Introduction to CAR-T Cell Therapy: Principles and Applications

Abstract

CAR-T cell immunotherapy involves the genetic modification of T lymphocytes to express a chimeric antigen receptor, enabling them to recognize tumor cells. There are five generations of CARs, and the production process of these cells involves several stages: collection, genetic modification, cell expansion, and subsequent infusion into the patient. This therapy can cause Cytokine Release Syndrome and neurotoxic events, which are both manageable and treatable. Its greatest efficacy has been observed in treating hematological tumors, with CD-19 and BCMA targets approved for four tumor types, though challenges related to antigen escape and relapses remain. In solid tumors, the immunosuppressive microenvironment, heterogeneity, and antigen escape present challenges to the efficacy of immunotherapy, which are being addressed with new CAR designs. CAR-T cell therapy is revolutionizing the treatment of hematological cancers, with ongoing advancements in genetic engineering and strategic collaborations, promising significant improvements despite the challenges in solid tumors. Thus, this literature review aims to cover the key concepts of CAR-T therapy, providing an overview of its current applications and the main challenges to be addressed.

Keywords: CAR-T cells; immunotherapy; solid tumors; hematological tumors.

Introduction

In 1993, Eshhar et al. developed a genetically modified therapy by creating chimeric genes that grant T lymphocytes the ability to recognize specific antigens of malignant

cells, leading to the advent of chimeric antigen receptor (CAR) T-cell immunotherapy. Since then, CARs have evolved, incorporating different domains and co-stimulatory elements that enhance their capacity to bind to cancerous cells and tissues (Kausar et al., 2023).

In CAR-T cell therapy, the patient's T lymphocytes are used, initially separated and purified from other blood cells. Subsequently, they are genetically modified by inserting specific receptors capable of recognizing tumor antigens, then expanded in the lab and transfused back into the patient (Soares et al., 2022). For a successful procedure, it is essential to identify the tumor target and ensure that the tumor-associated antigen is almost absent in normal cells (Huang, Wu, and Hu, 2020).

CAR-T cell therapy has demonstrated revolutionary innovation in treating hematological tumors, achieving remarkable results in inducing long-lasting remissions and receiving Food and Drug Administration (FDA) approval for treating diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), acute B-cell lymphoblastic leukemia (B-ALL), and multiple myeloma (MM) (Zhang et al., 2022). Moreover, research is underway to apply this immunotherapy to solid tumors. However, this approach faces significant challenges, such as the immunosuppressive environment around solid tumors, tumor heterogeneity, different anatomical locations, and the expression of solid tumor antigens by normal tissues (Soares et al., 2022).

In this context, CAR-T therapy has evolved over time, with extensive research being conducted to identify target antigens in various types of tumors and optimize the process. This study aims to address aspects related to this immunotherapy, discussing its concept, production process, CAR generations and structure, the most common adverse effects, and the results obtained in hematological tumors. Additionally, it examines the challenges and strategies associated with its implementation in solid tumors.

Methodology

This is a systematic review conducted in the electronic databases Scielo, BVS, and PubMed, using the descriptors "CAR-T cell therapy," "CAR generations," "tanCAR," "solid tumors," and "hematological tumors." Priority was given to fully accessible digital

works published in English and Portuguese. Given that CAR-T cell therapy is constantly evolving, articles from the last four years were emphasized to study the recent CAR generations, current efficacy data, and optimization strategies. For the study of basic concepts in immunology, biochemistry, and oncology, older yet still relevant articles in the field were also used.

Initially, article abstracts were analyzed to identify those that covered fundamental concepts of CAR-T therapy aligned with the study's objectives. From this screening, 44 articles were selected, which were later read and analyzed for the writing of this review. The article annotations included variables such as year of publication, objective, keywords, main themes addressed, and conclusions, providing a comprehensive view of the content.

This article aims to conceptualize and explore the current application of CAR-T therapy in hematological tumors, while discussing the challenges and strategies for its success in solid tumors. Therefore, exclusion criteria included lack of thematic relevance, lack of access to the full text, and studies in very preliminary research phases.

CAR Structure

CAR-T cell therapy involves modifying the patient’s T lymphocytes, which are genetically altered to express the chimeric antigen receptor (CAR), a type of synthetic receptor designed to identify and attack specific antigens associated with tumors (Yang et al., 2022). According to Soares et al. (2022), the CAR consists of four distinct parts: an extracellular region responsible for antigen recognition, an extracellular hinge region, a transmembrane region, and an intracellular signaling region.

The extracellular antigen recognition region is derived from monoclonal antibodies specific to the target antigen, where the Fab portion responsible for antigen recognition is isolated (Yang et al., 2022). The heavy chain (VH) and light chain (VL) of this portion are combined to form a single-chain variable fragment, known as ScFv (Subklewe, Bergwelt-Baildon, and Humpe, 2019). This fragment is capable of recognizing various surface antigens expressed by tumor cells, independent of major histocompatibility complex (MHC) molecular restriction (Soares et al., 2022).

