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CANCER IMMUNOTHERAPY: CHIMERIC ANTIGEN RECEPTOR (CAR)-T CELL THERAPY

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Abstract

Cancer remains one of the leading causes of morbidity and mortality worldwide, despite significant advances in conventional treatment modalities such as surgery, chemotherapy, and radiotherapy. In recent years, cancer immunotherapy has emerged as a transformative approach by harnessing the body's immune system to recognize and eliminate malignant cells. Among these strategies, Chimeric Antigen Receptor T-cell (CAR-T) therapy has demonstrated remarkable clinical success, particularly in the treatment of hematological malignancies, including acute lymphoblastic leukemia, diffuse large B-cell lymphoma, and multiple myeloma. CAR-T cell therapy involves the genetic modification.

Cancer still continues to be a leading cause of morbidity and mortality across the globe, despite the considerable developments in the field of traditional forms of cancer treatment, including surgical methods, chemotherapy, and radiation. Recently, a revolutionary method for cancer treatment has appeared, namely cancer immunotherapy, which is based on the stimulation of the patient's immune system to fight against malignant cells. Among the different immunotherapy techniques, Chimeric Antigen Receptor T-cell (CAR-T) therapy has shown great results in the treatment of hematological cancers, such as acute lymphoblastic leukemia, diffuse large B-cell lymphoma, and multiple myeloma. CAR-T cell therapy includes genetic engineering of a patient's T lymphocytes so that they can express receptors capable of binding with specific tumor markers without MHC presentation. The present paper discusses CAR-T cell therapy comprehensively.

The development of CAR-T cell generations is also reviewed, starting from the first generation of CARs to the fifth generation with increased efficiency and persistence. Moreover, the difficulties involved in CAR-T cell therapy are mentioned in the review, such as cytokine release syndrome, immune effector cell-associated neurotoxicity syndrome, antigen escape, inefficiency against solid tumors, manufacturing complications, and expensive therapy. Modern developments, such as gene editing, armored CAR-T cells, dual target CAR-T cells, allogenic off-the-shelf CAR-T cells, and combination therapies are covered in the review. These developments have contributed greatly to improving the therapeutic potential of CAR-T cells.

Key words

Activation of T cell, T-cell proliferation, CD19, BCMA, Immunotherapy, Neurotoxicity (ICANS), Immune Memory.

1 Introduction

Cancer is a broad group of diseases caused by the unchecked growth and multiplication of abnormal cells which invade nearby tissues and spread to other parts of the body via blood and lymph systems called metastasis. In a

healthy body, the growth, reproduction, and death of the body cells are controlled by some processes and regulations. Mutations in genes controlling these processes (oncogene, tumor suppressor gene, and DNA repair genes) can affect the balance of the body, leading to cancer development.

Cancer is one of the greatest problems of public health and causes many deaths globally. According to WHO data, there were almost ten million people who died due to cancer in 2022 and it is considered one of the leading causes of deaths worldwide. The most common types of cancer are breast, lung, colorectal, prostate, stomach, and liver cancers. There are many reasons of cancer development, such as smoking, drinking alcohol, eating unhealthy food, obesity, lack of physical activity, chronic infections, pollution, radiation, and hereditary mutations (Hanahan et al., 2022).

1.1 Over view of cancer

Traditional forms of cancer treatment techniques like surgery, chemotherapy, radiation therapy, hormone therapy, and target therapy have been greatly beneficial for increasing the lifespan of patients. Nevertheless, these types of therapies come with several drawbacks such as non-specific toxicity, unwanted side effects, resistance to medication, reoccurrence of the tumor, and ineffectiveness against advanced-stage or metastatic cancer. As a result of this, novel methods of treatments have been developed (Fesnak et al., 2016).

1.2 Burden of Cancer Globally

The development of cancer poses one of the biggest threats to global health care, its rate being constantly growing because of population growth, aging, urbanization, and lifestyle modification. It is a problem affecting all age groups, gender, and socioeconomic groups, thus creating immense pressure on the health care system and the national budget. In 2022 according to the Global Cancer Observatory (GLOBOCAN), there were 20 million newly detected cancer patients and 9.7 million cancer deaths around the world. The prediction is that nearly one out of five people would develop cancer in his or her life, and one in nine men and one in twelve women dies from the disease (Bray et al., 2024).

1.3 Need for Novel Immune therapies

Despite significant progress in cancer diagnosis and treatment, cancer still ranks as a leading cause of illness and death worldwide. Traditional treatments, including surgery, chemotherapy, radiotherapy, hormonal therapy, and targeted therapy, have improved patient outcomes for many types of cancer. However, these methods often have serious limitations, such as non-specific toxicity, severe side effects, treatment resistance, tumor recurrence, and limited effectiveness against metastatic or advanced-stage cancers. Additionally, many patients experience a return of the disease after initial treatment because resistant cancer cells survive and tumors find ways to avoid detection by the immune system (Fesnak et al., 2016).

