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Chimeric Antigen Receptor T Cells (CAR T-Cells): A New Frontier in Targeted Cancer Therapy

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Abstract

Introduction and Definition: The conventional approaches to cancer treatment—surgery, chemotherapy, and radiation therapy—while effective, often come with significant side effects and limitations, especially in advanced or metastatic disease. This has led to the rise of immunotherapy, a revolutionary approach that leverages the body's immune system to recognize and eliminate cancer cells.

Among immunotherapies, Chimeric Antigen Receptor (CAR) T-cell therapy has emerged as a ground breaking treatment. This therapy involves genetically modifying a patient's own T cells, key components of the adaptive immune response, to specifically target and destroy cancer cells. As a form of personalized medicine, CAR T-cell therapy is transforming cancer treatment by offering a novel, highly targeted strategy, particularly effective in certain hematologic malignancies. It represents a convergence of immunology and genetic engineering, creating T cells that act as potent cancer-killing agents. CAR T-cell therapy modifies T cells in the lab so they can find and destroy cancer cells, effectively turning a patient's T lymphocytes into cancer-fighting machines.

Body: The origins of CAR T-cell therapy trace back over six decades to the exploration of adoptive cell transfer (ACT). Early research focused on utilizing the anti-tumor potential of lymphocytes in animal models. However, a significant obstacle was overcoming the body's natural tolerance to self-antigens, which prevents immune responses against cancer cells that resemble healthy tissues. Advances in genetic engineering have since enabled the modification of T cells to recognize and attack cancer cells effectively. This breakthrough has overcome previous challenges and paved the way for CAR T-cell therapy's clinical success, offering new hope in the fight against cancer.

Keywords: CAR T-cell; immunotherapy; chimeric antigen receptor T-cell product

Introduction and Definition

CAR T-cell therapy, a subset of ACT (adoptive cell transfer). ACT is a form of immunotherapy that involves harvesting a patient's own immune cells, specifically T-cells, which are then modified and expanded in the lab before being reinfused into the patient to fight cancer. It addresses the limitations of conventional and earlier forms of ACT by equipping T-cells with synthetic receptors that combine the specificity of antibodies with the cytotoxic capabilities of T-cells, providing a highly targeted approach to cancer treatment [5]. Antibodies are proteins produced by the immune system to recognize and neutralize foreign substances, while antigens are molecules or parts of molecules that provoke an immune response by being recognized as foreign by the immune system. By modifying a patient's T lymphocytes to express chimeric antigen receptors (CARs), these cells can recognize and eliminate cancer cells more effectively.

CAR T-cells are created through a multi-step process that begins with the collection of the T-cells of a patient via leukapheresis. These T-cells are then genetically modified in the laboratory to express CARs on their surface. CARs are engineered synthetic receptors that consist of an extracellular antigen-recognition domain derived from antibodies, linked to intracellular signalling domains that trigger T-cell activation and proliferation upon antigen binding. This combination allows CAR T-cells to specifically target cancer cells expressing the relevant antigen, while bypassing the need for recognition by major histocompatibility complex (MHC) molecules, which are proteins on the surfaces of cells that present antigens to T-cells, playing a crucial role in the immune system's ability to recognize and respond to pathogens [6].

The manufacturing process involves transducing the T-cells with a viral vector that carries the CAR gene. The transduced T-cells are expanded in culture to increase their numbers and enhance their functionality. The manufacturing process involves genetically modifying the T-cells, referred to as transduction, by using a viral vector that carries the CAR gene. Once a sufficient number of CAR T-cells have been produced, they are infused back into the patient, where they can seek out and destroy cancer cells [7].

The mechanism of action of CAR T-cells involves the binding of the CAR to its target antigen on the surface of cancer cells. This binding activates the T-cell through its intracellular signalling domains, leading to the release of cytotoxic molecules that kill the cancer cells. Additionally, CAR T-cells proliferate upon activation, creating a sustained immune response against the cancer. The unprecedented success of anti-CD19 CAR T-cell therapy, which involves genetically modifying a patients T-cells to target CD19, a protein found on the surface of B-cells, led to its approval by the US Food and Drug Administration (FDA) in 2017. This therapy is important because it offers a highly effective treatment for B-cell malignancies, such as certain leukemias and lymphomas, that were previously difficult to treat. Anti-CD19 CAR T-cell therapy has shown remarkable results in clinical trials, achieving high rates of complete remission in patients who had exhausted other treatment options, thereby revolutionizing the approach to cancer immunotherapy and providing new hope for patients with these aggressive cancers [8].