The extracellular hinge region, also known as the spacer region, connects the ScFv to the CAR's transmembrane domain. This region is typically composed of crystallizable (Fc) fragments of immunoglobulin IgG, though CD8- and CD28-based spacers are also widely used (Kausar et al., 2023). Its presence provides the CAR with greater flexibility to access the target antigen and regulates the distance between the chimeric receptor and tumor cells.

The optimal length of the hinge region depends on the location of the target antigen: long spacers, derived from IgG1, IgG2, or IgG4, provide better access to antigens close to the membrane or complex glycosylated antigens. However, the binding of the CH2 domain to Fc receptors on myeloid cells can compromise the functionality of CAR T cells, necessitating the modification or removal of this domain (Guedan et al., 2019). On the other hand, short spacers are more effective in binding to antigens distant from the membrane and can be derived from IgG without the CH2-CH3 regions or from native CD28 and CD8 hinges (Guedan et al., 2019).

The transmembrane region connects the extracellular and intracellular parts, anchoring the CAR to the membrane and transmitting recognition signals to the signaling domain (Kausar et al., 2022). This region consists of a hydrophobic alpha-helix that spans the membrane, with designs based on CD4 and CD28, the latter being the most widely accepted and stable (Kausar et al., 2022).

The intracellular signaling region of the CAR is primarily composed of the CD3 ζ domain, responsible for transducing the primary antigen signal (Yang et al., 2022). Within CD3 ζ, there are three immunoreceptor tyrosine-based activation motifs (ITAMs), which act as phosphorylation sites, recruiting the ZAP70 protein, essential for triggering signaling cascades that lead to T cell activation (Jayamaran et al., 2020). Additionally, co-stimulatory domains mediate secondary and co-stimulatory signals, with CD28 playing a critical role in lymphocyte activation and proliferation. Other co-stimulatory domains, such as 4-1BB, ICOS, and CD134, are also used and can influence T cell differentiation pathways and metabolic cycles (Jayaraman et al., 2020). Based on the composition of this intracellular signaling region, CARs are currently classified into five generations, as shown in Figure 1.

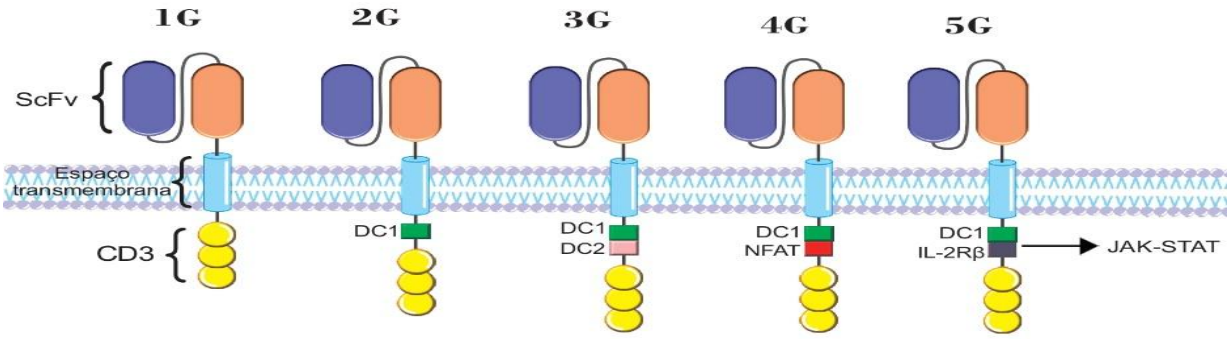

FIGURE 1- Representation of all CAR generations. Source: authors.

As illustrated in Figure 2, first-generation CARs were designed with an intracellular signaling region containing only the CD3ζ domain, which allows for primary signaling only (Jayaraman et al., 2020). Due to this limited configuration, cytokines are secreted insufficiently. For instance, the reduction in interleukin-2 (IL-2) secretion leads to inadequate proliferation of first-generation CAR-T cells and a shorter lifespan in vivo (Dejenie et al., 2022). Consequently, the antitumor efficacy of first-generation CARs is diminished, rendering them obsolete in current clinical practice (Dejenie et al., 2022).