1.4 Evolution of CAR-T Cell Therapy

The development of Chimeric Antigen Receptor T-cell (CAR-T) therapy is one of the biggest breakthroughs in cancer immunotherapy. Researchers first explored the idea of genetically engineering T lymphocytes to find and destroy tumor cells in the late 1980s and early 1990s. They aimed to overcome the limits of the natural T-cell receptor (TCR), which needs antigen presentation through the major histocompatibility complex (MHC). Tumor cells often avoid immune detection by reducing MHC molecules, which makes traditional T-cell responses less effective. CAR technology was created to get around this issue by allowing T cells to recognize tumor-associated antigens directly, without relying on MHC presentation (Kalos et al., 2013).

2 The Human Immune System and Cancer

The human immune system is a complex network of cells, tissues, organs, and signaling molecules that protects the body from infections, abnormal cells, and cancer. It is divided into the innate immune system and the adaptive immune system. The innate immune system offers the first line of defense with physical barriers, phagocytic cells like macrophages and neutrophils, dendritic cells, natural killer (NK) cells, and soluble mediators such as cytokines and complement proteins. In contrast, the adaptive immune system provides specific and long-lasting immunity by activating B lymphocytes and T lymphocytes. Together, these two systems help maintain immune stability and eliminate cells that show abnormal or cancerous traits (Abbas et al., 2021).

2.1 Immune Surveillance

Immune surveillance is defined as the activity of the immune system monitoring the body continuously to find and destroy abnormal, infected, or mutated cells that would otherwise progress into detectable cancers. First postulated by Burnet and Thomas in the middle of the last century, the idea states that the immune system is an essential part of cancer prevention due to its ability to recognize specific antigens of the tumor present on its surface. Immune surveillance is normally responsible for preserving homeostasis in tissues via destruction of potential tumor cells with involvement of both innate and adaptive immune systems (Dunn et al., 2004).

2.2 Escape from the Immune System of Cancer Cells

Even though the immune system has the ability to recognize and kill transformed cancer cells through the immune surveillance process, some cancer cells can develop various escape mechanisms to avoid immune recognition and killing. The escape of cancer cells from the immune system is referred to as immune escape of cancer cells and allows cancer cells to survive, grow, and metastasize even when there are functional immune cells. The escape of cancer cells from the immune system is one of the characteristics of cancer and poses great challenges in developing effective treatment for cancer (Hanahan et al., 2022).

2.3 Function of T Lymphocytes in Cancer

T lymphocytes (T cells) are the primary cells involved in the response of the adaptive immune system. Their role is to protect the body from pathogens and cancer. They arise from the hematopoietic progenitor stem cells that reside in the bone marrow and develop in the thymus after undergoing positive and negative selection. The role of T lymphocytes in immunity is recognition of peptide antigens presented by the major histocompatibility complex (MHC) using T cell receptors (TCRs). In the context of cancer, T lymphocytes play an important role in immune surveillance and elimination of the cancer cells (Abbas et al., 2021).

3. Cancer Immunotherapy

Cancer immunotherapy is a new type of treatment in which the body’s own immune system is harnessed and boosted to recognize and destroy cancer cells. While traditional treatments like surgery, radiation therapy, and chemotherapy are directly focused on the tumor cells, immunotherapy focuses on boosting the body’s natural defense system against the disease. The human body is inherently equipped to recognize and attack unusual cells; however, cancer cells have evolved ways to escape from the body’s defenses and form an immunosuppressive microenvironment around themselves (Hanahan, et al., 2022).

3.1 Definition of Cancer Immunotherapy

Cancer immunotherapy is a type of cancer treatment that harnesses and potentiates the body's own immune system to recognize, target, and eliminate tumor cells. Unlike conventional methods of cancer therapy, such as surgery, radiation therapy, and chemotherapy, that aim to remove or destroy cancer cells directly, cancer immunotherapy involves modulating immune cells to fight tumors. This modulation leads to an attack that specifically targets the

cancer while causing little harm to healthy tissues. Tumors evade immune attack by suppressing immune responses (Abbas et al., 2021).

3.2 Types of cancer immunotherapy

Cancer immunotherapy includes a variety of therapeutic strategies that stimulate or boost the immune system's capacity to recognize and kill cancerous cells. Rather than destroying tumors directly, immunotherapies awaken an immune response, overcome tumor-imposed suppression of the immune system, and provide long-term protection by inducing immunologic memory. Several forms of cancer immunotherapy are immune Checkpoint Inhibitors Immune checkpoint inhibitors are monoclonal antibodies which bind to receptors that are part of a negative signaling pathway that helps turn off immune cells. For example, PD-1, PD-L1, and CTLA-4 pathways typically help to maintain homeostasis and tolerance and suppress immune responses when needed to prevent the immune system from destroying normal tissue (Sharma & Allison, et al., 2015).

