Investigating chimeric antigen receptor T cell therapy and the potential for cancer immunotherapy (Review)

RAYANSH POOJARY, ANDY FANG SONG, BENNY SHONE SONG, CARLY SHAW SONG, LIQING WANG and JIANXUN SONG

Department of Microbial Pathogenesis and Immunology, Texas A&M University Health Science Center, Bryan, TX 77807-3260, USA

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Abstract. Immunotherapy has emerged as a crucial treatment option, particularly for types of cancer that display resistance to conventional therapies. A remarkable breakthrough in this field is the development of chimeric antigen receptor (CAR) T cell therapy. CAR T cells are generated by engineering the T cells of a patient to express receptors that can recognize specific tumor antigens. This groundbreaking approach has demonstrated impressive outcomes in hematologic malignancies, including diffuse large B cell lymphoma, B cell acute lymphoblastic leukemia and multiple myeloma. Despite these significant successes, CAR T cell therapy has encountered challenges in its application against solid tumors, leading to limited success in these cases. Consequently, researchers are actively exploring novel strategies to enhance the efficacy of CAR T cells. The focus lies on augmenting CAR T cell trafficking to tumors while preventing the development of CAR T cell exhaustion and dysfunction. The present review aimed to provide a comprehensive analysis of the achievements and limitations of CAR T cell therapy in the context of cancer treatment. By understanding both the successes and hurdles, further advancements in this promising area of research can be developed. Overall, immunotherapy, particularly CAR T cell therapy, has opened up novel possibilities for cancer treatment, offering hope to patients with previously untreatable malignancies. However, to fully realize its potential, ongoing research and innovative strategies are essential in overcoming the challenges posed by solid tumors and maximizing CAR T cell efficacy in clinical settings.

Correspondence to: Professor Jianxun Song, Department of Microbial Pathogenesis and Immunology, Texas A&M University Health Science Center, 8447 Riverside Parkway, MREB 2, Room 3344, Bryan, TX 77807-3260, USA
E-mail: jus35@tamu.edu

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Contents

1. Introduction

Cancer poses a substantial worldwide public health issue, and presently stands as the second primary contributor to mortality in the United States (1). Projections indicate that by the year 2040, the annual number of new cancer cases is expected to reach a staggering 29.5 million, with 16.4 million cancer-related deaths (2). The concept of the connection between immune cells and cancer was first suggested by Rudolf Virchow over 150 years ago (3). This observation laid the groundwork for exploring the potential of using immune cells as a therapeutic approach. Notably, in the late 19th century, William Coley conducted groundbreaking research where he injected heat-inactivated bacteria into tumor masses, resulting in a reduction of tumor size (4).

The process of immune-driven elimination of cancer cells encompasses a sequence of vital stages (5). Initially, local tissue disruption caused by stromal remodeling triggers the recruitment of innate immune system cells such as NK cells, macrophages, and neutrophils (6). These NK cells recognize the developing tumors and initiate the process of tumor cell killing (7). After tumor cell death, tumor-associated antigens (TAAs) from the deceased cancer cells are taken up by antigen-presenting cells (APCs) such as dendritic cells (DCs) (8). The activated DCs then migrate to draining lymph nodes where they present the tumor antigens to naïve CD4+ and CD8+ T-cells through major histocompatibility complex (MHC) class I and II death (8). Additionally, the DCs release cytokines that regulate T-cell responses and convert naïve CD8+ T cells into cytotoxic T-cells. As a result, these cytotoxic T-cells leave the lymphoid organs, enter the bloodstream, infiltrate the tumor site, and effectively induce tumor cell death (8).

Tumors have developed multiple mechanisms to evade immune-mediated elimination (9). They employ inhibitory

cytokines such as TGF-β and IL-10, which activate inhibitory signals, leading to the attenuation of antitumor immunity (10). Furthermore, TGF-β plays a role in converting CD4+T cells that infiltrate the tumor into Foxp3+Tregs, which possess highly immunosuppressive properties (11). Moreover, most tumors downregulate the expression of costimulatory molecules necessary for effective T-cell activation. Additionally, myeloid-derived suppressor cells (MDSCs) also contribute to inhibiting the antitumor response (9). Another crucial aspect of T cell dysfunction in cancer is related to a phenomenon known as T cell exhaustion, wherein T cells lose their tumor-killing ability and express inhibitory receptors such as PD1, Tim3, LAG3, CTLA4, etc (12). As a result of these strategies, tumors create an immunosuppressive tumor microenvironment (TME) (9).