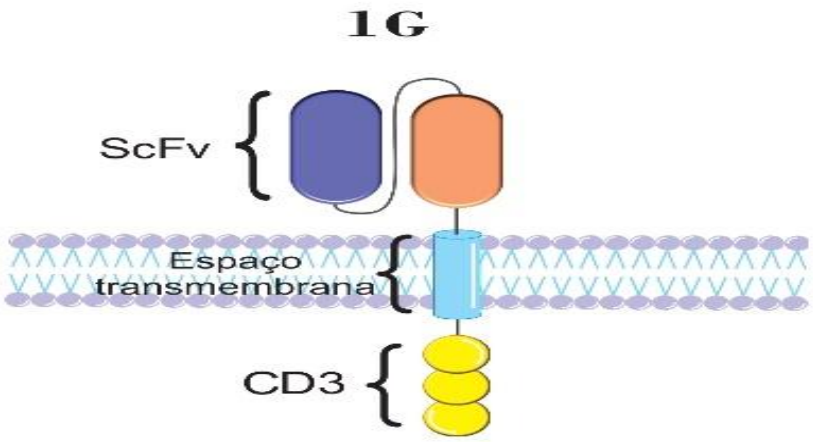

FIGURE 2- Representation of the 1st generation CAR. Source: authors

Second-generation CARs enhance the capability of the intracellular signaling region by incorporating, in addition to CD3ζ, a co-stimulatory domain, allowing for dual signaling

pathways (Dejenie et al., 2022), as illustrated in Figure 3. The most commonly used co-stimulatory domains are CD28 or 4-1BB, both FDA-approved (Kausar et al., 2023). CARs with CD28 domains promote greater T lymphocyte proliferation, along with faster phosphorylation and memory cell formation (Dejenie et al., 2022). On the other hand, the 4-1BB domain (CD130) promotes prolonged T cell persistence, despite less intense signaling compared to CD28 (Dejenie et al., 2022). Thus, CD28-based CARs are more suited for diseases requiring rapid tumor elimination, while 4-1BB-based CARs are preferable for treating conditions requiring sustained CD8 T cell persistence (Guedan et al., 2019). Other co-stimulatory domains include ICOS, which enhances CD4 T cell persistence (Guedan et al., 2019), and CD134 (OX-40), which strengthens IL-2 production, thereby supporting proliferation (Zhang et al., 2017).

Currently, all FDA-approved products available on the market are second-generation CAR-T cells. Patients in clinical trials receiving treatment with these products are monitored for up to 15 years to evaluate long-term effects, safety, and the risk of new malignancies (Food and Drug Administration, 2023). Despite significant advancements, challenges remain related to the persistence and recurrence of CAR T cells that utilize only one co-stimulatory domain. This has driven the evolution toward third-generation CARs, aimed at addressing these issues and further improving the performance of this technology (Dejenie et al., 2022).

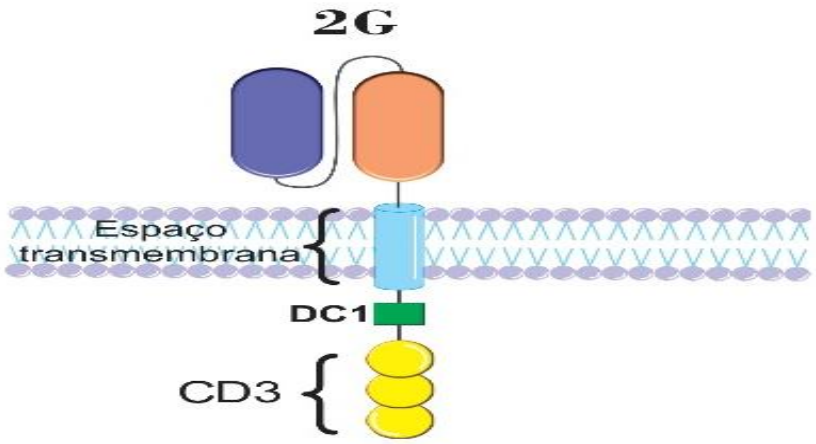

FIGURE 3- *Representation of the 2nd generation CAR. Source: authors.*

Third-generation CARs have enhanced their intracellular signaling region by incorporating, in addition to CD3ζ, two co-stimulatory domains (Jayaraman et al., 2020), as illustrated in Figure 4. The most commonly used combination of these domains in third-generation CARs is CD3ζ-CD28-4-1BB (Dejenie et al., 2022). According to preclinical results from Zhao et al. (2015) and Quintarelli et al. (2018), this combination has been tested against various targets such as CD19, PSMA, GD2, and mesothelin, demonstrating stronger signal transduction, more efficient tumor eradication, and greater persistence of CAR-T cells compared to second-generation CARs. Other combinations are also used, such as CD28-OX40-CD3ζ, which showed strong proliferation and expansion, and COS-4-1BB-CD3ζ, which significantly increased antitumor potency and CAR-T cell persistence (Jayaraman et al., 2020). Additionally, various other co-stimulatory domains are utilized in different combinations, such as NKG2D, CD27, and TLR2 (Dejenie et al., 2022).

However, the major challenge with this generation, according to Dejenie et al. (2022), arises from the increased risk of serious side effects and T cell exhaustion, caused by the excessive activation of multiple signals mediated by the two co-stimulatory domains in conjunction with CD3ζ.