3.3 Monoclonal antibodies

Monoclonal antibodies (mAbs) are laboratory-produced antibodies which specifically recognize and bind to certain antigens displayed on cancer cells. The introduction of monoclonal antibodies in the field of clinical oncology heralded a breakthrough in cancer immunotherapy due to their ability to target malignant cells specifically while largely preserving normal cells. Monoclonal antibodies are derived from a single clone of B lymphocytes by utilizing recombinant DNA and hybridoma technology and they bind to the same epitope on a target antigen (Rosenberg et al., 2015).

3.4 Cancer Vaccines

A cancer vaccine is a type of immunotherapy aimed to trigger the body's immune system to recognize, target, and eradicate cancer cells by eliciting responses against tumour-associated antigens (TAAs) or tumor-specific antigens (TSAs). Unlike prophylactic vaccines used to prevent infectious diseases by provoking immunity against pathogens, cancer vaccines are used to prevent virus-induced cancers or treat existing cancers by boosting anti-tumour immune responses. Cancer vaccines represent a key component of precision medicine as they are able to evoke very specific immune responses, while minimizing the collateral damage to normal tissues. Cancer vaccines can be categorized as prophylactic (preventive) or therapeutic cancer vaccines (Russell et al., 2012).

3.5 Cytokine Therapy

Cytokine therapy was among the earliest approaches to cancer immunotherapy and involves administration of naturally occurring or recombinant cytokines to stimulate the immune system to combat malignant cells. Cytokines are small protein signaling molecules secreted by immune as well as non-immune cells that modulate cell proliferation, differentiation, activation, migration and survival. They are essential to the orchestration of innate and adaptive immunity by acting as the key communication molecules of immune cells. Unfortunately, treatment is generally associated with flu-like symptoms, depression, fatigue, myelosuppression and hepatotoxicity, making it poorly tolerated. Other cytokines have been explored for potential use in experimental and clinical trials. Interleukin-12 (IL-12) induces proliferation and activation of NK cells and cytotoxic T cells and production of interferon-gamma (IFN-) (Waldmann et al., 2018).

3.6 Adoptive Cell Therapy (ACT)

Adoptive cell therapy (ACT) represents an advanced type of cancer immunotherapy, where the patient's or a donor's immune cells are retrieved, modified or genetically enhanced outside the body in vitro, and then reinfused into the patient to strengthen anti-tumor immunity. This type of therapy relies on the premise that potent tumor-destroying immune cells can be selectively targeted to improve their persistence, specificity, and cytotoxic activity. Unlike

traditional methods of cancer therapy that directly target cancer cells, ACT utilizes living immune cells to attack cancer, and holds promise for lasting immunity and enduring clinical benefits. The process ofACT entails multiple sequential steps (June et al., 2018).

4. Chimeric Antigen Receptor (CAR)-T Cell Therapy

Chimeric Antigen Receptor (CAR)-T cell therapy is a novel adoptive cellular immunotherapy that involves genetically modifying a patient's own T lymphocytes to express synthetic receptors, called chimeric antigen receptors (CARs). The CARs endow the T cells with the ability to recognize and kill cancer cells specifically, without dependence on major histocompatibility complex (MHC) antigen presentation. This distinctive ability overcomes a primary mechanism of tumor immune escape, establishing CAR-T therapy as one of the most profound developments in modern oncology (June et al., 2018).

4.1 Definition of Chimeric Antigen Receptor (CAR)-T cell Therapy

Chimeric Antigen Receptor (CAR)-T cell therapy is a personalized form of adoptive cellular immunotherapy where patients' T lymphocytes are genetically engineered in vitro to express synthetic receptors called chimeric antigen receptors (CARs) capable of mediating antigen-specific targeting and elimination of cancer cells independent of MHC. The basic structure of the chimeric antigen receptor consists of an extracellular antigen binding domain, commonly the single chain variable region fragment (scFv) of a murine monoclonal antibody; a hinge domain that provides space for positioning the antigen-binding domain; a transmembrane domain that inserts the CAR into the T cell membrane; and intracellular signaling domains including CD3 zeta chain and one or more costimulatory motifs such as 4-1BB and CD28 that promote T cell activation, expansion, survival and persistence (Kalos & June, et al., 2013).

4.2 History of CAR-T Cell Therapy

The history of CAR-T cell therapy began in 1987, when Yoshihisa Kuwana and his colleagues developed the first chimeric antigen receptor (CAR) by combining an antibody-binding domain with a T-cell signaling domain. In 1989, Zelig Eshhar and Gideon Gross further advanced this technology and introduced the term "Chimeric Antigen Receptor (CAR)." Later, Carl H. June and his team demonstrated the first successful clinical application of CAR-T cell therapy in leukemia patients in 2010–2011. Finally, in 2017, the U.S. Food and Drug Administration approved the first CAR-T cell therapy, Tisagenlecleucel (Kymriah), marking a major breakthrough in cancer immunotherapy. This concept was born from the recognition that tumor immune escape often relies on the downregulation of MHC expression to circumvent endogenous T-cell recognition(Eshhar et al., 1993).

