

CAR-T Cell Therapy: Structure, Mechanism of Action, Adverse Effects, and Current Challenges

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Abstract

The Chimeric antigen receptor T-cells are genetically modified T-cells which are used to as powerful therapeutic treatment of patients with chemotherapy-refractory/relapsed blood cancers, acute lymphoblastic leukemia, diffuse large B-cell lymphoma, follicular lymphoma and other types of cancers. In CAR-T cell therapy, the T-cells are collected from the tumor patients and prepared. The CAR-T cells lies in appropriate T-cells genetically processed in laboratory conditions. By helping the CRISPR/Cas9 technique used instead of retroviral vectors to integrate the CAR gene into specific sites in the genome of T-cells. CAR-T cells are special genetically modified T-cells which wear chimeric antigen receptor (CAR) protein on its cell membrane. The composition of these CAR proteins consists of three parts such as the recognition region which consist of a single chain antibody domain. These domains are responsible for recognition and binding to specific antigen present on cancerous cells. The middle parts of these antibodies are embedded in the membrane and third parts are the internal signaling region. The single chain variant antibody (ScFv) recognition domains which have properties anti-tumor effects through the secretion of inflammatory cytokines.g., IL-2, IFN- γ , and TNF- α , cytolytic effector function via perforin and granzyme. TNF-related apoptosis-inducing ligand, which binds to death receptors such as DR4 and DR5 on tumor cell surface to activate graft-versus tumor effect. Also, tumor cell apoptosis can be initiated via the activation of caspase-8 and the formation of death-inducing signaling complex (DISC) leading to cell death mediated by mature caspase-3 subsequent cleavage of over 500 cellular substrates as a result of Fas and Fas ligand (FasL) pathway activation.

Keywords: CAR-T Cell Therapy, Single Chain Variant Antibody (ScFv), Death-Inducing Signaling Complex (DISC), TNF-Related Apoptosis-Inducing Ligand, Tumor Cell Apoptosis

1. INTRODUCTION

Cancer is a group of diseases involving uncontrolled cell growth typically in tumor and more than 100 types of cancer affects human life-style (Anand P et al., 2008). It is the second worldwide cause of death. The biological reconstruction of the resulting defects, depending on several patient's tumor-related factors (TRF) is usually necessary (D. Andreue et al., 2015). As far as health issues are concerned, cancer causes one out of 6 deaths around the globe. The potential therapeutic are still awaited for the successful treatment of cancer, some unconventional treatment like radiotherapy, surgical therapy and chemotherapy are used but some advance technology like gene therapy, stem cell therapy, natural antioxidants, targeted therapy and photodynamics therapy, nano particles and precision medicine are available to diagnose and treat cancer (Rasanpreet Kaur et al., 2023). Apart from surgical treatment, patients suffering from cancer specially Osteosarcoma and Ewing sarcoma cancer require a preoperative as well as postoperative chemotherapy, while Ewing sarcoma patients often undergo radiation therapy (D. Andreue et al., 2015). Targeted therapy is also now available to arrest the growth and development of cancer cells without damaging healthy tissues.

The photodynamic therapy (PDT) has more potential to treat cancer with minimal damage and does not limit other technology as in case of chemotherapy and radiotherapy (Rasanpreet Kaur et al., 2023). The novel targeted therapies of interesting by FDA-approved targeted cancer drugs in recent years, block biological transduction pathway or specific cancer patients to induce the death of cancer cells by apoptosis and specific cell signaling stimulation of the immune system or specifically deliver chemotherapeutic agents of cancer cells minimizing the undesirable side effects (Edger-Perez-Herrero et al., 2015). The potential of the current method of investigation and the diagnostic and treatment of which still present some unsolved problems (F. Schajowicz et al., 1983).