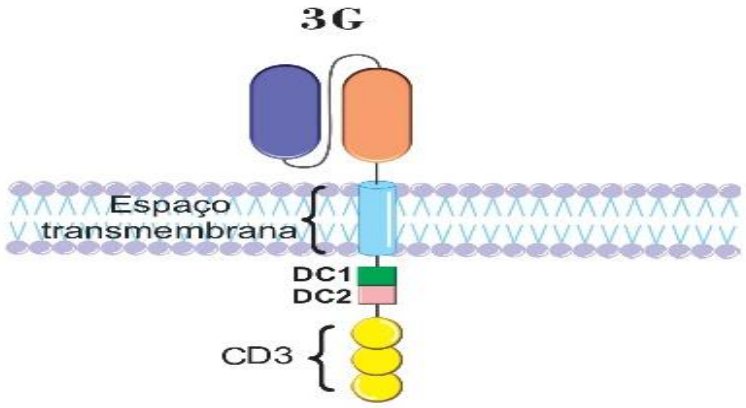

FIGURE 4- Representation of the 3rd generation CAR. Source: authors.

Fourth-generation CARs, also known as T cells Redirected for Universal Cytokine Killing (TRUCKs), feature an advanced intracellular signaling configuration. In addition to the CD3ζ and a co-stimulatory domain, they incorporate a special domain called the nuclear factor of activated T cells (NFAT) (Dejenie et al., 2022), as illustrated in Figure 5. NFAT is composed of transgenes that enhance cytokine production (primarily IL-12) with the aim of not only increasing antitumor activity through T cell cytotoxicity but also leveraging other components of the immune system to modulate this response and make the tumor microenvironment less hostile (Huang et al., 2020).

Thus, IL-12 accumulates at the tumor site after being recognized by CAR-T cells, recruiting innate immune cells (macrophages and NK cells). These cells then both destroy the tumor and modulate the tumor environment to enhance the immune response (Yang et al., 2022). Despite the advantages of TRUCKs—such as greater expansion, persistence, antitumor activity, and fewer side effects—their effectiveness in solid tumors remains limited. They are often activated outside the tumor target, releasing transgenic cytokines into healthy tissues, which leads to adverse effects (Chmielewski and Abken, 2015).

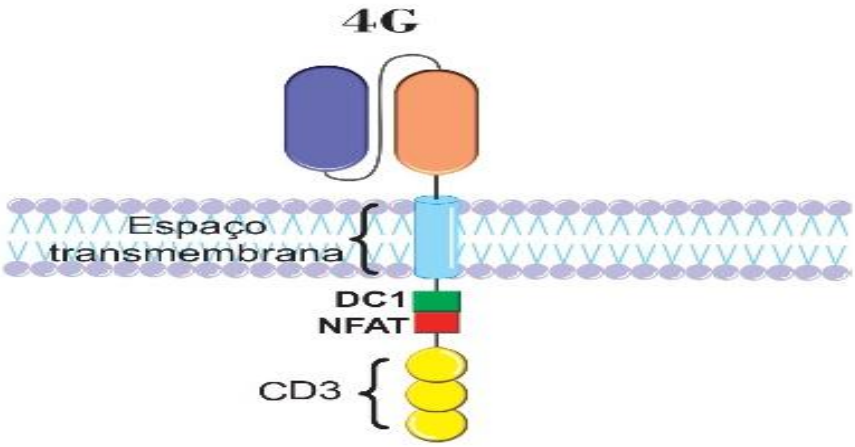

FIGURE 5- Representation of the 4th generation CAR. Source: authors.

Fifth-generation CARs contain the CD3ζ domain, a co-stimulatory factor, and a special domain derived from an extra fragment of the β-chain of the IL-2 receptor, known as IL-2Rβ, in their intracellular region (Yang et al., 2022), as illustrated in Figure 6. The IL-2Rβ has a YXXQ STAT3 binding site that activates the JAK-STAT pathway, inducing cytokine production (Dejenie et al., 2022). This way, when the tumor antigen binds to the CAR, the T cell receives three simultaneous activation signals: from the CD3ζ domain, the co-stimulatory domain, and the JAK-STAT pathway (Smirnov et al., 2024). This combination promotes greater activation, proliferation, and persistence of the CAR-T cells (Kausar et al., 2023).

Moreover, like fourth-generation CARs, this type is also effective in creating a favorable microenvironment for the immune response and in restoring the patient's immune system after infusion (Huang et al., 2020). However, the main limitation of fifth-generation CARs is their reduced efficacy in solid tumors, along with potential adverse effects (Dejenie et al., 2022).