Immunotherapy encompasses strategies aimed at overcoming immune suppression by blocking inhibitory receptors such as CTLA-4 and PD-1 (immune checkpoint inhibitors) and stimulating antitumor immune responses through adoptive transfer T cell therapy (13-15). The introduction of checkpoint inhibitors, such as ipilimumab targeting CTLA-4, has revolutionized the field and shown promising results. Similarly, chimeric antigen receptor (CAR) T cell therapy has demonstrated remarkable clinical success in treating hematological malignancies. One significant advantage of CAR T cell therapy is its independence from antigenic peptide-bound major histocompatibility complex (MHC) recognition. This is crucial because tumor cells often evade immune responses by losing MHC-associated antigen presentation, making traditional T cell responses less effective. CAR T cells directly target tumor-specific antigens, bypassing the MHC-related limitations.

In this review, we provide a comprehensive overview of the development and mechanisms of Chimeric Antigen Receptor (CAR)-T cell therapy. Additionally, we address the limitations of CAR T cell therapy in treating solid tumors and explore potential strategies to manage cytokine release syndrome, a common adverse effect associated with CAR T cell therapy (Fig. 1).

2. CAR design

CARs consist of four distinct parts, each serving specific functions (16) (Fig. 2). The first part is the antigen-binding domain, which is the extracellular component conferring antigen specificity. This domain is formed by connecting variable heavy (VH) and light (VL) chains of monoclonal antibodies through a flexible linker, creating a single-chain variable fragment (scFv) (17). The scFvs bind to cancer antigens on the cell surface, leading to T cell activation independent of the major histocompatibility complex (MHC) (17,18).

Next is the hinge region, linking the antigen-binding domain to the transmembrane region. Typically derived from CD28 and CD8, the hinge imparts flexibility, overcoming steric hindrance, and allowing the antigen-binding domain to access targeted tumor antigens more effectively (16).

The transmembrane domain spans the T cell membrane's lipid bilayer, anchoring the CAR to the cell membrane. While its primary function is anchoring, some evidence suggests it may also influence CAR T cell function (16). Transmembrane domains are often derived from natural proteins such as CD3ζ, CD4, CD8α, or CD28 (16).

The fourth component, the intracellular signaling domain, consists of an activation domain and one or more costimulatory domains (Fig. 2). Most CARs utilize CD3ζ-derived immunoreceptor tyrosine-based activation motifs (ITAMs) for T cell activation, but this signaling alone is insufficient (19). A costimulatory signal is essential for optimal T cell function and persistence (20). Notably, all FDA-approved CAR T cells include either a CD28 or 4-1BB costimulatory domain (16).

Upon recognizing specific tumor antigens through their ScFv, CAR T cells trigger the phosphorylation of ITAM domains on the CD3ζ chain, initiating signaling through the tyrosine kinase ζ-associated protein of 70 kDa (ZAP70). Consequently, CAR T cells become activated, proliferate, release cytokines, undergo metabolic changes, and exhibit cytotoxicity, unleashing a potent T cell effector response.

CARs can be categorized based on the number of signaling domains they contain (Fig. 3) (1) First-generation CARs: Contain only the CD3ζ activation domain. They show limited persistence and efficacy (2) Second-generation CARs: Incorporate a costimulatory domain (e.g., CD28 or 4-1BB) in addition to the CD3ζ activation domain. These CARs exhibit enhanced T cell function and persistence (3) Third-generation CARs: Have multiple costimulatory domains. The combination of costimulatory domains aims to improve CAR T cell function and therapeutic efficacy (4) Four-generation CARs: Also known as armored CAR T cells, co-express key cytokines, such as interleukins and chemokines, or suicide genes that can significantly enhance the efficacy and safety of CAR T therapy (5) Five-generation CARs: Contain an extra intracellular domain than their predecessors. The CARs comprise truncated intracellular domains of cytokine receptors (e.g., IL-2R chain fragment) with a motif for binding transcription factors such as STAT-3/5 (21-23). It should be noted that there weren't widely recognized fourth or fifth generation CAR T cells. However, researchers might have explored additional modifications and generations to further enhance CAR T cell therapy's efficacy, persistence, and safety.

The choice of antigen-binding domain, costimulatory domain, and the overall CAR architecture depends on various factors, including the target tumor antigen, the type of cancer, and the desired T cell response. Ongoing research aims to optimize CAR design to enhance specificity, efficacy, and safety in CAR T cell therapy.

3. Generation of CAR T cells

The selection of the target tumor antigen is a crucial initial step in the development of CAR T cells (24). The ideal scenario is to have CAR T cells that solely target tumor cells while sparing normal, healthy cells. However, a number of tumor antigens are self-antigens, which means they are also present on normal cells but are often overexpressed in tumors (24). In CAR T-cell therapy for CD19- and CD20-positive hematologic malignancies, CD19 and CD20 have become widely used tumor antigens (17). These antigens have shown promising results in treating certain blood cancers.