Among the immune system, the chimeric antigen receptor T-cells are a special subpopulation of T lymphocytes that are genetically engineered to express specific antigen receptors and effectively recognize and destroy cancer cells (Pan K et al., 2022). The cancer cells lack specific antigens or displaying heterogeneity in antigen expression can impair the antigen selectivity of CAR-T cells (Mejzner RG et al., 2018). CAR-T cells have provided promising advances as a novel immunotherapeutic approach for cancer treatment. CAR-T cell therapy has shown stunning results especially in B-cells malignancies, however, it has shown less success against solid tumors and related to the specific characteristics of the tumor (Reza Elahi et al., 2018). The impressive efficacy of chimeric antigen receptor T-cells against hematologic malignancies focusses the attention of oncologists to extend these efforts for the treatment of solid tumors. T cells, genetically modified by chimeric antigen receptors (CAR-T), are endowed with specificity to a desired antigen and are cytotoxic to cells expressing the targeted antigen. CAR-T-based cancer immunotherapy is a promising therapy for curing hematological malignancy, such as acute lymphoid leukemia, and is promising for extending their efficacy to defeat solid tumors. To date, dozens of different CAR-T cells have been evaluated in clinical trials to treat tumors; this necessitates the establishment of guidelines for the production and application of CAR-T cells.

In recent years CAR-T cell therapy has been a very interesting topic for the biologist, which is also known as the "living drugs" (Wu W et al., 2020). Chimeric antigen receptor (CAR)-T cells have recently emerged as a powerful therapeutic approach for the treatment of patients with chemotherapy-refractory or relapsed blood cancers, including acute lymphoblastic leukaemia, diffuse large B cell lymphoma, follicular lymphoma, mantle cell lymphoma and multiple myeloma (Marco Ruella et al., 2023). This therapy is a novel immunotherapy in clinical practices treating B-cells associated leukemia and lymphoma cancer. CRISPR/CAS systems, an efficient and simple method for precise gene editing offer new possibilities for optimizing CAR-T cells by increasing the function of CAR-T cells and reducing manufacturing costs

(Ruiyu Tao et al., 2024). In therapeutic engineered T-cell and effective responses of CD19-directed chimeric antigen receptor T-cell immunotherapy particularly CAR-T cells therapy become a rapidly growing field in cancer therapy (Hasan Mallonoori et al., 2018), which could be produced by disrupting T-cell receptor (TCR) and β -2 microglobulin in CAR-T cells by knocking in a CAR at the disrupted TRAC locus (Xiaojun Liu et al., 2018). Chimeric antigen receptor T-cell therapy which uses T-lymphocytes to eliminate cancer cells can be improved by combining it with CRISPR technology (Pegah Kavousinia et al., 2024). CAR-T cell therapy is an approach to immune cell therapy which works by modification in the T-cells of a patient to express the chimeric antigen receptor (CAR) protein on the outer surface of T-cells which induce their recognition and destruction process against cancer cells (Dahua Sun et al 2023). The resistance mechanisms to CAR-T cell immunotherapy by analysing CAR-T cell dysfunction, intrinsic tumor resistance and the immunosuppressive tumor microenvironment.

2. PREPARATORY MECHANISM OF CAR-T CELLS IN LABORATORY

The process of creating CAR-T cells is a complicated and multifaceted procedure that requires several key steps, each of which play an important step in the development of these powerful immunotherapy agents. During the process of preparation of CAR-T cell therapy, the T-cells are collected from the tumor patients and prepared CAR-T cells lies in appropriate T-cells genetically processed in laboratory conditions (Davenport AJ et al., 2015). First of all, the extraction of T-cells from a patient's blood in a process called leukapheresis, and the activation of the cells using the aid of some antibodies or synthetic antigen-presenting cells. A lentiviral vector capable of generating CAR-T cells *in-vivo* by the incorporation of T-cell activation and costimulatory signals onto the surface of VivoVec particles (VVPs) in the form of a multidomain fusion protein and show enhanced *in vivo* transduction and improved CAR T-cell antitumor functionality (Christopher J. Nicolai et al., 2024). The expanded T-cells are purified and then transduced with a gene encoding the engineered CAR through a retroviral vector by an integration the vector of gamma-retrovirus (γ -RV) or lentivirus (LV) (Christopher Perry et al., 2021).

