are prepared to give their permission to such experimental therapy (1). Hence, the quantity of medicinal goods now being development and undergoing evaluation in clinical trials is rapidly increasing. This exemplifies the capacity of research in this domain to be used in practical settings. The first clinical results documented so far are very promising for certain hematological cancers. Nevertheless, obstacles and restrictions might be encountered in both the pre-clinical research and clinical implementation of CAR T cells (30).

Cancer treatment has been a major health challenge for countless millennia, with only minor degrees of progress being made for the vast majority of human existence. However, thanks to intense research and perseverance, great strides in therapeutic development have been made over the last few hundred years. These treatments have focused on

the disease with the entire objective identification and eradication of abnormal tumor cells, and they have roused interest from the public, healthcare professionals, and regulatory, and funding agencies ever since (31). Individual therapeutic modalities are superior to others for certain cases, with some modalities assuming first-line treatments while others are used as adjuvant therapies or utilized when disease recurs. With its demonstrated antitumor potential, T-cell therapy offers an important tool for countering cancer aggressiveness while offering the possibility of reducing many of the long-term, late events associated with conventional cancer treatments currently in use. T cells can be engineered to express chimeric antigen receptor-modified autologous T cells that are seamlessly integrated into the clinical oncological portfolio. They more effectively target and lyse the cells of most hematological malignancies which express the target antigens at levels that are considered to be safe (32).

Novel therapies for non-Hodgkin's B-cell lymphoma have the potential to greatly enhance long-term results for patients, even those with unfavorable prognoses. Chimeric antigen receptor T-cell (CAR-T cell) therapy is revolutionizing the treatment approach for afflicted patients. Due to its exceptional ability to enhance a patient's immune response against cancer and exhibit strong T-cell killing mechanisms in the body, CAR-T cell therapy has been shown to greatly improve survival rates in hematological malignancies that express CD19, especially when traditional treatments are ineffective. However, like any novel areas of medicine, CAR-T cell treatment has certain hurdles that are directly linked to its commercialization and the progress of clinical-grade practice (33).

Obstacles and Constraints in CAR T Cell Therapy

Antigen evasion

An important challenge in CAR-T cell therapy is the development of tumor resistance to CAR designs that specifically target a single antigen. Although individual antigen-targeting CAR-T cells can at first offer impressive outcomes, a significant proportion of individuals who receive these cells experience either a complete or partial inhibition of target antigen expression in their cancerous cells. The phenomenon being referred to is known as antigen escape. For example, a significant number of patients with recurrent and/or persistent acute lymphoblastic leukemia (ALL) have shown long-lasting favorable responses to CAR-T cell treatment that targets CD19, ranging from 70-90%. However, current follow-up information suggests that a common mechanism of resistance to treatment is starting to emerge. During the return of the illness after therapy, 30-70% of individuals report a reduction or absence of the CD19 antigen. Moreover, a reduction or lack of BCMA production has been observed in those with multiple myeloma who are receiving therapy with BCM-targeted CAR-T cells. Similar patterns of resistance to antigen escape have been discovered in solid malignancies (13, 34). A concrete example is a research study on CAR-T cell therapy that specifically investigated the role of IL13Ra2 in treating glioblastoma. The study showed that there was a decrease in the expression of IL13Ra2 in tumor recurrences. In order to reduce the frequency of recurrence in CAR-T cell treatment for both hematologic malignancies and solid tumors, many strategies are now relying on the specific targeting of distinct antigens. These use either dual CAR constructs or tandem CARs, which are single CAR constructions containing two single-chain variable fragments (scFvs) to concurrently target multiple tumor proteins. From a clinical perspective, it seems that both of these techniques have the potential to achieve durable rates of remission. Ongoing clinical trials are investigating the efficacy of combining CD19 with either CD20 or CD22 (35). Initial results from clinical trials using dual-targeted CAR-T cells (targeting CD19/CD22 or CD19/BCMA) demonstrate promising results. Specifically, first results from trials using CD19/CD22 CAR-T cell therapy indicate promising efficacy in adult individuals diagnosed with acute lymphoblastic leukemia (ALL) and diffuse large B cell lymphoma. Furthermore, preliminary results on the use of BCMA/CD19 targeted CARs for the treatment of multiple myeloma demonstrate their significant efficacy and favorable safety profile. Early-stage models of solid cancers, including glioblastoma and breast cancer, have been used to study the effects of sequential CARs targeting HER2 and IL13Ra2 in glioblastoma and HER2 and MUC1 in breast cancer (36). Simultaneous targeting in both cases resulted in superior anti-tumor reactions in comparison to therapy that addressed just one factor. The study on glioblastoma showed that CARs targeting HER2 and IL13Ra2 had improved effectiveness in fighting tumors and decreased the ability of antigens to evade therapy. These results were better than those of two other therapies that targeted two other factors simultaneously. This study highlights the importance of selecting specific antigens that not only improve the body's response to tumors but also minimize the development of antigen avoidance processes, therefore preventing the recurrence of the illness (37).