Various other tumor antigens have been explored for CAR T cell therapy, and some examples are listed in Table I.

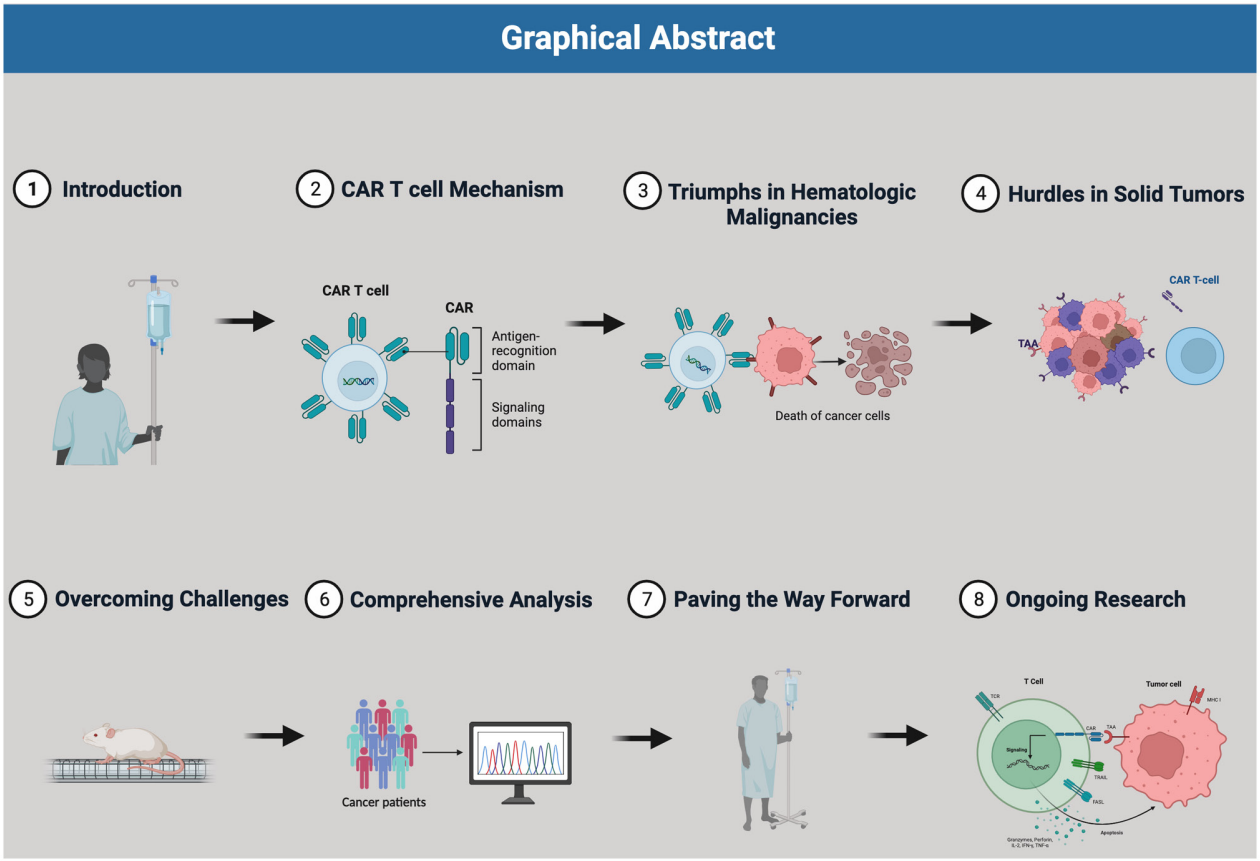

Figure 1. Graphical abstract of the present study. i) Introduction. Immunotherapy has revolutionized cancer treatment, with CAR T cell therapy standing out as a breakthrough approach. ii) CAR T cell mechanism. CAR T cells are engineered T cells with receptors that recognize tumor antigens, demonstrating success in treating hematological malignancies. iii) Triumphs in treating hematological malignancies. Impressive outcomes seen in diffuse large B cell lymphoma, B cell acute lymphoblastic leukemia and multiple myeloma, showcasing the potential of CAR T cell therapy. iv) Hurdles in solid tumors. CAR T therapy encounters challenges in treating solid tumors due to limited tumor infiltration and CAR T cell exhaustion. v) Overcoming challenges. Researchers are actively working to enhance CAR T cell trafficking, prevent exhaustion and improve efficacy against solid tumors. vi) Comprehensive analysis. The present review provided an insightful analysis of the achievements and limitations of CAR T cell therapy in cancer treatment. vii) Paving the way forward. Understanding successes and hurdles helps drive further advancements in CAR T cell research, holding promise for untreatable malignancies. viii) Ongoing research. Continued exploration and innovative strategies are crucial for maximizing CAR T cell efficacy in clinical settings. CAR, chimeric antigen receptor.