4.3 The Basic Principle of Chimeric Antigen Receptor (CAR)-T Cell Therapy

The basic principle of CAR-T cell therapy is to genetically engineer a patient's T lymphocytes to allow for specific and enhanced recognition of tumor cells. While natural T cells recognize tumors via an antigen-presentation mechanism requiring major histocompatibility complex (MHC) presentation, CAR-T cells engage tumor-associated antigens on the cell surface via an artificial receptor independent of MHC antigen presentation. Thus, CAR-T cells can circumvent MHC-independent immune evasion and demonstrate profound activity against a number of hematologic cancers. CAR-T cell therapy involves obtaining peripheral blood mononuclear cells from the patient via leukapheresis and subsequently isolating and transducing their T lymphocytes with an viral vector encoding a chimeric antigen receptor (CAR) (Maude et al., 2018).

5 Structure of the Chimeric Antigen Receptor (CAR)

The Chimeric Antigen Receptor (CAR) is a bioengineered T cell receptor, that empowers T cells to identify and eradicate tumors. It incorporates features of both antibody specificity and T-cell cytotoxicity to identify tumour-

specific antigens on cancer cells independently of the major histocompatibility complex (MHC). CAR construction design determines T cell proliferation, persistence, and the expression of functions that contribute to tumor clearance. It typically consists of four major components: (1) an extracellular antigen-binding domain, (2) a spacer/hinge domain, (3) a transmembrane domain, and (4) intracellular signaling motifs (Sadelain et al., 2013).

5.1 Antigen Recognition Domain (Single-Chain Variable Fragment, scFv)

The antigen recognition domain, which is the extracellular part of the Chimeric Antigen Receptor (CAR), recognizes tumor-associated antigens expressed on the surface of tumor cells in a highly specific manner. It is the most crucial element of CAR because it dictates the specificity, sensitivity and efficacy of CAR-T cells. The most common form of the antigen recognition domain used in CAR design is the single-chain variable fragment (scFv), generated from the variable regions of the heavy (VH) and light (VL) chains of a monoclonal antibody (Majzner & Mackall et al., 2018).

5.2 Hinge (Spacer) Region

The hinge (spacer) region is a key component of the Chimeric Antigen Receptor (CAR) that connects the extracellular antigen recognition site (single-chain variable fragment, scFv) to the transmembrane domain. While not directly involved in antigen binding or intracellular signaling, the hinge region critically affects the flexibility, accessibility, stability, and overall functionality of the CAR-T cell. The length, sequence composition, and structural properties of the hinge significantly influence CAR-T cell activation, immune synapse formation, and antitumor responses (Guedan et al., 2019).

5.3 Transmembrane Domain

The transmembrane domain is an essential part of the CAR structure, responsible for tethering the receptor into the plasma membrane of the modified T cell. Bridging the extracellular hinge region (spacer) and the intracellular signaling modules, it provides structural support and ensures that antigen recognition at the extracellular face of the cell leads to the transmission of activation signals to the interior of the cell. Despite its relatively small size, the transmembrane domain plays a vital role in modulating the expression level of the CAR, the receptor's stability, its signaling capabilities, and, ultimately, its clinical efficacy(Bridgeman et al., 2014).

5.4 Intracellular Signalling Domain

The intracellular signalling domain is the powerhouse of the Chimeric Antigen Receptor (CAR), and is responsible for relaying the signal of activation to the genetically engineered T cell after it has recognised its target antigen. Following antigen recognition by the extracellular domain of the CAR (usually a single-chain variable fragment, scFv) binding to a tumor-associated antigen, the intracellular signalling domain transmits a cascade of molecular events that ultimately results in T cell activation, proliferation, secretion of cytokines, cytotoxic effectors, and the establishment of long-lasting immune memory. The architecture of this component largely dictates the potency, persistence and efficacy of CAR-T cell therapy (June et al., 2018).

5.5 Costimulatory Domains (CD28 and 4-1BB)

Costimulatory domains are crucial elements in modern Chimeric Antigen Receptor (CAR) constructs that enable robust T cell activation, proliferation, persistence and anti-tumor effects. In normal T-cell biology, a complete activation requires the delivery of two signals. Signal 1 arises from the engagement of the T-cell receptor (TCR) with the peptide-MHC complex, and Signal 2 is generated by co-stimulatory molecule-antigenic receptor pairs such as CD28-B7 molecules interaction on antigen-presenting cells. Failure of the second signal results inT cell anergy, apoptosis or unresponsiveness to the antigen (June et al., 2018).

6 Generation of CAR-T Cells

Generating Chimeric Antigen Receptor (CAR)-T cells is a complicated multi-step process that requires collecting a patient's T cells, genetically modifying them to become the special CAR-T cells, growing them in quantity in the lab, and then putting them back into the patient to kill cancer. All of these steps have to take place in a good manufacturing practice (GMP) facility to ensure the product is safe, sterile, and works (Tokarew et al., 2019).