By helping the CRISPR/Cas9 technique used instead of retroviral vectors to integrate the CAR gene into specific sites in the genome (Justin Eyquem, et al., 2017) and these CAR gene is synthesized. The CAR proteins synthesized by the lentivirus vector, are displayed on the outer surface of the T-cells. (Aynur Abdulla, et al., 2024). The composition of the CAR is usually an important part consisting of extracellular domain for binding to antigens, transmembrane segment and internal signaling segments, altogether in the ability of the modified T cells to identify and target specific cancer antigens (A X Wang et al., 2023). The genetic alteration of CAR-T cells undergoes in a phase to augment their quantity, and are then subjected to rigorous quality assessment testing to ensure their safety, purity and effectiveness. The fabricated CAR-T cells are then cryopreserved for conservation. Before the infusion of CAR-T cells into the body of patients, the patient goes through a process of lymphodepletion chemotherapy for detecting the patient's immune system to accommodate the CAR-T cells (Theresa L Hunter, et al., 2025). This lymphodepletion have a variable chemo-therapeutic agents such as cyclophosphamide, fludarabine, pentostatin or bendamustine and interleukin-2 or total body irradiation (A X Wang et al., 2023). Ultimately, the CAR-T cells are frozen and the phase of treatment begins by introducing the CAR-T cells back into the bloodstream of the patient.

3. STRUCTURE OF CAR-T CELLS

CAR-T cells are special genetically modified T-cells which wear a chimeric antigen receptor (CAR) protein on its cell membrane (Schubert MT et al., 2021). The composition of these CAR protein consists of three parts. The external part works as a recognition region and consists of a single chain antibody domain. These domains are responsible for recognition and binding to specific antigen present on cancerous cell (Bao C et al., 2021). The middle region of CAR protein is transmembrane domain embedded in the cell

membrane of T-cells which are tightly packed with lipid bilayers. The internal signaling region typically includes the signaling molecules which are required to activate T-cells which starts the antigen specific activation and induce proliferation of CAR-T cells (Delip S. et al., 2020) and cell culture conditions can influence CAR-T cell differentiation and anti-tumor efficacy (W-C Pei et al., 2021) (Figure 1).

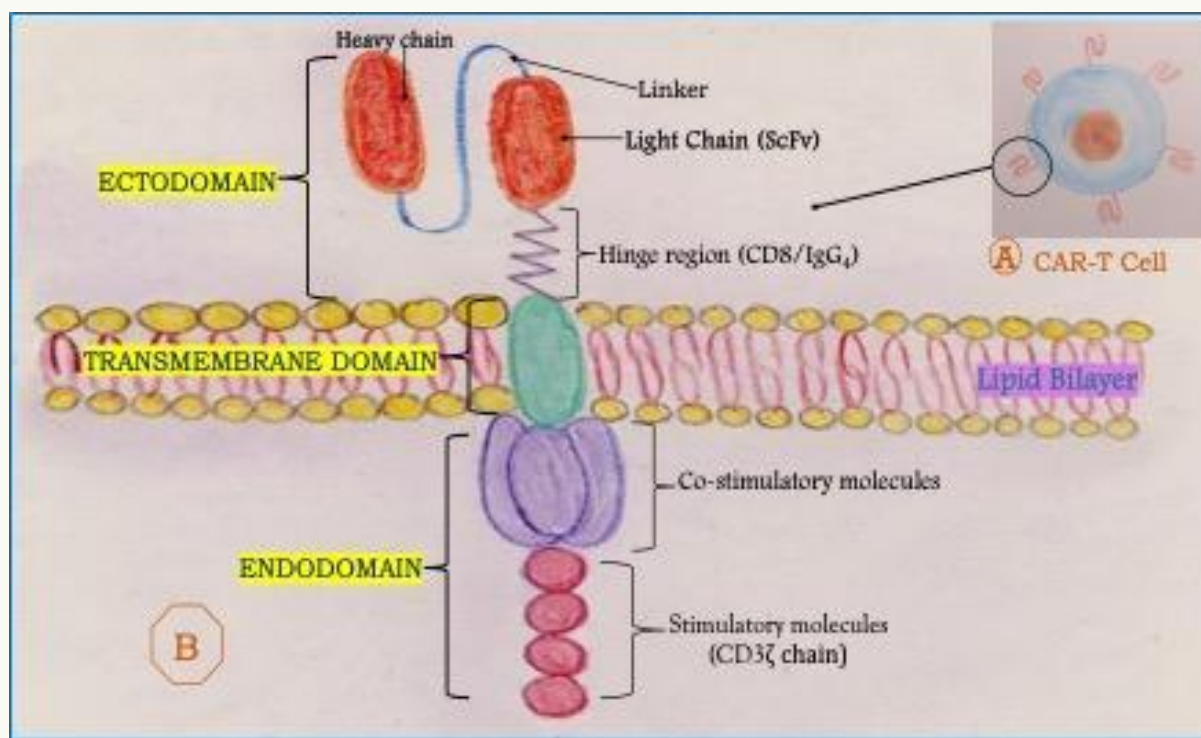


Figure 1: Structure of CAR-T cell and their Chimeric Antigen Receptor (CAR) protein on the cell membrane and their component domains.