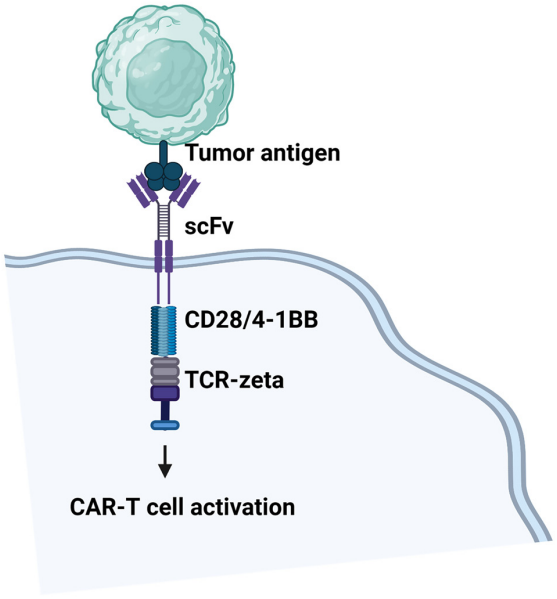

Figure 2. Tumor-specific CARs. CARs have at least four parts with distinct functions: i) The antigen-binding domain; ii) the hinge; iii) the transmembrane domain; and iv) the intracellular signaling domain. CAR, chimeric antigen receptor; TCR, T cell receptor; scFv, single-chain variable fragment.

The subsequent step in CAR T cell therapy involves isolating the patient's T cells through a process called leukapheresis. During this procedure, the patient's blood is withdrawn, and leukocytes (white blood cells) are collected, while the rest of the blood components are returned to the patient. T cells, including both $CD4^+$ and $CD8^+$ T cells, are then isolated from the collected leukocytes using specific antibody bead conjugates, which enriches for T cells.

In the laboratory, the isolated T cells undergo genetic engineering to express CARs on their surfaces. This process typically involves the use of retroviral or lentiviral vectors for transfection (1) Transduction: The retroviral or lentiviral vectors carry the genetic information necessary for the CAR's expression. These vectors are engineered to be non-replicative and safe for use in gene transfer. The T cells are exposed to the viral particles, and the vectors enter the T cells (2) Integration: Once inside the T cells, the viral vectors integrate the genetic material encoding the CAR into the T cell's genome. This integration ensures stable and long-term expression of the CAR as the T cells divide and proliferate (3) CAR expression: With the CAR's genetic material now integrated into the genome, the T cells start to express the

Table I. Tumor antigens targeted for CAR T cell therapy.

Target antigen	Cancer type	(Refs.)
CD22	B-cell acute lymphoblastic leukemia	(17)
CD30	Hodgkin lymphoma	(18-20)
CD33	Acute myeloid leukemia	(21-23)
Estrogen-related receptor β type 2	Prostate cancer, breast cancer	(24,25)
Prostate-specific membrane antigen	Prostate cancer	(26-28)
Carbonic anhydrase IX	Renal cell carcinoma	(29)
Carcinoembryonic antigen	Colon cancer	(30)
Mesothelin surface glycoprotein	Malignant pleural mesothelioma, pancreatic adenocarcinoma, breast cancer, ovarian cancer	(31-34)