On-target, off-tumor impacts

A challenge in addressing solid tumor antigens is that they are often expressed in normal tissues at varying amounts. Therefore, the meticulous selection of antigens is crucial in the development of chimeric antigen receptors (CARs), as it ensures both the efficacy of the treatment and the reduction

of potential toxicity that may arise when CARs target healthy cells. An effective strategy to tackle the problem of antigens appearing in both solid cancers and healthy tissue is to selectively target distinct alterations that are only prevalent in tumors. The alterations consist of truncated O-glycans, namely Tn (GalNAca1-O-Ser/Thr) and sialyl-Tn (STn) (NeuAca2–6-GalNAca1-O-Ser/Thr), which are prominently present in solid tumors (38). Four notable targets for CAR-T cell therapy have been investigated, such as TAG7228, B7-H3, MUC1, and MUC16. Although the first CAR-T cells that aimed at TAG72 in colorectal cancer did not elicit any anti-tumor reaction, scientists are now investigating novel versions of second-generation TAG72-CAR-T cells and other modifications that specifically target tumors after protein production. In order to broaden the clinical application of CAR-T cell therapies in both hematological malignancies and solid malignancies, it is crucial to continue creating novel methods that can reduce antigen evasion and select antigens that can effectively trigger an immune response against tumors, while minimizing concerns regarding toxicity (39, 40).

CAR-T cell transportation and tumor invasion

The efficacy of CAR-T cell treatment for solid cancers is impeded by the challenge of CAR-T cells in accessing and infiltrating solid tumors, in contrast to blood malignancies. The limited mobility and penetration of CAR-T cells inside tumors may be attributed to the immunosuppressive conditions and physical obstacles, like the tumor stroma, present in the tumor microenvironment (38). One method to enhance these constraints is by use alternative distribution channels, like local management, instead of systemic delivery. This approach has two benefits: firstly, it obviates the need for CAR-T cells to migrate to the sites of illness, and secondly, it diminishes the likelihood of on-target off-tumor effects. By precisely targeting the CAR-T cells' activity towards tumor cells, the impact on normal tissue is reduced. Preclinical research has shown that administering CAR-T cells, which specifically target HER2 and IL13Ra2, by intraventricular administration is a more potent approach for treating brain metastases of breast cancer and glioblastoma (41). Although targeted injection might appear to be more successful in principle, this approach is only appropriate for treating specific tumor disorders or oligometastatic disease. A novel strategy has been developed to significantly improve the mobility of CAR-T cells. This involves adding chemokine receptors onto the CAR-T cells that specifically recognize and respond to chemokines released by tumors (43). Latest study indicates that altering integrin αvβ6-CAR-T cells to exhibit CXCR2 or enhancing the expression of CXCR1 or CXCR2 in CAR-T cells both enhance cellular mobility and significantly enhance their efficacy in combating malignancies. The effectiveness of CAR-T cell therapy is impeded by physical barriers, like the tumor stroma, which restrict the penetration of the tumor. The stroma mostly comprises extracellular matrix, whereby heparin sulfate proteoglycan (HSPG) serves as the principal part that CAR-T cells must degrade in order to infiltrate the tumor (43).

CAR-T cell-related toxicity

Although the application of CAR-T cells has a chance to revolutionize cancer treatment, its widespread adoption as the major therapeutic choice has been impeded by a range of adverse effects, notably death. The architecture of the CAR, the specific goal, and the type of tumor are important factors that are likely to affect the frequency and extent of cytokine release syndrome (CRS), hemophagocytic lymphohistiocytosis/macrophage activation syndrome (HLH/MAS), and immune effector cell-associated neurotoxicity syndrome (ICANS) (44). Thus far, the adverse effects linked to CAR-T cell therapy have been extensively examined by individuals who have undergone the first FDA-approved CAR-T cell therapy, which is those with CD19-directed CARs. Among the most influential clinical trials, individuals have had serious and sometimes fatal consequences. When it comes to those with ALL/LBL who are receiving CAR-T cell treatment, almost all patients report minor toxicity signs. However, a considerable number of individuals (ranging from 23% to 46%) show significant overproduction of cytokines and large growth of T cells in their bodies (45). The overabundance of cytokines being released into the circulation and the ensuing stimulation of immune cells in some people result in a few detrimental consequences: CRS is defined by an overproduction of cytokines and a substantial increase in T cell growth inside the body. HLH and MAS are two severe conditions characterized by excessive inflammation. These conditions are characterized by the presence of CRS, elevated levels of serum ferritin and hemophagocytosis, kidney failure, elevated liver enzymes, enlarged spleen, fluid accumulation in the lungs, impaired natural killer (46) cell activity, and ICANS. ICANS is characterized by a high level of cytokines in the CSF fluid and impairment of the blood-brain barrier (47, 48).