The outer CAR protein domain is mainly linked together as a single chain of variable fragments (ScFv). The Single chain antibodies (ScFv) consist of an antigen-binding portion and second a structural domain connected to CD3 ζ and signaling domains such as CD28, CD19 4-1BB and OX40 domains (van de Donk et al., 2021). The Single chain antibody (ScFv) is a chimeric protein having light (VL) and heavy (VH) chains of immunoglobins. These light and heavy chains are linked together with short linker peptides of serine-glycine or glutamate-lysine (M. Nakajima, et al., 2019). The structure and design of CARs contribute to their signaling mechanisms, effector functions, efficacy, and toxicity. The ligand recognition and signaling of CARs are affected by both the single chain variable fragment (ScFv) and cytoplasmic domains. However, the transmembrane and spacer domains (nonsignaling) affect the function of CARs (Jayaraman et al., 2020). The ScFv introduced the antigen to T-cells by binding to it and activate the antitumor effects of T-cells (Duan Y et al., 2021). The transduction domain of CAR-T cell is located inside and it help in the transmission of external antigen recognition signal and activate the T-cells and triggering immune response (Weber EW et al., 2021). The most common transduction domain is CD3 ζ and this domain is involved in signaling pathway for T-cell activation (Tausley AM et al., 2023). The structure and design of CARs contribute to their signaling mechanisms, effector functions, efficacy, and toxicity. The ligand recognition and signaling of CARs are affected by both the single chain variable fragment (scFv) and cytoplasmic domains; however, the transmembrane and spacer domains (nonsignaling) affect the function of CARs (Jayaraman et al., 2020). Generally, CAR T-cells can specifically recognize cancer cells and lyse them (Maggs et al., 2021) (Figure 1).

The intracellular domain classifies CARs into five generations: First has a single activation domain, a cytoplasmic domain mostly CD3 zeta (CD3 ζ), and some studies used the gamma chain (γ) of the Fc

receptors. The second generation has CD3 ζ plus one costimulatory domain, obtained from costimulatory molecules such as 4-1BB or CD28 connected to an activator domain (CD3 ζ / γ chain of Fc receptor) to enhance both cell proliferative and cytotoxic competences of CAR-T cells (Finney et al., 1998; Hombach et al., 2001; Acuto and Michel, 2003). The third generation is similar to the second generation but has multiple costimulatory domains with CD3 ζ , such as 4-1BB and CD28, CD134, and CD137 (Sadelain et al., 2013; Zhang et al., 2017; Guedan et al., 2019). The fourth generation CARs, known as T-cells redirected for universal cytokine-mediated killing (TRUCKs), were engineered to release transgenic cytokine-like interleukin 12 (IL-12) upon CAR signaling in the tumor tissue to overcome TME immunosuppression and endorse robust therapeutic outcomes (Chmielewski et al., 2014; Chmielewski and Abken, 2015, 2020). Apart from antibodies, other approaches or sources are also used in CARs as antigen recognition domain such as TNF receptor, innate immune receptors, cytokines, structural proteins and growth factors. (S.S. Chandran et al., 2019). Innovations in the structure and manufacturing of CAR-T cells have resulted in significant improvements in efficacy and persistence, particularly with the development of fourth-generation CAR-T cells. Paired with an immune modifier, the use of fourth-generation and next-generation CAR-T cells will not be limited because of cytotoxic effects and will be an efficient tool for overcoming the tumor microenvironment (Ruihao Huang et al., 2020).