6.1 First Generation CAR-T Cells

The earliest form of gene-engineered T cell-based therapy for cancer, the first-generation CAR-T cells, established the basis for modern CAR-T cell immunotherapy. These CARs, which were designed during the late 1980s and early 1990s after Zelig Eshhar and colleagues developed the concept of the chimeric T cell receptor to merge the antigen specificity of the monoclonal antibody with the cytotoxic capacity of T cells, were intended to recognize tumor-associated antigens regardless of MHC to overcome the prevalent tumor immune escape mechanism (Tokarew et al., 2019).

6.2 Second-Generation CAR-T Cells

The advent of second-generation Chimeric Antigen Receptor (CAR)-T cells is a major development in cancer immunotherapy and forms the basis of all current CAR-T cell therapies. Second-generation CAR-T cells were developed to improve upon the shortcomings of their first-generation counterparts, which, with only the CD3 signaling domain, failed to efficiently proliferate, persist long-term, and produce sufficient quantities of cytokines to achieve clinically relevant efficacy. The incorporation of a single costimulatory molecule, such as CD28 or 4-1BB (CD137), in conjunction with the CD3 activation domain significantly enhanced CAR-T cell activation, survival, and sustained antitumor efficacy (June et al., 2018).

6.3 Third-generation CAR-T Cells

To further enhance the therapeutic activity of second-generation CAR-T cells, third-generation CAR-T cells were created to include two intracellular costimulatory domains in addition to the CD3 activation domain. While second-generation CARs have one costimulatory domain, third-generation CARs express two costimulatory signals-commonly CD28 and 4-1BB (CD137) or CD28 and OX40 (CD134)- to bolster T cell activation, proliferation, persistence, cytokine secretion and antitumor immunity long term. This design strategy aims to take advantage of the differential but synergistic biological activities of the co-stimulatory pathways to develop a more effective immune response (Tokarew et al., 2019).

6.4 Fourth-Generation CAR-T Cells (TRUCKs)

Fourth generation Chimeric Antigen Receptor (CAR)-T cells, also known as TRUCKs (T cells Redirected for Universal Cytokine-mediated Killing), are an improved version of CAR-T cells designed to address the limitations of the first- and second-generation CAR-T cells, specifically in targeting solid tumors. The first and second generation CAR-T cells have proven successful in treating hematological malignancies but have had limited success against solid tumors due to low T-cell infiltration, tumor heterogeneity, and an immunosuppressive tumor microenvironment (TME). Fourth generation CAR-T cells are engineered to enhance targeted tumor cell elimination by simultaneously delivering a broad range of localized cytokine signals. The structural organization of fourth-generation CAR-T cells are based on that of the second generation CAR (Sterner & Sterner, et al., 2021).

6.5 Fifth-generation CAR-T Cells Fifth-generation

Chimeric Antigen Receptor (CAR)-T cells are the most advanced development of the CAR-T cell technology. This next generation of CAR-T cells were engineered to address some of the limitations of earlier generation CAR-T

cells such as limited persistence, T-cell exhaustion, antigen escape, and poor efficacy against solid tumors. The next-generation CARs combine benefits of the second- and fourth-generation CAR-T cells with additional intracellular signaling domains that better replicate the physiologic signaling of T lymphocyte activation. Their aim is to elicit potent and long-lasting highly regulated tumor immune responses that improve efficacy and safety (Hartmann et al., 2017).

7 Process of CAR-T cell Production in Chimeric Antigen Receptor (CAR)-T cell therapy

CAR-T cell production process is a complex process and it means the patients' own T lymphocytes are modified with genetically engineering immune cells, which can identify and kill cancer cells. This is the critical process in the therapy of CAR-T cell therapy and it is divided into several steps such as collecting of T-cell, activation of T-cell, gene editing of T-cell, expansion of cells, quality testing, infusion to patient. Since CAR-T cell is personalized therapy, each product of CAR-T cell must be produced only for one patient (Neagu & Constantin, et al., 2024).(Figure.1).