CRS occurs as a result of the stimulation of CAR-T cells, which triggers the creation of significant amounts of cytokines. Mild CRS is characterized by clinical signs such as fever, lethargy, diarrhea, headache, rashes, arthralgia, and myalgia. Individuals with serious illnesses may exhibit symptoms such as low

blood pressure, heart disease, circulation problems, respiratory difficulties, renal impairment, multiple organ dysfunction, and possibly fatal outcomes. A substantial proportion of leukemia patients who received CAR-T cell treatment encountered CRS, with rates varying from seventy-s to ninety-three In the same way, individuals diagnosed with lymphoma who underwent CAR-T cell treatment had CRS rates ranging from 37% to 93%. Out of the patients who had tisagenlecleucel treatment for relapsed/refractory B-ALL, 46% of them suffered CRS of any severity. The incidence of severe CRS in patients with diffuse large B-cell lymphoma was 13-18% for axicabtagene ciloleucel and tisagenlecleucel, accordingly (49, 50). From a pathophysiological standpoint, it is widely believed that CRS is mostly induced by IL-6. Consequently, the treatment for CRS entails administering tocilizumab and corticosteroids, that inhibit the IL-6 receptor. Although tocilizumab, an FDA-approved drug for treating severe CRS, is used, serious CRS and fatality rates remain high. HLH/MAS after CAR-T cell therapy may exhibit resistance to IL-6 inhibition and might require chemotherapy as an alternative therapy (51).

The precise incidence of HLH/MAS after CAR-T cell therapy remains unknown because to the challenge of differentiating it from serious CRS. Nevertheless, CAR-T cell therapy has been recorded to occur in around 1% of patients. The exact origin and factors contributing to neurotoxicity are not well understood. The clinical manifestations of ICANS may exhibit variability and include disorientation, cephalalgia, cognitive impairment, aphasia, localized neurological deficits, or encephalopathy. In extreme instances, it might result in life-threatening cerebral edema, transient coma, or convulsions. Neurotoxicity often arises during CAR-T cell treatment, impacting approximately 67 percent of leukemia patients and 62% of lymphoma individuals (52). Corticosteroids are primarily used to treat neurotoxicity, since IL-6 inhibitors are often inefficient in treating neurotoxicity associated with CAR-T cell therapy. At present, there are no officially sanctioned medicines available to prevent the hazards stated before. Hence, it is essential to optimize CAR engineering and use additional strategies to mitigate the toxicities associated with CAR treatment. In this discussion, we explore the insights gained from constructing Chimeric Antigen Receptor (CAR) therapies with the aim of reducing toxicity, as well as other strategies to enhance the management of adverse reactions in CAR-T cell therapy (52).