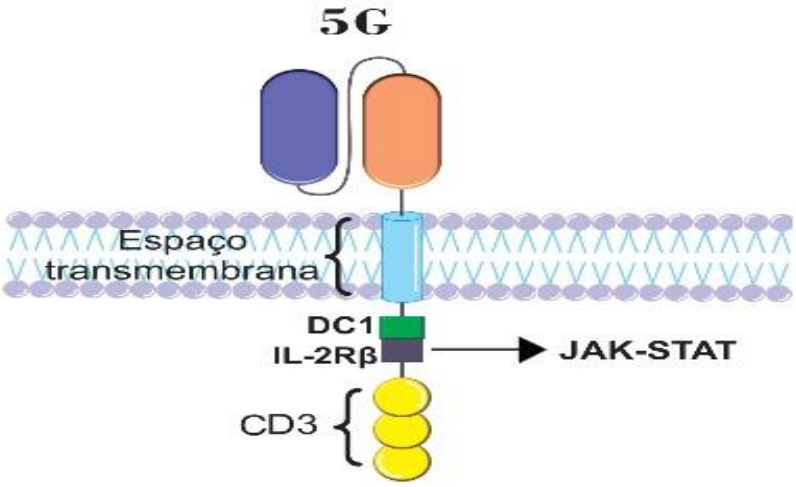

FIGURE 6- Representation of the 5th generation CAR. Source: authors.

CAR-T Cell Production Process

CAR-T cells are developed through genetic engineering from T lymphocytes, which are genetically modified to express a chimeric antigen receptor (CAR) on their surface, enabling them to target tumor cells. The CAR-T cell production process involves several stages and quality control measures to ensure safety and efficacy (Kausar et al., 2023), as shown in the flowchart in Figure 7.

The first step involves therapeutic leukocyte removal (leukapheresis) by collecting blood from the patient (for autologous products) or from a healthy donor (for allogeneic products) (U.S. Department of Health and Human Services, Food and Drug Administration, and Center for Biologics Evaluation and Research, 2024). Next, T cells are enriched and washed for isolation, while the remaining blood is reintroduced into the patient (Zhang et al., 2017).

T cell isolation can be performed by various methods, such as removing red blood cells and platelets using density gradients (elutriation), separating cells by size, removing monocytes, and isolating lymphocytes (Subklewe, Bergwelt-Baildon, and Humpe, 2019). Finally, CD4/CD8 T cell subsets are magnetically separated using conjugates or bead markers (Zhang et al., 2017). The entire extraction, enrichment, and T cell separation process takes approximately 2 to 3 hours (Powell Jr et al., 2009).

After isolating T cells, gene transfer is required to express the CAR receptor. This can be done using viral or non-viral vectors. The most common method is genetic transduction using viral vectors such as retroviruses, lentiviruses, adenoviruses, or adeno-associated viruses (Dejenie et al., 2022). These vectors introduce CAR RNA into T cells, which is reverse transcribed into DNA, integrated into the T cell genome, and permanently incorporated (Soares et al., 2022). Lentiviral vectors are the most widely used, though they have complex accessibility, prompting research into alternatives such as non-viral vectors, including nanoparticles, liposomes, electroporation, and CRISPR/Cas9 technology (Dejenie et al., 2022).

Subsequently, CAR-T cells are expanded *ex vivo* in bioreactors that provide ideal gas exchange and factors that stimulate cell proliferation, such as monoclonal antibodies, APCs, and IL-2 (Dejenie et al., 2022). This gene transfer and expansion process can take from 10 days to several weeks (Dejenie et al., 2022).

The cells are then prepared for infusion, frozen in a cryopreservation medium, and sent to the treatment center, where they are thawed and infused into the patient (Alzubi et al., 2021). Before infusion, the patient must undergo cytotoxic therapy for lymphodepletion and meet certain prerequisites, such as tolerance to chemotherapy and the ability to wait for the CAR-T cell production process (Nardo et al., 2021). The usual interval between leukapheresis and CAR-T cell infusion is around 4 to 5 weeks, and the total time from referral to infusion can take up to 2 months (Kausar et al., 2023).