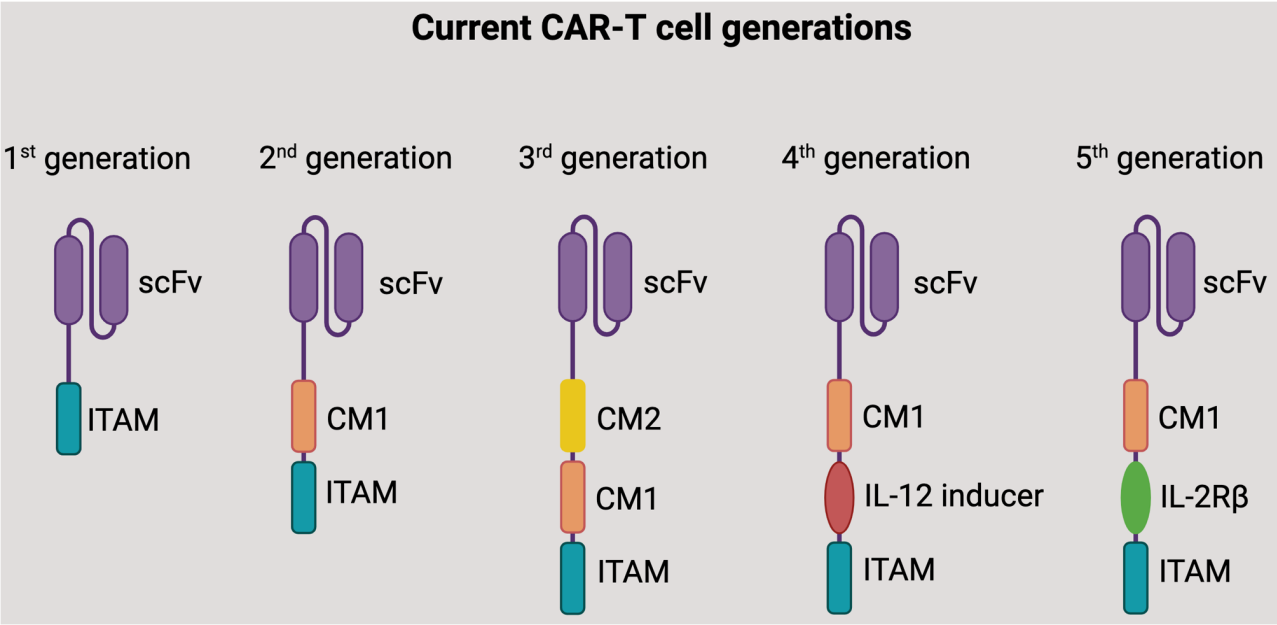

Figure 3. A diagram of five generations of CARs. CARs are structurally divided into three key regions: i) The antigen-binding domain, derived from antibodies, facilitates antigen recognition. This domain typically incorporates a single-stranded variable fragment sourced from antibodies. ii) The transmembrane domain provides anchoring support to the plasma membrane, ensuring stability. iii) The signaling domain triggers T-cell activation. In first-generation CARs, this domain contains a CD3ζ-derived signaling module. In second-generation CARs, an additional co-stimulatory domain is included. Meanwhile, third-generation CARs feature two co-stimulatory domains, including CD28, 4-1BB (CD137), CD27 and OX40 (CD134). Furthermore, there are advanced iterations of CAR T cells: Fourth-generation CAR T cells, also referred to as TRUCKs, are designed to induce expression of chemokines such as IL-12, enhancing their therapeutic potential. Fifth-generation CARs introduce a novel co-stimulatory domain that activates specific signaling pathways. scFv, single-chain variable fragment; ITAM, immunoreceptor tyrosine-based activation motif; CM, co-stimulatory molecule; IL-2Rβ, interleukin-2 receptor β; CAR, chimeric antigen receptor.

chimeric antigen receptor on their cell surfaces. The CAR allows the T cells to recognize and bind to specific antigens present on cancer cells (4) Expansion: The genetically modified T cells are then cultured and expanded in the laboratory. This expansion process helps generate a large population of CAR-expressing T cells that can be used for the patient's treatment. By using retroviral or lentiviral vectors, researchers can efficiently introduce the CAR into the patient's T cells, ensuring sustained CAR expression and enabling the cells to target and attack cancer cells when infused back into the patient's body during CAR T cell therapy. This process has shown significant promise in treating various hematological malignancies and is being explored for potential applications in solid tumor therapies as well.

Once the viral particles carrying the CAR's genetic material enter the T cells, they integrate the CAR into the T cell's genome. This integration ensures stable and heritable expression of the CAR as the T cells divide and proliferate. As the T cells multiply and expand in culture, the CAR expression is retained in all the daughter cells, resulting in a large population of CAR-expressing T cells. This is a crucial step in CAR T cell therapy as it allows for the production of a sufficient number of engineered T cells for infusion back into the patient. The long-lasting expression of the CAR enables the CAR T cells to recognize and target cancer cells effectively, leading to the desired antitumor immune response when these modified T cells are reinfused into the patient for therapy. The ability of CAR expression to persist as the T cells undergo division and

expansion is essential for the success of CAR T cell therapy in treating various types of cancers.

Once the CAR T cells have undergone significant expansion and are ready for therapeutic use, they are sent back to the hospital for infusion into the patient. However, before the CAR T cell infusion, a preparatory step known as 'lymphodepletion' is often performed.

Lymphodepletion involves the administration of chemotherapy or other agents to temporarily suppress the patient's immune system. This procedure serves several important purposes (1) Create Space: By reducing the number of existing immune cells, lymphodepletion creates space for the infused CAR T cells to expand and exert their antitumor effects without competition from the patient's endogenous immune cells (2) Facilitate Persistence: The temporary suppression of the patient's immune system may help the infused CAR T cells persist and survive for a more extended period in the body, increasing the treatment's efficacy (3) Reduce Rejection: Lymphodepletion also helps reduce the risk of the patient's immune system recognizing the CAR T cells as foreign and launching an immune response against them (rejection). This enhances the chances of successful CAR T cell therapy. Once lymphodepletion is completed, the expanded and engineered CAR T cells are infused into the patient. These CAR T cells then target and attack the cancer cells, leveraging the patient's immune system to fight the disease effectively.