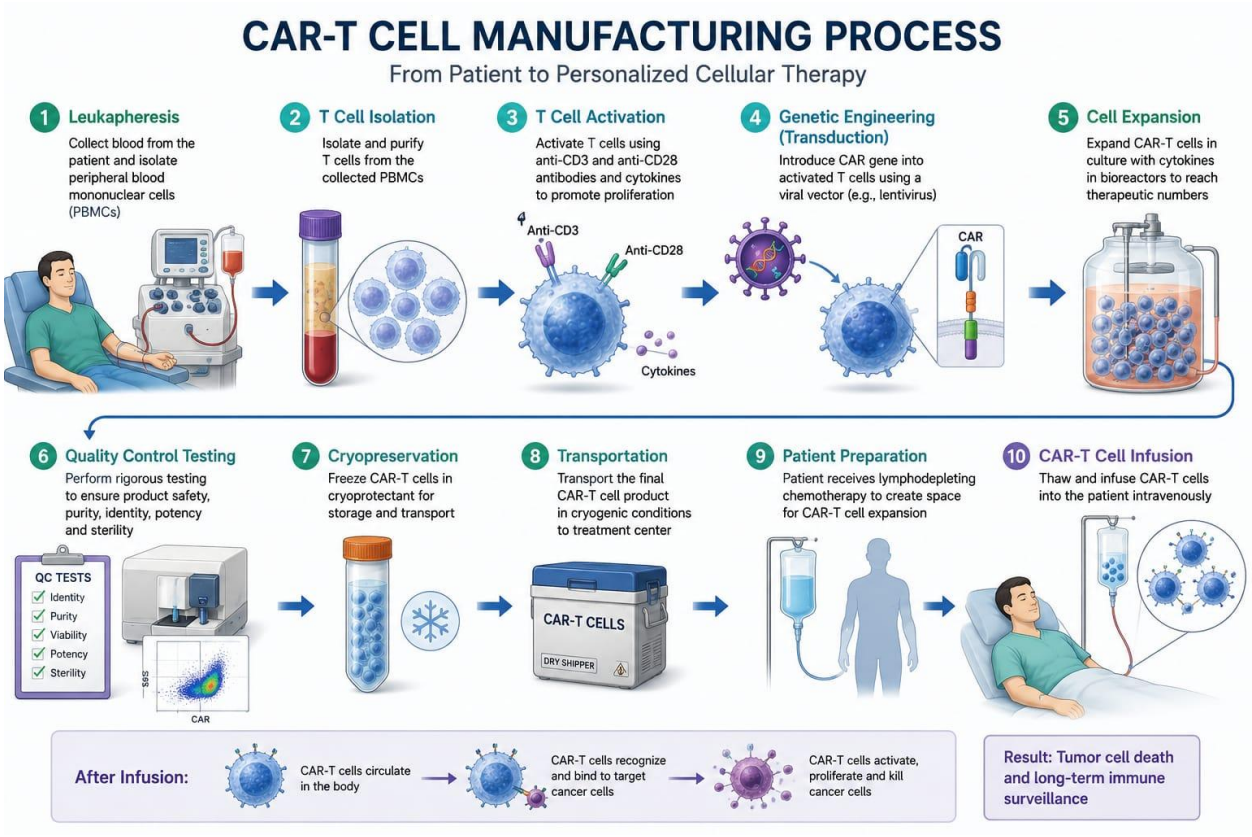


Figure.1 CAR-T cell manufacturing process.

7.1 Leukapheresis in Chimeric Antigen Receptor (CAR)-T CellTherapy

Leukapheresis is the first and arguably the most crucial step in the process of Chimeric Antigen Receptor (CAR)-T cell therapy. Leukapheresis is a medical procedure in which T-cells, a type of white blood cell that play a critical role in the immune system, are separated from the blood. For CAR-T therapy, the patient's own T-cells are used, making leukapheresis an essential preliminary procedure. Leukapheresis not only helps to secure sufficient numbers of viable T-cells needed for manufacturing but also determines the quality of CAR-T-cells which directly impact

therapeutic efficacy. Fortunately, this process is generally safe and minimally invasive, resulting in a good safety profile and tolerability in most patients (Syrigou et al., 2023).

7.2 T-Cell Isolation in Chimeric Antigen Receptor (CAR)-T Cell Therapy

T-cell isolation is a pivotal manufacturing stage in the production of Chimeric Antigen Receptor (CAR)-T cell therapy. This process commences following the leukapheresis procedure, where a patient's peripheral blood mononuclear cells (PBMCs) are gathered from their blood sample. The purpose of T-cell isolation is to procure T lymphocytes from a diverse collection of blood cells to genetically engineer them for CAR expression. Since CAR-T cell therapy hinges on the viability of these T cells, a pure and high-quality T-cell collection is imperative for successful therapy development. The isolated T cells will later undergo gene insertion, multiplication, and are intended to eliminate cancer cells following administration (Neagu & Constantin, et al., 2024).

7.3 Methods for gene transfer in chimeric antigen receptor (CAR)-T cell therapy

Gene transfer is an essential step in the manufacturing process of Chimeric Antigen Receptor (CAR)-T cells. Through gene transfer, CAR gene is introduced into T cells collected from the patient, which will reprogram the cells to recognize and destroy cancer cells expressing tumor-specific antigen. The efficacy, stability and safety of the gene transfer method used are key factors in determining the success of CAR-T cell therapy. Multiple gene delivery methods have been explored including viral vectors and non-viral vectors, each with its own advantages and disadvantages (Neagu & Constantin, et al., 2024).