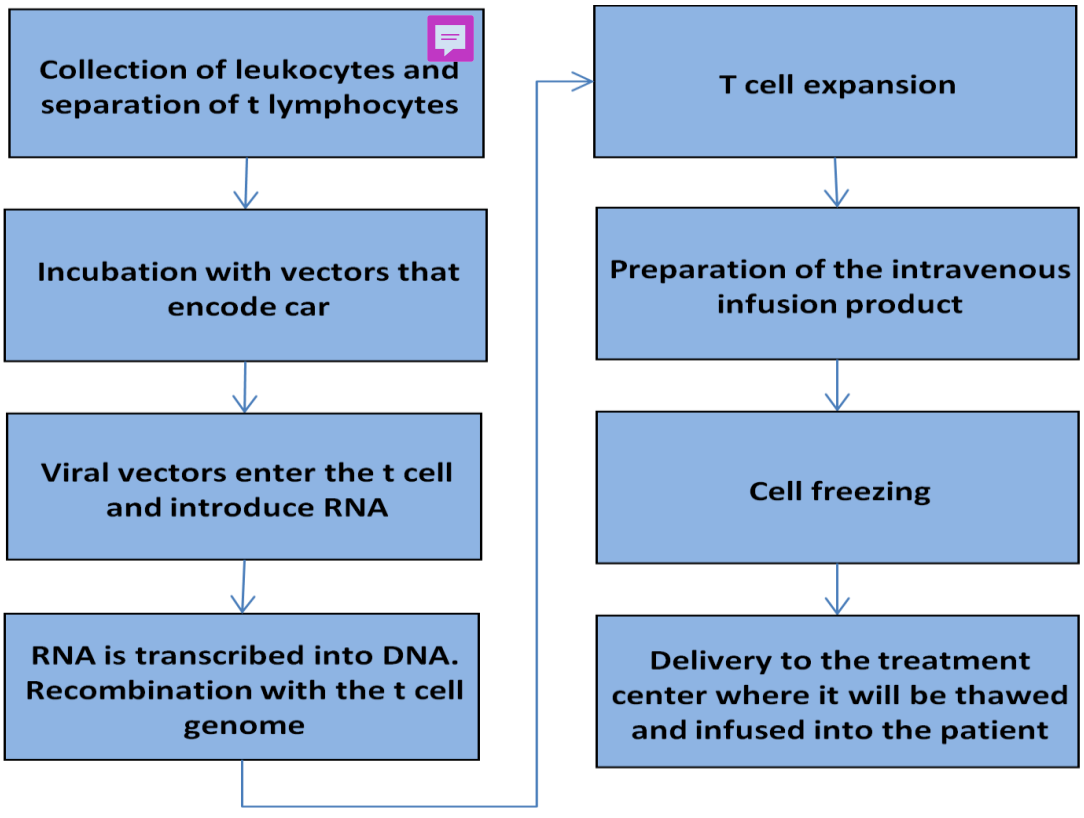

FIGURE 7- CAR-T cell treatment process, from production to infusion into the patient. Source: authors.

CAR-T Therapy Targets and Immune Response

Currently, the most common targets in CAR-T cell therapy are CD19 and BCMA, but antigen escape has driven research into identifying new targets (Zhang et al., 2022). When CAR-T cells recognize the tumor-associated surface antigen, the signaling cascade is activated, leading to cytokine secretion and proliferation (Nardo et al., 2021). This activation enhances CAR-T cells' ability to attack tumor cells by releasing cytotoxic products such as perforins and granzymes. Additionally, CAR-T cells stimulate other immune cells to contribute to immune responses against tumors (Kausar et al., 2023).

Adverse Effects

According to research by Ma et al. (2024), CAR-T cell therapy can trigger significant adverse effects, the most common being **Cytokine Release Syndrome (CRS)** and **Neurotoxic Events (NEs)**.

Cytokine Release Syndrome (CRS) is characterized by a systemic inflammatory response caused by the excessive release of pro-inflammatory cytokines like IL-1, IL-2, and IL-6 by CAR-T cells. In mild cases, CRS may present flu-like symptoms, but in severe cases, it can lead to multiple organ dysfunction and coagulopathy, requiring immediate interventions such as hemodialysis, mechanical ventilation, and cryoprecipitate replacement (Soares et al., 2022). This "cytokine storm" typically occurs within the first week of transfusion, peaking within one to two weeks when CAR-T cells reach maximum expansion. C-reactive protein (CRP) is a reliable indicator for measuring CRS activity (Soares et al., 2022).

The FDA-approved anti-interleukin-6 antibody **tocilizumab**, approved in 2017, can control most cases of CRS (Soares et al., 2022). Tocilizumab blocks IL-6 receptors, quickly alleviating symptoms in moderate to severe patients without impairing CAR-T cell efficacy (Roex et al., 2020). Another proposed strategy is the early application of

granulocyte colony-stimulating factor (G-CSF), which, according to some studies, does not affect CAR-T cell toxicity or efficacy (Ma et al., 2024).

Neurotoxicity, also known as **CAR-T Cell-Related Encephalopathy Syndrome (CRES)**, is the second most common adverse reaction in CAR-T therapy. This syndrome occurs because CAR-T cells, after recognizing the target antigen, activate other immune cells like macrophages. These macrophages release large amounts of cytokines, causing the breakdown of blood-brain barrier junctions, increasing its permeability (Mackall and Miklos, 2017). As a result, cytokines infiltrate the central nervous system, leading to toxic encephalopathy. Patients may experience seizures, confusion, aphasia, and cerebral edema (Deng et al., 2020). Treatment involves corticosteroids and, when available, IL-1 blockers. Due to the syndrome's rapid progression, patients require strict monitoring (Roex et al., 2020).

Ma et al. (2024) reported that the incidence and severity of CRS and NEs were similar in patients treated with and without G-CSF in CAR-T therapy. Therefore, G-CSF use may be a viable strategy for managing these adverse effects, along with anti-inflammatory, immunosuppressive medications, and supportive therapies like hydration and close patient monitoring.

Moreover, most target antigens, like CD19 and BCMA, are expressed on both tumor and healthy B cells, leading to **B cell aplasia** and **hypogammaglobulinemia** as normal cells are also attacked (Pfeiffer et al., 2018). Additionally, the lymphodepleting chemotherapy administered before CAR-T infusion can lead to infections due to impaired immunity (Zhang et al., 2022). Therefore, long-term monitoring for biomarkers and immunoglobulin supplementation is essential (Zhang et al., 2022).