Design and development of CAR-T cells

Modification of TCR-related elements

The entire effector actions and longevity of regulatory T cells depend on the appropriate stimulation, which is achieved by well-balanced signaling via both the TCR-CD3 complex (signal 1) and the co-stimulatory substances (signal 2). Co-stimulation is often accomplished by the cooperation of CD28 receptors on T-cells with B7 molecules (CD80 and CD86) on antigen-presenting cells (APCs). This relationship is essential to avoid the suppression of activated T cells via the induction of anergy. However, antigen-presenting cells (APCs) in cancer patients, particularly in the TME, often persist in a rudimentary state characterized by the absence of CD80 and CD86 expression. Consequently, this results in the dysfunction of effector T cells (54). In order to tackle this issue, some TCR-T cells were modified to enhance co-stimulation via CD3ζ signaling utilizing various techniques. The development of Artificial T-cell-activating adapter molecules (ATAMs) aimed to enable the transmission of both CD3ζ signaling and co-stimulation signals. This was achieved by inserting either the CD28 or the 4-1BB intracellular domain (ICD) into the CD3ζ chain. The TCR:CD28ε complex had a higher binding strength to pMHC, leading to enhanced T-cell activity upon peptide stimulation in comparison to regular TCRs. The distinctive arrangement of TCR:CD28ε effectively avoids the erroneous combination of TCRs with native TCRs, hence ensuring the persistent existence of TCR:CD28ε and mitigating any potential unanticipated off-target toxicity. In addition, a mandated increase in the expression of co-stimulatory molecules improves the transmission of signals in T-cells. 4-1BB is a transiently expressed co-stimulatory molecule that is synthesized by T cells in response to TCR engagement. The ligand, 4-1BBL, is produced by antigen-presenting cells (APCs) and, in some cases, by cancerous cells. In relation to this issue, the incorporation of 4-1BB into TCR-T cells through genetic transfer and its excessive expression in melanoma patients who received vaccination with the associated antigen led to enhanced efficacy in combating tumors, as observed in a chick-embryo chorioallantoic membrane (CAM) model system (56). Tumor cells often evade immune system recognition by downregulating the production of peptide-major histocompatibility complex (pMHC), thereby diminishing the affinity between T cell receptors (TCR) and tumor cells. Ahmadi et al. performed an experiment to investigate the problem of reduced efficacy in TCR-T-cell therapy caused by the CD3 density. The researchers investigated the co-transduction of all four CD3 chains (CD3ε, δ, γ, and ζ) together with the TCR in T cells. The findings demonstrated a significant increase in TCR expression and tetramer interaction. The co-transfer of CD3 chains together with the TCR was associated with an improved affinity of T lymphocytes, enabling them to recognize low-density pMHC and exhibit greater efficacy against malignancies in live beings (57).

Alteration of CAR-related components

The addition of co-stimulatory domain from 4-1BB and/or CD28 to initial-generation CARs, which contain just one CD3ζ signaling domain, greatly enhances the efficacy of CAR-T-cell therapy. The limited efficacy of CAR-T-cell therapy in solid cancers underscores the need for precise modulation of signaling pathways for overcoming obstacles, as previously mentioned (refer to the "The co-stimulatory domain and CD3ζ signaling domain" section). The efficacy of CAR-T-cell treatment in various scenarios is mostly determined by the engraftment and durability of CAR-T-cells. According to Feucht et al., the second-generation CAR with the CD28 co-stimulatory domain has the ability to strongly activate T-cells but has limited lifespan. This CAR also causes distinct changes in the activation of T-cells (58). They successfully modified CD3ζ signaling by altering ITAMs in the CD3ζ chains. They specifically altered the ITAM arrangement in 1XX by interfering with the second and third distal ITAMs. This alteration enhanced the enduring efficacy of CAR-T cells by preserving a harmonious equilibrium among their capacity to replicate as memory cells and their proficiency in efficiently combating malignancies. Conversely, Guedan et al. demonstrated that a single modified amino acid in the CD28 co-stimulatory domain (specifically, changing asparagine to phenylalanine, CD28-YMFM) improves the enduring anti-tumor reaction by decreasing T-cell distinctions and exhaustion, while also promoting the formation of Th17-like T cells (59). CD28-YMFM was thought to interfere with the binding between the CD28 domain and the SH2 domain of Grb2. As a consequence, there was an elevation in the stimulation of AKT and a decline in the activation of VAV1 and PLCγ1, leading to a decrease in the entry of calcium into CAR-T cells upon antigen stimulation. These notable alterations in gene expression are linked to decreased T-cell differentiation and exhaustion. Considering the significance of T-cell exhaustion in cancer immunotherapy, namely in TCR/CAR-T-cell treatment, it is essential to prioritize the production of TCR/CAR-T cells that possess resistance to fatigue. Recent study has shown that replacing the CD3ζ chain with a CD3ε chain that includes both activating and inhibitory motifs as a signaling domain in CARs improves the ability of CAR-T cells to fight tumors (60). This implies that CD3ε has the capacity to function as an inherent signal modulator, enhancing the durability of CAR-T cells by averting depletion. Moreover, there have been reports indicating that the temporary cessation of CAR signaling may enhance the functionality of depleted CAR-T cells by altering their epigenetic characteristics. Dasatinib treatment leads to a decrease in CAR expression, causing the cells to undergo a metamorphosis resembling memory formation. This transformation is accompanied by alterations in their gene expression and epigenetic processes. This procedure eventually rejuvenates the capacity of depleted CAR-T cells to combat malignancies (61).