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4. MECHANISM OF KILLING THE TARGETED CANCEROUS CELLS

The action mechanism of chimeric antigen receptor CAR-T cells is due to the presence of Chimeric antigen receptor (CAR) protein target antigen present on the outer surface of tumor cell. CAR-T cell therapies to treat cancer especially hematologic and solid cancers, as well as other indications such as autoimmune diseases, is dependent on effective CAR-T cell manufacturing that impacts not only product safety and efficacy but also overall accessibility to patients in need (Melanie Ayala Ceja et al., 2024). These binding of single chain variant antibody (ScFv) recognition domains which have properties anti-tumor effects through the secretion of inflammatory cytokines.g., IL-2, IFN- γ , and TNF- α , cytolytic effector function via perforin and granzyme (Benmebarek et al., 2019), TNF-related apoptosis-inducing ligand, which binds to death receptors such as DR4 and DR5 on tumor cell surface to activate graft-versus

tumor effect (donor T-cells) (Watanabe et al., 2021). Also, tumor cell apoptosis can be initiated via the activation of caspase-8 and the formation of death-inducing signaling complex (DISC) leading to cell death mediated by mature caspase-3 subsequent cleavage of over 500 cellular substrates as a result of Fas and Fas ligand (FasL) pathway activation (Waring and Müllbacher, 1999; Nagata and Tanaka, 2017) CAR-T cells use the CARs on their surface to specifically recognize and bind to complementary antigens found on the surface of the cancer cells. Subsequent to this binding event the signaling cascade within the CAR-T cell is activated, which then activates the phosphorylation of immunoreceptor tyrosine-based activation motifs (ITAMs) located within CD3 ζ (V. Durante et al 2025). These expanded cells are the final CAR-T cell therapy product, which is sent back to the hospital to be returned to the patient as a single infusion (Mangle JL et al., 2021) (Figure 2).

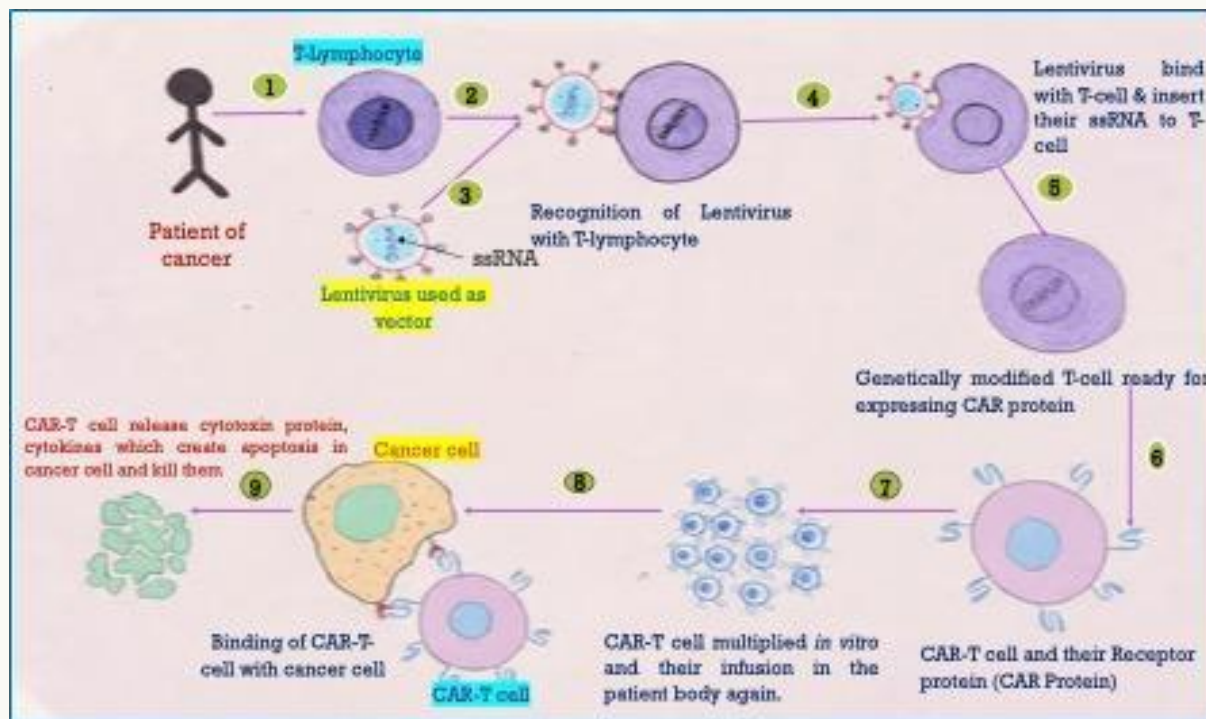


Figure 2: The preparatory and action mechanism of CAR-T cell to create apoptosis in Cancerous cells.