Early symptom identification and proactive management of adverse effects are crucial to ensuring the safety and efficacy of CAR-T cell therapy. A multidisciplinary approach and careful patient evaluation are essential to minimize risks and optimize therapeutic outcomes (Ma et al., 2024).

CAR-T Cell Immunotherapy in Hematological Tumors

CAR-T cell therapy has shown promising results in treating hematological tumors, particularly those affecting B cells (Pang et al., 2018). Currently, FDA-approved CAR-T cell products target **CD19** for **acute B-cell lymphoblastic leukemia (B-ALL)**, **diffuse large B-cell lymphoma (DLBCL)**, and **follicular lymphoma (FL)** (Zhang et al., 2022), as this surface protein is widely expressed in most B-cell lineages and is almost absent in normal cells (Soares et al., 2022). FDA-approved CAR-T products for **multiple myeloma (MM)** target **BCMA** (Zhang et al., 2022).

In **B-cell acute lymphoblastic leukemia**, CD19-targeted immunotherapy has achieved remarkable results, with complete remission in about 90% of cases (Soares et al., 2022). However, antigen escape in some cases drives the investigation of new therapeutic targets to combat this neoplasia (Zhang et al., 2022). In this context, **CD22** has emerged as a promising target, as it is expressed in B-cell tumor cells in B-ALL (Olejniczak et al., 2009). A study conducted at Beijing Boren Hospital, China, demonstrated that anti-CD22 CAR-T therapy led to high remission rates, but also a high recurrence rate without additional treatments (Pan et al., 2019). Therefore, the development of **bispecific CARs** or **tanCARs**, designed to simultaneously recognize two tumor antigens, such as CD19/CD22, is a promising strategy for treating this neoplasia (Han et al., 2024).

In patients with **diffuse large B-cell lymphoma**, CARs targeting CD19 lead to complete remission in 40% to 58% of cases (Soares et al., 2022). Similar to B-ALL, CD19 is an essential therapeutic target, but frequent antigen escape has driven the search for new antigens in CAR-T therapy (Zhang et al., 2022). In this context, **CD20**, which is overexpressed in this neoplasm, has shown promise. In a study conducted by Wang et al. (2014), the overall response rate was 86%, while in a study by Zhang et al. (2017), 17 patients treated with anti-CD20 CARs exhibited a complete remission rate of 54.5%, with 12 patients maintaining remission over a median follow-up of 20 months. Bispecific or tanCARs that simultaneously recognize **CD19 and CD20** are emerging as a safe and viable option for treating resistant lymphomas, offering an innovative approach to overcoming antigen escape and improving therapeutic outcomes (Sang et al., 2020).

In **follicular lymphoma**, CD19-targeted immunotherapy has demonstrated high efficacy and good tolerance. In a study conducted by Hirayama et al. (2019), the complete remission rate was 88%, with remission lasting up to 3 years post-CAR cell infusion. Although clinical trial participation remains low, there is evidence that anti-CD19 CAR therapy can be a highly effective option for patients with FL, resulting in high and prolonged remission rates (Bishop, 2019).

Patients with **multiple myeloma** treated with BCMA-targeted CAR-T cells demonstrated a positive response rate of 86%. However, relapses still occur, and the down regulation of BCMA under therapeutic pressure highlights the need for new target antigens (Zhang et al., 2022). **CD138** (Syndecan-1) is highly expressed in multiple myeloma cells. A study by Guo et al. (2016) showed a positive response rate of 80% in patients treated with anti-CD138 CARs. Other antigens, such as **CD229**, **APRIL**, and **GPRC5D**, are also being studied as potential targets (Zhang et al., 2022).

Although not yet approved, various CAR-T therapy approaches are being explored for cancers like **T-cell acute lymphoblastic leukemia (T-ALL)**, **chronic lymphocytic leukemia (CLL)**, **Hodgkin lymphoma (HL)**, and **acute myeloid leukemia (AML)**.

In T-ALL, **CD7**, **CD4**, **CD99**, and **CCR9** are being investigated as promising targets due to their high expression in T lymphoid and leukemic cells, with recent studies demonstrating targeted efficacy and cytotoxicity (Zhang et al., 2022; Shi et al., 2021; Maciocia et al., 2022).

In CLL, the main target remains **CD19**, but the complete response rate remains limited, requiring further advancements (Amatya et al., 2024). In HL, targets like **CD30** and **CD123** are being studied, with promising preliminary results in tumor eradication (Haslauer et al., 2021; Ruella et al., 2017). Finally, in AML, **CD123**, **CD33**, **CD70**, and **Siglec-6** are under investigation. These antigens are challenging due to their expression in normal hematopoietic stem cells, but preclinical studies show promising potential (Zhang et al., 2022; Eissenberg et al., 2024).