It's important to note that lymphodepletion is not used in all CAR T cell therapy protocols, and its use may vary depending on the specific cancer type and the CAR T cell product being used. The decision to include lymphodepletion is based on the clinical trial protocol and the medical team's judgment to optimize the therapy's effectiveness and safety (25) (Fig. 4).

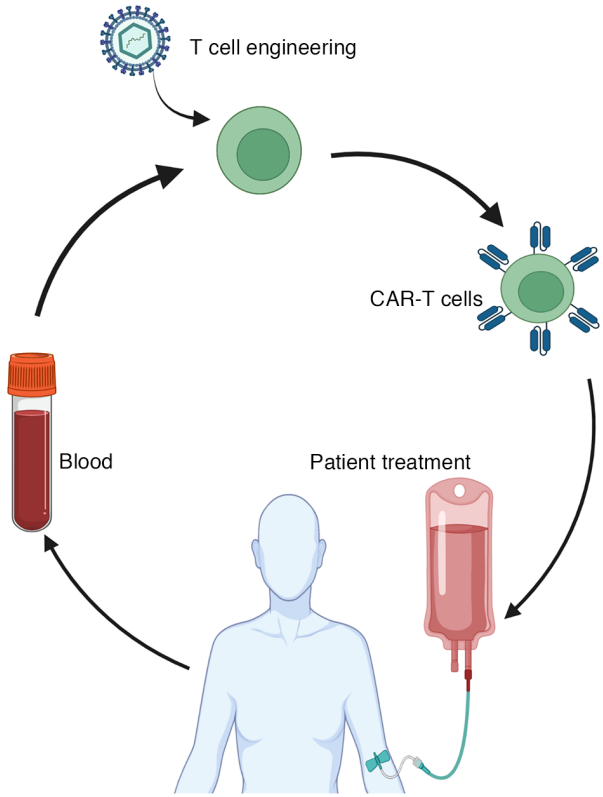

Figure 4. Cancer immunotherapy using CAR T cells. T cells are extracted from the patient, modified in the lab to express CARs that recognize cancer-specific antigens and then infused back into the bloodstream of the patient, leading to a highly targeted immune response against the cancer. The treatment has shown significant success in treating certain blood cancers and offers new hope for patients who have not responded to conventional therapies. CAR, chimeric antigen receptor.

4. Limitation of CAR T cells and toxicity

The development of resistance to the targeted single antigen is a significant limitation of CAR T cell therapy. In some cases, cancer cells in patients treated with CAR T cells either reduce or completely lose the expression of the target antigen. This phenomenon is commonly known as antigen escape and has been observed in patients treated with CD19-targeted CAR T cells for acute lymphoblastic leukemia (ALL) (26,27). Similar reduced expression of BCMA has been reported in multiple myeloma (MM) patients treated with BCMA-targeted CAR T cells (28,29). Moreover, glioblastoma patients treated with CAR T cells targeting IL13Ra2 have shown tumor recurrences with decreased IL13Ra2 expression (30). Of note, the potential for CAR T cells to suppress cancer stem cells (CSCs) by specifically homing in on their cell surface markers has been investigated. This approach holds promise for enhancing the effectiveness of treatments in individuals with different types of cancer (31).

To overcome this hurdle, one strategy is to target multiple antigens simultaneously. Clinical trials using dual-targeted CAR T cells, such as CD19/CD22 or CD19/BCMA, have shown promising results (32-35). In solid tumors, tandem CARs have also demonstrated potential in preclinical models. For example, targeting HER2 and IL13Ra2 in glioblastoma or HER2 and MUC1 in breast cancer has shown encouraging outcomes (36,37). By targeting multiple antigens, CAR T cells have an improved chance of recognizing and attacking cancer cells, reducing the likelihood of resistance due to antigen escape. This approach opens up new possibilities for enhancing the effectiveness of CAR T cell therapy against various types of cancer.

The immunosuppressive tumor microenvironment (TME) poses another significant challenge to CAR T cell therapy, particularly in solid tumors. Within solid tumors, various immune cells with inhibitory functions, such as myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs), infiltrate and hinder CAR T cell activity (38). Moreover, immune checkpoint pathways, including PD-1, Tim3, Lag3, and CTLA-4, play a crucial role in suppressing antitumor immunity and promoting CAR T cell exhaustion (38). As a result, researchers are actively exploring strategies to combine CAR T cell therapy with checkpoint blockade, both in hematological malignancies and solid tumors (39). By blocking these inhibitory checkpoint pathways, the aim is to enhance the function and persistence of CAR T cells, enabling them to better combat the immunosuppressive effects of the TME. This approach holds significant promise in overcoming the limitations posed by the immunosuppressive TME and improving the efficacy of CAR T cell therapy in various cancer types.