Body

Historical Development

The conceptual groundwork for CAR T-cell therapy was laid in 1989 by Zelig Eshhar, who developed the first chimeric antigen receptors (CARs) [9]. These early CARs combined the antigen-binding domains of monoclonal antibodies with T-cell receptor signalling domains, creating a synthetic receptor capable of directing T-cell activity towards specific cancer antigens. Initial studies demonstrated the potential of these engineered T-cells to target tumor cells, but they faced significant challenges, including limited persistence, meaning the CAR T-cells did not survive or remain active long enough in the patient's body to provide sustained cancer-fighting effects. Additionally, there was suboptimal anti-tumor activity in vivo, where the CAR T-cells were not as effective at killing cancer cells within the body as they were in laboratory conditions. These limitations highlighted the need for improvements in CAR T-cell durability and functionality to enhance their effectiveness in real-world cancer treatments. [10]

The early 2000s saw significant advancements in CAR T-cell design with the introduction of costimulatory domains such as CD28 and 4-1BB. These costimulatory domains are protein regions that enhance T-cell activation and persistence. CD28 and 4-1BB are critical for providing the necessary secondary signals that promote T-cell proliferation, survival, and cytokine production, thereby improving the overall efficacy and longevity of CAR T-cell therapies. These domains enhanced T-cell activation, proliferation, and survival, addressing the earlier limitations and leading to the development of second-generation CARs [11]. The incorporation of these costimulatory signals was pivotal in improving the clinical efficacy and durability of CAR T-cell responses.

A major breakthrough occurred with the development of anti-CD19 CAR T-cells, which target the CD19 antigen present on B-cells. In 2010, the first successful clinical trials of anti-CD19 CAR T-cell therapy were conducted by Carl June and his team at the University of Pennsylvania. These trials demonstrated remarkable efficacy in treating B-cell malignancies, including chronic lymphocytic leukaemia (CLL) and acute lymphoblastic leukaemia (ALL) [12]. The unprecedented success of these trials led to the approval of the first CAR T-cell therapy, Kymriah (tisagenlecleucel), by the U.S. Food and Drug Administration (FDA) in 2017 for the treatment of relapsed or refractory B-cell precursor ALL in children and young adults [13].

Following the approval of Kymriah, another significant milestone was reached with the approval of Yescarta (Axicabtagene ciloleucel) in 2017 for the treatment of relapsed or refractory large B-cell lymphoma in adults [14]. These approvals marked the beginning of a new era in cancer treatment, validating the potential of CAR T-cell therapy as a transformative approach to immunotherapy.

The evolution of CAR T-cell therapy continued with ongoing research aimed at improving the safety and efficacy of the treatment. Third-generation CARs, which incorporate multiple costimulatory domains, enhance T-cell activation and persistence, allowing them to remain functional for longer periods and improving their ability to sustain an anti-tumor response. Fourth-generation CARs, known as T-cells Redirected for Universal Cytokine-mediated Killing (TRUCKs), deliver cytokines directly into the tumor microenvironment. This helps counteract the immunosuppressive environment created by tumors, allowing the CAR T-cells to function more effectively within hostile conditions [15]. These advancements address challenges such as antigen escape, where tumors evade detection by losing the targeted antigen, by enabling T-cells to maintain their activity through enhanced stimulation. They also reduce off-target effects by improving the precision of T-cell activity, making the therapy safer by minimizing damage to healthy tissues.

Fundamental Concepts

CAR T-cell therapy represents an approach in the treatment of cancer, harnessing the body's immune system to target and destroy malignant cells. The core concept of CAR T therapy revolves around chimeric antigen receptors (CARs), which are synthetic constructs that equip T-cells

with the ability to recognize specific antigens on the surface of tumour cells. This capability is crucial as it allows for the direct targeting of cancer cells while sparing healthy tissue.