CAR-T Cell Immunotherapy in Solid Tumors

Solid tumors present various barriers that have hindered the success of CAR-T cell trials and treatments, such as the immunosuppressive microenvironment, antigen heterogeneity, and antigen escape (Nardo et al., 2021).

The main challenge for CAR-T cells is the hostile microenvironment created by tumor cells. In addition to being highly hypoxemic, immune cells within the tumor, such as myeloid-derived suppressor cells (MDSCs), regulatory T cells (Tregs), and tumor-associated macrophages (TAMs), produce growth factors (TGF-β), cytokines, and immunosuppressive chemokines. These not only inhibit CAR-T cell antitumor effects but also accelerate their exhaustion and reduce the therapy's efficacy (Guzman et al., 2023). Several strategies are being developed to prevent CAR-T cell exhaustion, including inhibiting the inhibitory marker **PD-1** with monoclonal antibodies and using **CRISPR/Cas9** to eliminate the **TGF-β receptor II** (Guzman et al., 2023). To overcome the hypoxemic tumor environment, hypoxia-inducible CARs (**HiCARs**) are being developed, which are activated specifically under low oxygen conditions (Liao et al., 2020).

Tumor antigen heterogeneity in solid tumors means that there is no ideal target antigen for technology and studies. Tumor-expressed antigens vary among patients with the same cancer type, between primary and metastatic stages, and even within a single tumor, where different neoplastic subpopulations each have distinct characteristics and antigens. This complexity complicates treatment (PharmD et al., 2021). Even when an antigen is uniformly expressed, **antigen escape or loss** remains a common issue (Nardo et al., 2021). To address this, multivalent CAR-T therapy is being explored, utilizing advanced CAR designs like **tandem CARs (TanCARs)**, which feature two distinct scFvs in a single CAR. This enables the CAR-T cell to simultaneously recognize two surface antigens, increasing tumor efficacy and reducing the likelihood of antigen escape (Guzmán et al., 2023). Additionally, **bispecific CARs**, which equip a single T cell with two CARs targeting two different tumor antigens, are also under investigation (Mohanty et al., 2019).

Another effective strategy against tumor heterogeneity is **epitope spreading**. When some tumor cells are destroyed by CAR-T cells, they release epitopes, which are presented by antigen-presenting cells to the immune system. This process creates targets for endogenous tumor-infiltrating lymphocytes (TILs), generating a secondary immune response (Nardo et al., 2021).

Furthermore, many tumor antigens are also found in normal tissues, creating another challenge. In solid tumors, antigens like **CEA, GD2, and MUC1** are often overexpressed in tumor cells but also present at lower levels in normal tissues (Nardo et al., 2021).

Despite these challenges, CAR-T therapy has shown promising results in certain solid tumors, such as **small cell lung cancer**, **malignant pleural mesothelioma**, **cholangiocarcinoma**, **epithelial ovarian cancer**, **glioblastoma**, and **sarcomas** (Soares et al., 2022). Advances have also been observed in **breast cancer**, with a focus on targeting **HER2, HER1**, and **EFR** (Yu-huan et al., 2022). Although several clinical trials for solid tumor treatment with CAR-T cells are ongoing, and some cases of temporary remission have been observed, only a few results have been made public, and the published data is inconsistent (Soares et al., 2022).

Conclusion

CAR-T cell therapy represents a significant innovation in the treatment of hematological cancers. The evolution of CARs across generations, from simple first-generation receptors to sophisticated fifth-generation CARs and alternative designs, reflects advancements in genetic engineering and our understanding of T cell activation and signaling mechanisms. The main challenges remain in solid tumors, where the immunosuppressive microenvironment, tumor heterogeneity, and antigen escape limit the effectiveness of CAR-T therapy. However, many research strategies are being adopted to overcome these issues, as well as to reduce adverse effects.

As CAR-T cell therapy advances, continued investment in research is essential to overcome technical and clinical challenges, thereby expanding the therapeutic potential

of these innovative approaches in cancer treatment. Collaboration between researchers, clinicians, and the industry plays a key role in translating these advancements into tangible benefits for patients. Additionally, finding ways to reduce treatment costs is crucial for making CAR-T therapy more globally accessible. An encouraging example of this collaboration is the recent partnership between **Oswaldo Cruz Foundation (Fiocruz)** and **Caring Cross**, established in March 2024. This partnership aims to transfer CAR-T cell and lentiviral vector production technology to Brazil, which has the potential to significantly reduce the cost per dose. This will not only increase treatment accessibility for Brazilian patients but could also facilitate its inclusion in the **Brazilian Unified Health System (SUS)**, benefiting more people battling cancer.

Therefore, ongoing innovation in genetic engineering and a deeper understanding of T cell activation mechanisms point to a promising future. The advancement of CAR-T cell immunotherapy holds the potential to offer increasingly better outcomes in cancer treatment, expanding therapeutic options and improving patients' quality of life.

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