Clinical Trials

At present, multiple studies are examining the use of CAR T cell therapy in the management of solid cancers and other medical ailments. Studies indicate that T cells, which have been genetically engineered with mesothelin-specific CAR mRNA, may activate the immune system to target solid malignancies (6). Furthermore, the use of CAR methods for organ donation has included the utilization of two cutting-edge HLA-A2-specific CARs. One chimeric antigen receptor (CAR) has a CD28-CD3d signaling domain, whereas the other does not include an intracellular signaling domain (dCAR). Transferring Tregs that are particular to the donor (allospecific Tregs) is more efficacious in avoiding the rejection of transplanted tissue compared to transferring Tregs that are derived from several sources (polyclonal Tregs). The inclusion of the ICOS signaling domain in a chimeric antigen receptor (CAR) has been shown to be very successful in fighting against glioma that expresses the epidermal growth factor receptor variant III (EGFRvIII) (63). Preclinical studies demonstrate the efficacy of CAR T cell therapies in targeting the tumor antigen 5T4 for the treatment of ovarian cancer. Remarkably, an unexpected revelation was uncovered during the examination of CAR treatment for autoimmune disorders. A chimeric autoantibody receptor (CAAR) that includes the pemphigus vulgaris autoantigen, desmoglein (Dsg3), together with CD137-CD3d signaling domains, was able to precisely target and destroy cells that produce anti-Dsg3. This targeted approach showed precise toxicity against these cells (64). Ongoing research is focused on investigating CAR targets for certain malignant neoplasms.

Conclusion and Future Directions

CAR T cells are an innovative therapeutic strategy that represents a unique and separate way of treating diseases. The current CARs are constructed with a modular structure, enabling targeted enhancements to address the challenges posed by the tumor microenvironment (TME). Scientists can improve the safety and effectiveness of CAR T cells by using synthetic biology and gene-editing techniques. In addition, CAR T cells may function as living T cell 'micropharmacies' to selectively deliver immunomodulatory drugs to the tumor microenvironment (TME). Increasing the complexity

of CAR designs and changing the genes of T cells may significantly amplify the potential risks associated with CAR T cell therapy. For example, the process of viral transduction and the use of gene-editing machinery might potentially result in the unintended disruption of genes that are not the intended target. The transformation of T cells into malignant clones, resulting from the introduction of mutations that either activate oncogenes or disrupt tumor suppressor genes, is a well recognized inherent risk associated with gene therapy. Currently, there is no evidence of insertional mutagenesis leading to transformation in patients. Nevertheless, clinical data have shown the occurrence of viral insertion into genes in lentivirally transduced CAR T cells used for the treatment of a patient with CLL (67). Specifically, the CAR gene was inserted into the TET2 locus, leading to the development of a cluster of genetically identical T cells (67). Nevertheless, the population of T cells in this community gradually diminished in size without any external interference. This incident highlights the inherent risk involved in using engineered cells as a therapeutic approach for patients. As researchers get more familiar with adoptive cell transfer, they are growing more comfortable with the inherent danger. As a result, a study has been started (NCT03399448) to test the use of NY-ESO-1-directed transgenic TCR T cells that have undergone numerous CRISPR-Cas9 gene edits to remove the natural TCR and PD-1. The complex procedure of genetically modifying biological products exogenously also adds complexity to the manufacturing process.

Prior research on the existing CAR T cell production procedure has shown that the CAR design may inadvertently alter both tumor cells and lymphocytes found in the apheresis result. This might result in the concealment of antigens via the attachment of CARs generated by these cancer cells to tumor-associated antigens (TAA) on the same cells. Consequently, there may be an unregulated proliferation of these neoplastic cells and their dissemination throughout the organism (68). The CAR T cell technique is the first gene treatment to be granted clinical approval. Regular surveillance of gene editing-induced difficulties in clinical trials of CAR T cell products will contribute to a more comprehensive comprehension of the potential long-term hazards linked to the developing domain of gene editing in the medical profession. This monitoring may also aid in identifying remedies for certain issues. Introducing novel technological approaches may also increase the already high costs linked to the manufacturing of CAR T cells. One of the reasons that adds to the total cost of CAR T cell therapy is the high price and time-consuming process of manufacturing clinical-grade retroviruses. However, using non-viral vectors to decrease manufacturing costs has the potential to improve affordability (69). Ultimately, there are now ongoing efforts to improve the efficacy and scope of CAR T cell therapies, while simultaneously boosting their safety and optimizing their production processes. These novel engineering solutions, designed to optimize CAR T cell biology, will lead to the broader use of this technology in the therapy of cancer, hence enhancing the benefits of CAR T cells for patients.

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Authors' Contribution

Mahtab Dolatabadi and Shabnam Radbakhsh were involved in the conceptualization, design and writing of the manuscript draft. All authors read and confirmed the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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