7.4 Cell Culture & Expansion in Chimeric Antigen Receptor (CAR)-TCell Therapy

Cell culture and expansion are a crucial part of the manufacturing process for chimeric antigen receptor (CAR)-T cell therapy. Once a patient's T cells are isolated from their blood and modified to express chimeric antigen receptors, they need to be grown in a lab and expanded in vitro until a sufficient amount of therapeutic cells can be generated for treatment in the clinic. Given the relatively small number of T cells collected in leukapheresis and introduced to a gene transfer protocol, an immense expansion process is needed to grow enoughCAR-T cells – in the millions to billions – that are needed to elicit a successful anti-tumor response upon infusion into the patient. The quality and number of cells produced in the expansion phase determine how well the CAR-T treatment is likely to work, how long it will last, and whether it's safe for the patient (Neagu & Constantin, et al., 2024).

7.5 Quality Control in Chimeric Antigen Receptor (CAR)-T Cell Therapy

Quality control (QC) is a fundamental element in the production of Chimeric Antigen Receptor (CAR)-T cell therapy. It involves testing to ensure the safety, efficacy, sterility, and usability of the final CAR-T cell product. Since CAR-T therapy involves the genetic manipulation of live cells and their subsequent expansion, strict quality control procedures are implemented throughout the manufacturing process to ensure product uniformity and regulatory compliance. The overarching goal of quality control in CAR-T cell therapy is to ascertain that the engineered cells exhibit the desired biological properties and effectively target and destroy cancer cells while minimizing the risk of adverse events (Bishop et al., 2025).

7.6 Cryopreservation in CAR-T Cell Therapy

Cryopreservation plays a crucial role in the manufacturing and clinical delivery of Chimeric Antigen Receptor (CAR)-T cell therapy, which involves genetically engineered T cells that recognize and target tumor cells. This process preserves the CAR-T cells at ultra-low temperatures, maintaining their viability, function, and therapeutic efficacy until they are needed for a patient's treatment. Due to the complexity of the CAR-T cell manufacturing process, which can take several weeks, cryopreservation provides a reliable way to store and ship the final cellular product without compromising the biological activity of the cells. This allows for flexibility in scheduling treatments

and shipping CAR-T cell products from specialized manufacturing facilities to clinical centers worldwide (Wei et al., 2024).

8 Applications of CAR-T Therapy in Medicine

Chimeric Antigen Receptor (CAR)-T cell therapy is a groundbreaking technique in cancer treatment that has radically improved treatment for a variety of blood cancers. In this technique, patient's own immune T cells are used. These T cells are reprogrammed to target and kill cancer cells before being returned to the patient. The clinical advantage of CAR-T therapy lies in the capacity of engineered T cells to recognise cancer cell specific markers without the need for any MHC (Major Histocompatability Complex) recognition. This ensures highly specific and potent cancer cell targeting. The firstCAR-T cell therapy was approved some years back, and this therapy is already proving very useful in the treatment of blood cancers resistant to traditional treatments (Maakaron & Hu, et al., 2022)(Table.1).

(**Table.1.** Applications of CAR-T Therapy in Medicine)

Clinical Trial	CAR-T Product	Target Antigen	Disease Indication	Key Findings
ELIANA Trial (2018)	Tisagenlecleucel	CD19	CD19 | B-cell Acute Lymphoblastic Leukemia (ALL)	81% remission in pediatric and young adults with relapsed/refractory ALL.
JULIET Trial (2019)	Tisagenlecleucel	CD19	Diffuse Large B-Cell Lymphoma (DLBCL)	Durable response and long-term remission for relapsed/refractory DLBCL.
ZUMA-1 Trial (2017)	AxicabtageneCiloleucel	CD19	Large B-cell Lymphoma and DLBCL	High response rate, durable complete remissions for pretreated patients.
ZUMA-2 Trial (2020)	BrexucabtageneAutoleucel	CD19	Mantle Cell Lymphoma (MCL)	Significant clinical responses, high remission rates for relapsed/refractory MCL.
TRANSCEND NHL 001 Trial (2021)	LisocabtageneMaraleucel	CD19	Relapsed/Refractory Large B-cell Lymphoma	Durable responses, favorable safety profile, reduced severe toxicities.
ZUMA-7 Trial (2022)	AxicabtageneCiloleucel	CD19	Second-line Large B-cell Lymphoma	Better event-free survival vs standard chemo/stem cell transplant.
BELINDA Trial (2022)	Tisagenlecleucel	CD19	Aggressive B-cell Lymphoma	CAR-T in second-line settings, provided key efficacy and safety data.
KarMMa Trial (2021)	IdecabtageneVicleucel	BCMA	Multiple Myeloma	High response rates, durable remission in pretreated multiple myeloma.
CARTITUDE-1 Trial (2021)	CiltacabtageneAutoleucel	BCMA	Relapsed/Refractory Multiple Myeloma	Very high response rates, deep and durable remission for many.
ROCKET Trial	Experimental CAR-T Product	BCMA	Multiple Myeloma	Investigated new CAR-T methods for effectiveness and relapse prevention.
ZUMA-3	BrexucabtageneAutol	CD19	B-cell Acute	Substantial remission rates for