The fundamental mechanism of CAR T-cell therapy involves several key steps. First, T-cells are extracted from the patient's blood and then genetically engineered in the laboratory to express CARs on their surface. These receptors are designed to bind to a specific protein, or antigen, that is found on cancer cells. This process of genetic modification typically utilises viral vectors to insert the CAR gene into the T-cells, thereby enabling them to recognize and attach to the target antigen [16].

Once engineered, the CAR T-cells are expanded in the laboratory to create a large population and then reintroduced into the bloodstream of the patient. Upon infusion, these modified T-cells circulate throughout the body, identifying and attaching to cancer cells that express the target antigen. Binding of the CAR to the antigen triggers a cascade of internal signals in the T-cells, activating them to attack and kill the cancer cells. This activation also promotes the proliferation and survival of CAR T-cells, enhancing their therapeutic efficacy [17].

A critical aspect of CAR T therapy is the selection of target antigens. The effectiveness of this treatment largely depends on the presence of antigens that are highly expressed on cancer cells but have limited or no expression on healthy cells. These target antigens are typically tumor-associated antigens (TAAs) or tumor-specific antigens (TSAs). TAAs, such as CD19 in B-cell malignancies, are proteins that are overexpressed in cancer cells but also found at lower levels in normal cells. TSAs, on the other hand, are unique to cancer cells and result from mutations in tumor DNA. This selective targeting of TAAs and TSAs helps minimize side effects associated with the therapy, making it a potent option for treating various hematological cancers and, potentially, solid tumors [18].

Recent Developments

CAR T-cell therapy has emerged as a new approach in cancer treatment, particularly for haematological malignancies. However, extending its success to solid tumors remains a challenge due to the complex tumor microenvironment. For example, solid tumors often create physical barriers, such as dense stromal tissue, that prevent CAR T-cells from effectively infiltrating the tumor. Additionally, tumor cells in solid tumors can secrete immunosuppressive molecules, such as TGF-β, which inhibit the activity of CAR T-cells and reduce their effectiveness. These factors make it more difficult for CAR T-cells to penetrate, survive, and function within solid tumors compared to blood cancers thereby enabling them to recognize and attach to the target antigen as seen in Figure 1 [16]. Emerging advancements in CAR T-cell therapy encompass a variety of innovative approaches aimed at overcoming these limitations and broadening its therapeutic potential.

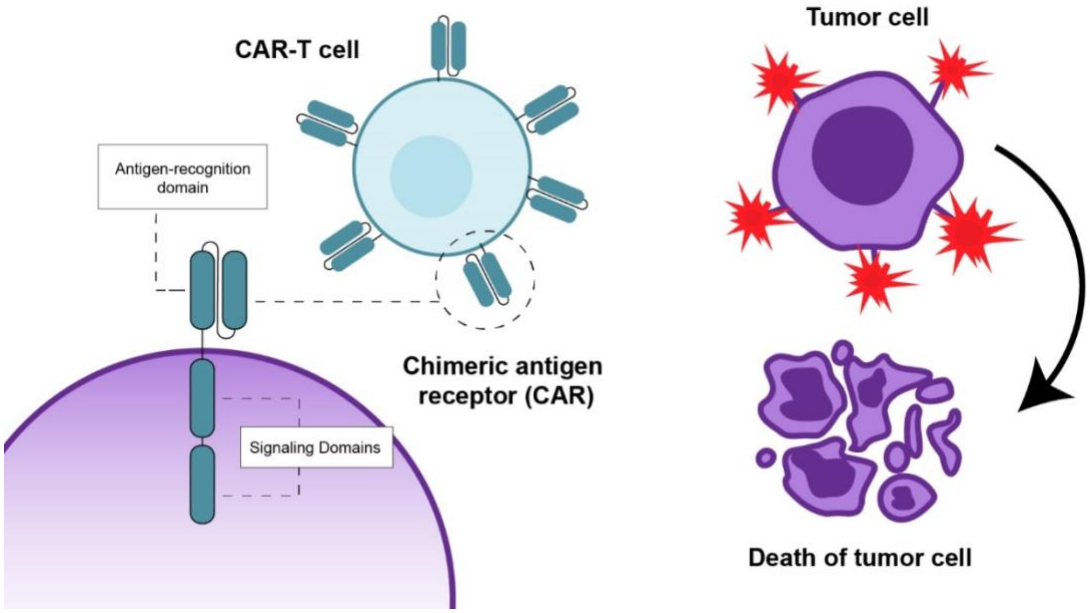

Figure 1. Illustration of CAR T-cell Mechanism on Tumour Cell. Figure was created by our medical illustrator, Joseph Liao, using Canva.