In this process, time plays a very important role in the successful treatment for manufacturing the CAR-T cells in the body of patients which expand the cells and testing quality (Zhang X et al., 2023). Typically, CAR-T cells therapy target tumor-specific antigens which are present on the outer surface of cancer cell are recognized by CAR-T cell and CAR protein binds with this specific antigen molecules through a single chain variant antibody (ScFv) on the CAR protein (Wu W et al., 2020). When CAR-T cells and tumor cells come to close with other, the CAR protein binds with the target antigen of tumor cells and signaling domains such as CD3 ζ activate and triggering the cascade protein which is a type of intracellular signaling molecules (Feng Q et al., 2022). The activated CAR-T cells killing mechanism completed in the following ways-

- The CAR-T cells released cytotoxin protein specially targeted enzymes and perfin which induce apoptosis in cancerous cells and destroyed them (Yuying Liu et al., 2020).
- CAR-T cells secrete cytokines molecules such as interferon- γ , factor α , which create necrosis in tumor cells.
- The CAR-T cells could recruit other immune cells such as natural killer (NK) and macrophase cells. These two cells after activation killed tumor cells in the patient's body (Figure 3)

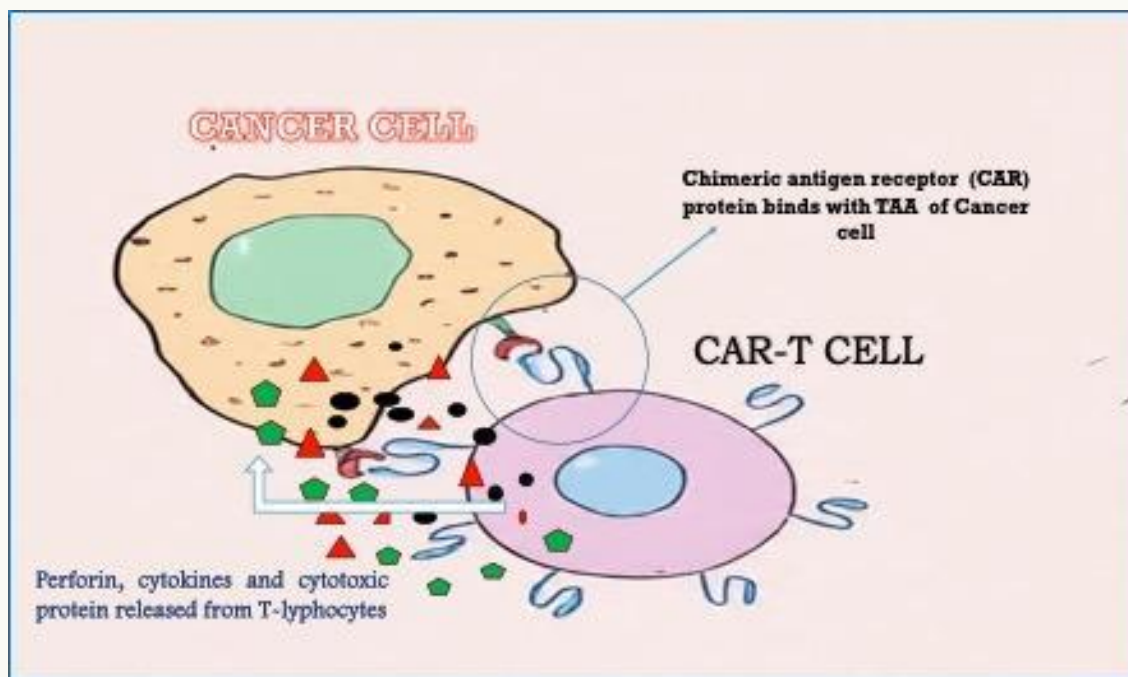


Figure 3: The binding of CAR-T cells with Cancerous cell by CAR protein and Antigen protein by cancer cell which stimulates to released cytokines, cytotoxic protein perforin etc. by T-lymphocytes and induce apoptosis in cancer cell in the body of cancer patients.