Another major limitation of CAR T cells is toxicity which can be broadly classified under two categories (1) Systemic

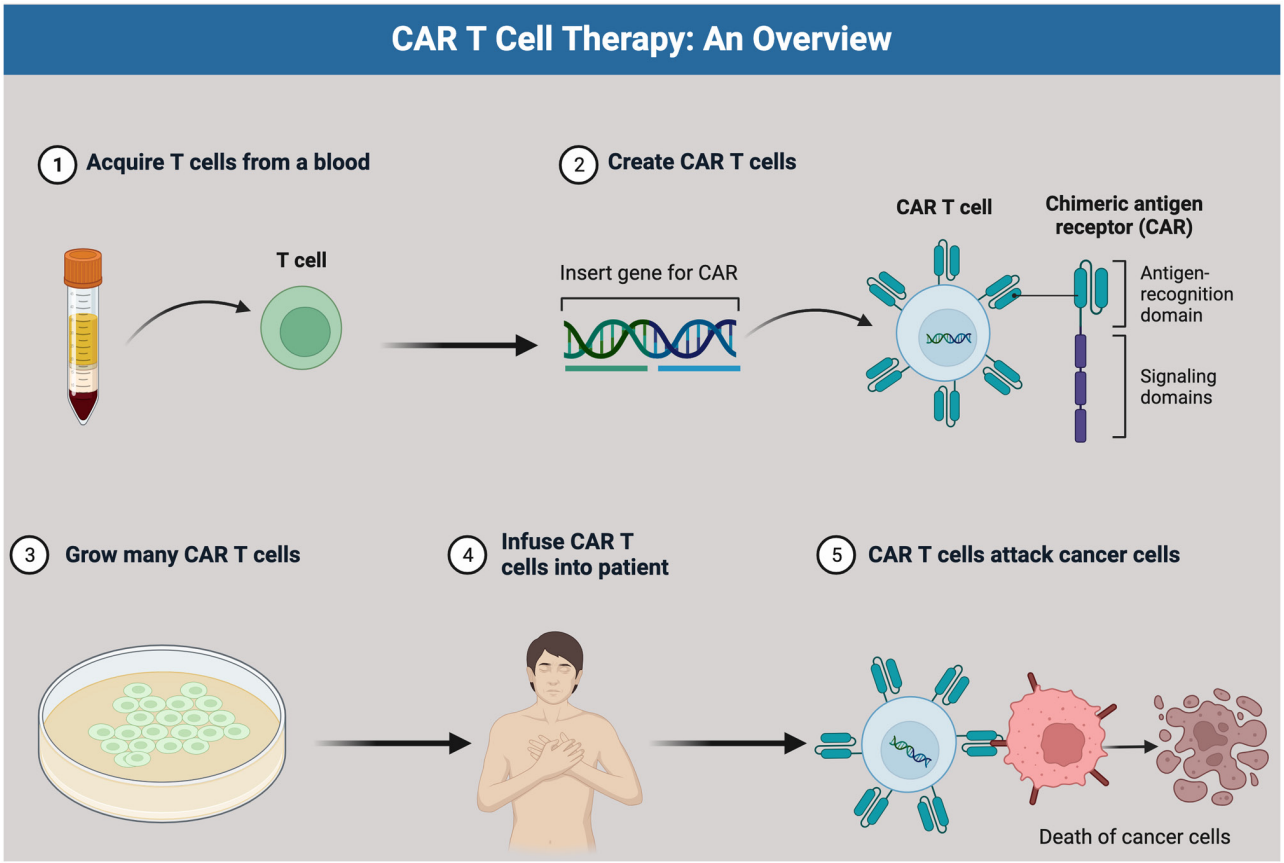

Figure 5. CAR T cell therapy overview. CAR T cell therapy is a groundbreaking immunotherapy for treating certain types of cancer. It involves genetically modifying a patient's own T cells to express a CAR that targets cancer cells. Once infused back into the patient, these modified CAR T cells can recognize and destroy cancer cells, offering a highly targeted and potent approach to cancer treatment. CAR, chimeric antigen receptor.

toxicities: These occur as a result of the robust activation of T cells, leading to excessive cytokine production. This phenomenon is known as cytokine-release syndrome (CRS) and can manifest as severe and potentially fatal increases in cytokine levels. CRS may also be accompanied by other complications, such as macrophage activation syndrome (MAS) and neurotoxicity (2) On-target but off-tumor toxicities: This type of toxicity occurs when CAR T cells recognize and attack not only tumor cells but also healthy cells expressing the same target antigen. This can lead to adverse effects on normal tissues.