One significant development is the creation of dual-targeting CARs, which recognize two distinct antigens on cancer cells. This enhanced specificity improves treatment precision, minimises off-target effects, and reduces the likelihood of tumour escape variants [19]. Additionally, research is exploring the use of gene-editing technologies

like CRISPR to enhance CAR T-cell functionality and persistence. CRISPR can be used to knock out inhibitory genes or introduce genes that boost anti-tumor activity, paving the way for more effective and durable CAR T-therapies with fewer side effects [20].

Another promising area of research involves the development of allogeneic CAR T-cells derived from healthy donors. They are obtained from peripheral blood like umbilical cord blood or derived from induced pluripotent stem cells. These "off-the-shelf" products offer advantages such as standardized quality, reduced production time and cost, and improved accessibility for patients [21]. Additionally, advancements in bioprocessing technologies, including automated cell culture systems and optimized protocols for T-cell expansion, are being developed to ensure higher efficiency and scalability in CAR T-cell manufacturing, meeting the increasing demand for these therapies. For example, the CliniMACS Prodigy® system automates the process of cell separation, activation, transduction, and expansion, significantly reducing manual labor and contamination risks. Research by Mock et al. (2016) demonstrated that using this system resulted in consistent and high-quality CAR T-cell products. Moreover, studies have shown that optimized T-cell expansion protocols, such as those involving the use of IL-2 and IL-15 cytokines, enhance the proliferation and persistence of CAR T-cells, leading to improved therapeutic outcomes.

Furthermore, research is investigating the combination of CAR T-cell therapy with complementary treatments like checkpoint inhibitors, which are drugs that help to release the brakes on the immune system, allowing it to attack cancer cells more effectively, and oncolytic viruses, which are viruses that selectively infect and kill cancer cells while stimulating an anti-tumor immune response. These combination therapies aim to overcome the immuno suppressive tumour microenvironment and enhance the overall anti-tumor response in solid tumours. Early results from these studies suggest promising potential for improving patient outcomes.

Finally, there is growing interest in the use of CAR T-cells for the treatment of non-cancerous conditions, such as autoimmune diseases and chronic infections. Early research is exploring the potential for CAR T-cells to selectively target immune cells that drive autoimmune conditions, such as in systemic lupus erythematosus (SLE), where modified CAR T-cells are designed to eliminate B-cells producing harmful autoantibodies. In chronic infections, researchers are investigating how CAR T-cells could be engineered to target infected cells, such as those harboring HIV, by attacking viral reservoirs that are resistant to standard therapies. While this research is still in its early stages, it holds promise for expanding the applications of CAR T-cell therapy beyond oncology, offering potential new treatment avenues for these challenging diseases.

List of Abbreviations
ACT: adoptive cell transfer
ALL: acute lymphoblastic leukaemia
CAR: chimeric antigen receptor
CLL: chronic lymphocytic leukemia
CRISPR: clustered regularly interspaced short palindromic sequences
FDA: food and drug Administration
MHC: major histocompatibility complex
TRUCK: T cells redirected for universal cytokine-mediated killing

Conflicts of Interest
The author(s) declare that they have no conflict of interests.

Authors' Contributions
DW: Contributed to the conceptualization of the study, conducted the literature review, drafted the initial manuscript, participated in formatting and editing, and gave final approval of the version to be published.
HW: Assisted in the literature review, contributed to writing the manuscript, involved in formatting and revising the content for important intellectual input, and gave final approval of the version to be published.
KR: Participated in the research and data analysis, contributed to writing and reviewing the manuscript, and was responsible for the final formatting and proofreading, and gave final approval of the version to be published.

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