5. SIDE EFFECTS OF CAR-T CELL THERAPY

Like all cancer treatments, CAR T-cell therapies can cause severe side effects, in which most common for these immunotherapies are infections and a mass die-off of antibody-producing B cells. These therapy remains limited by significant toxicities, including cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) (Nicolus Gazeau et al., 2023). Currently, CAR-T cell therapy is approved for the treatment of B cell relapsed or refractory leukemia and lymphoma, and most recently, multiple myeloma (MM) (Karan L Chohan, et al., 2023; Jennifer N Brudno et al., 2024). The treatment of CAR-T cell-associated complications such as cytokine release syndrome, immune effector cell associated neurotoxicity syndrome, hematotoxicity, hypogammaglobulinemia, and CAR-T cell-induced pseudo-progression amongst others for physicians treating patients with CAR-T cell-associated complications and intensivists (Torsten Schroeder, et al., 2024).

In the case of CRS, the infused T-cells flood the bloodstream with cytokines, chemical messengers that help stimulate in immune response (Sanam Shahid et al., 2025). Beyond hematologic malignancies, CAR T-cell therapies are now increasingly being repurposed to tackle B-cell-mediated autoimmune diseases (AIDs). This is primarily achieved through broad B-cell depletion, but more targeted approaches such as the selective elimination of autoantibody-producing B-cell sub-populations are also being explored (Dimitrios E Katsaros et al., 2025). The treatment of neuro-immunological diseases, such as multiple sclerosis, myasthenia gravis, neuromyelitis optica spectrum disorders, and myelin oligodendrocyte glycoprotein antibody disease (Aiden Haghikia et al., 2024).

In many patients, both children and adults, CRS can be managed with tocilizumab which can be initially used to treat inflammatory conditions like juvenile arthritis, blocks the activity of IL-6, a cytokine that is often secreted in large amounts by immune cells. Some of the hallmark signs of ICANS include confusion, excessive sleepiness, and impaired speech. In the other studies it is clear that the anakinra shortly after CAR T-cell therapy may be helpful to prevent the ICANS or decrease its severe conditions (Karan L Chohan et al., 2023).

6. CONCLUSION

There are several challenges that researchers must face when treating solid cancers with CAR-T cells, these include choosing an ideal target, promoting efficient trafficking and infiltration, overcoming the immunosuppressive microenvironment, and avoiding associated toxicity (Bing-Lan Zhang et al., 2016). However, it is challenging to standardize CAR-T cancer therapy because it involves a combination of gene therapy and cell therapy. In this review, we compare the existing guidelines for CAR-T cells and discuss the challenges and considerations for establishing guidance for CAR-T-based cancer immunotherapy (Wei Wang et al., 2016). Chimeric Antigen Receptor T-cell (CAR-T cell) therapy is a promising cancer treatment that has shown success in treating certain type of cancer. It has demonstrated remarkable efficacy, particularly in hematologic malignancies such as certain types of leukemia and lymphomas (Mahesh Kumar Posa et al., 2026). CAR-T cell therapies to treat cancer especially hematologic and solid cancers, as well as other indications such as autoimmune diseases, is dependent on effective CAR-T cell manufacturing that impacts not only product safety and efficacy but also overall accessibility to patients in need (Melanie Ayala Ceja et al., 2024).

The T-cells are first isolated by the infected person after the complete checkup including type and stages of cancer, physical health and their immune system status of the patient (Fabrizio VA et al., 2022). By the cell culture process in laboratory *in vitro* condition, the T-cells increased their number by proliferation process. So, T-cells are able to bear CAR protein in its membranous outer surface and are unable to recognize and kill the specific tumor cells (Bupha-intr O, et al., 2021). The CARs help the cells to latch on to specific proteins, known as antigen which are specific in nature and are present on cancer cells (and some normal cells). They also enhance the T cells' ability to kill cancer cells. These cells are then sent to one of the treatment manufacturer's laboratories, CAR-T cell therapies to treat cancer especially hematologic and solid cancers, as well as other indications such as autoimmune diseases, is dependent on effective CAR-T cell manufacturing that impacts not only product safety and efficacy but also overall accessibility to patients in need (Melanie Ayala Ceja et al., 2024). Once the CAR-T cells are expanded a sufficient number. These expanded cells are the final CAR T-cell therapy product, which is sent back to the hospital to be returned to the patient as a single infusion (Mangle JL et al., 2021). In this process, time plays a very important role in the successful treatment for manufacturing the CAR-T cells in the body of patients which expand the cells and testing quality (Zhang X et al., 2023).

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