To mitigate the detrimental effects of systemic toxicities, therapeutic antibodies blocking the IL-6 pathway, such as tocilizumab or siltuximab, are now being utilized. These antibodies help reduce the harmful effects of excessive cytokine release without compromising the antitumor activity of CAR T cells. Managing toxicity is a critical aspect of CAR T-cell therapy, and ongoing research and advancements aim to optimize the therapy's safety and effectiveness (40).

The implementation of 'off-switches' in CAR T cell therapy is an emerging and promising strategy to mitigate toxicity. These off-switches are designed to selectively block or deactivate CAR T cells in response to adverse events, providing a way to rapidly control the therapy's effects when needed. One such example is the use of inducible cas9, which has shown significant efficacy in a clinical trial. This approach resulted in the elimination of over 90% of engineered T cells within just 30 min, offering a swift and controllable means of attenuating the CAR T cell response when necessary (41). Another strategy involves utilizing protease-based small molecule-assisted shutoff CARs (SMASh-CARs) or switch-off CARs (SWIFF-CARs) (42). These engineered CARs incorporate specific protease cleavage sites, allowing for external control over CAR T cell activity through administration of appropriate small molecules.

However, despite the potential benefits of these off-switch strategies in reducing toxicity, a challenge remains in finding a balance between temporary inhibition of CAR T cells and timely reactivation to resume antitumor activity. Abruptly stopping therapy can be a concern, particularly if cancer progression occurs rapidly during the period when CAR T cells are deactivated.

In addition, other approaches have been developed (1) Tunable CARs: Designing CARs with tunable activation thresholds can enable the control of CAR T cell function. These CARs respond to specific signals or concentrations of antigens, allowing for fine-tuning of CAR T cell activity based on the tumor burden and potential toxicity (2) Localized delivery: Developing methods for localized delivery of CAR T cells to the tumor site can minimize systemic toxicity. By targeting CAR T cells directly to the tumor, it reduces the risk of damage to healthy tissues and decreases the likelihood of severe adverse effects (3) Combination therapies: Combining CAR T cell therapy with other treatments, such as immune

checkpoint inhibitors or targeted therapies, may help modulate CAR T cell responses and improve safety and efficacy.

To further advance this approach, new and more sophisticated strategies need to be developed. These strategies should enable the temporary inhibition of CAR T cell function while allowing for CAR T cell therapy rescue once the toxicity subsides. Achieving this delicate balance is essential for CAR T cell therapy to progress towards becoming a viable first-line treatment option for both hematological and solid tumors.

Research in these areas is ongoing, and these innovative strategies hold great promise for overcoming the current limitations of CAR T cell therapy and moving it closer to becoming a frontline treatment for both hematological and solid tumors. With continued advancements in CAR design and safety measures, CAR T cell therapy has the potential to transform cancer treatment and significantly improve patient outcomes.

5. Summary

CAR T cell therapy (Fig. 5) has demonstrated remarkable potential in treating hematologic malignancies. Despite this promise, its widespread clinical application has been hindered by several challenges, including target antigen escape, a tumor-suppressive microenvironment, and adverse reactions. To overcome these obstacles, it is crucial to gain a comprehensive understanding of the intricate interactions among engineered T cells, endogenous immune cells, tumor cells, and other tumor-associated factors. Such knowledge is paramount for enhancing the antitumor effects and minimizing the occurrence of adverse reactions.

Excitingly, recent advancements in genome editing, proteomics, and metabolomics present an opportunity for adopting multilayered approaches that address multiple critical aspects in unison. This multi-faceted strategy holds great promise for further improving CAR T cell therapy. The next generation of CAR T cells must also address practical concerns such as the high cost of treatment and lengthy preparation times. By tackling these issues, accessibility to this groundbreaking therapy can be significantly enhanced, making it more accessible to patients in need.

One significant limitation of current CAR T cell therapy is its potential for severe and occassionally life-threatening side effects, particularly in the form of CRS and neurotoxicity. These adverse events are caused by the robust activation and proliferation of CAR T cells upon encountering their target antigen. Researchers and clinicians are actively working to mitigate these limitations through improved patient selection, improved CAR T cell design, and refined treatment protocols. These challenges still pose significant hurdles in the broader adoption and application of CAR T cell therapy.

Overall, a comprehensive approach that combines cutting-edge technologies with a deep understanding of the complex interactions within the immune-tumor microenvironment holds the key to advancing CAR T cell therapy and unleashing its full potential in the fight against cancer.

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Authors' contributions

RP, AFS, BSS and CSS wrote and prepared the original draft. LW reviewed and edited the manuscript. JS supervised the study and acquired funding. All authors have read and approved the final version of the manuscript. Data sharing is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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