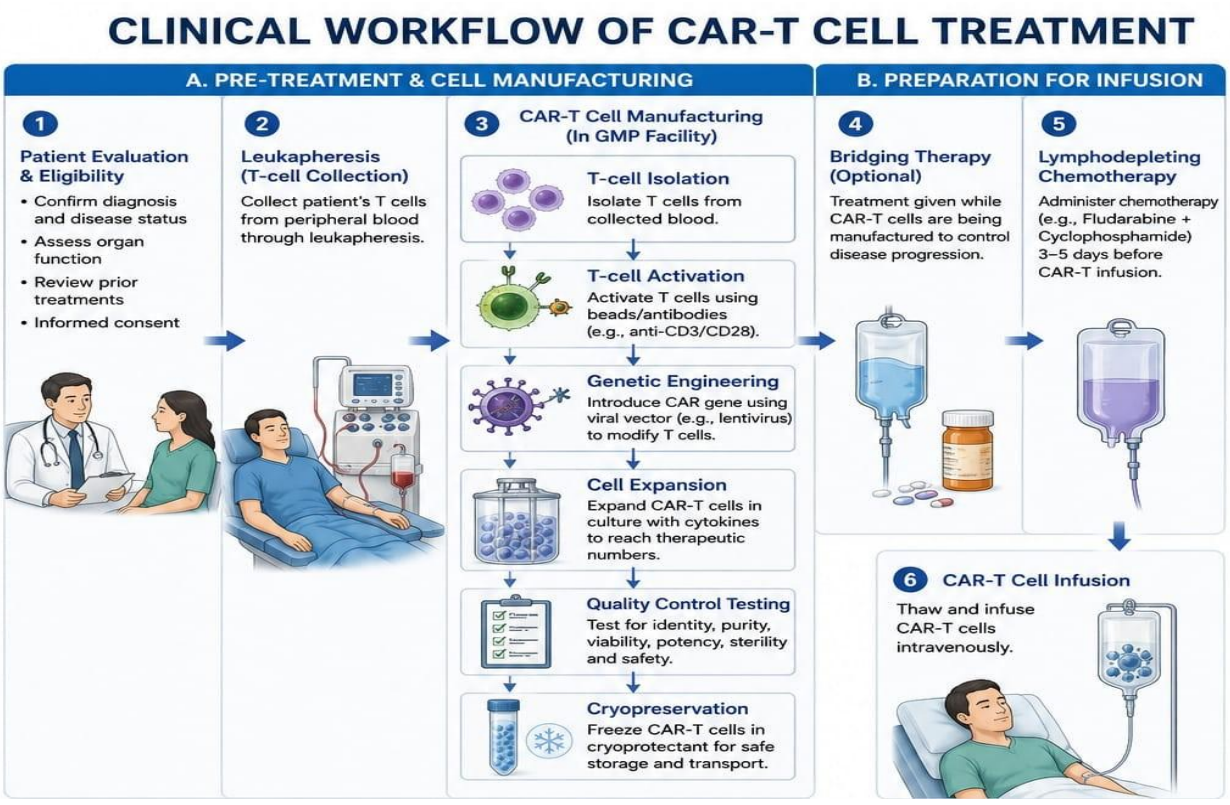
Trial (2021)	eucel		Lymphoblastic Leukemia (ALL)	adults with relapsed/refractory ALL.
PILOT Trial (2022)	LisocabtageneMaraleucel	CD19	Large B-cell Lymphoma	Effective for patients not candidates for stem cell transplant.

8.1 Acute Lymphoblastic Leukemia (ALL) and CAR-T Cell Therapy

Acute Lymphoblastic Leukemia (ALL) is a cancer of the blood and bone marrow that is rapid-growing and involves uncontrolled proliferation of imature lymphoid cells called lymphoblasts. These cells accumulate in the bone marrow, inhibiting normal production of other cells, and they may also travel through the blood and other parts of the body. ALL is one of the most common childhood leukemia, but adults can develop ALL too. The usual treatment includes chemotherapy, target therapy, radiation, and stem cell transplantation. However, some of the people diagnosed with ALL eventually relapse or they may not respond to treatments and end up being refractory. For those with refractory or relapsed ALL, Chimeric Antigen Receptor (CAR)-T cell therapy has proven itself to be a highly successful and new type of treatment (Maakaron & Hu, et al., 2022).

8.2 Diffuse Large B-Cell Lymphoma (DLBCL) and CAR-T Cell Therapy

Diffuse Large B-Cell Lymphoma (DLBCL) is a prevalent and aggressive form of non-Hodgkin lymphoma that originates from mature B lymphocytes. It is defined by the accelerated proliferation of large, cancerous B cells in lymphoid organs (such as lymph nodes, spleen) and often in other areas (extranodal tissue, bone marrow). Typical symptoms associated with DLBCL include swelling of lymph nodes, fever, night sweats, unexplained weight loss, and fatigue (Weber et al., 2024). (Figure.2).



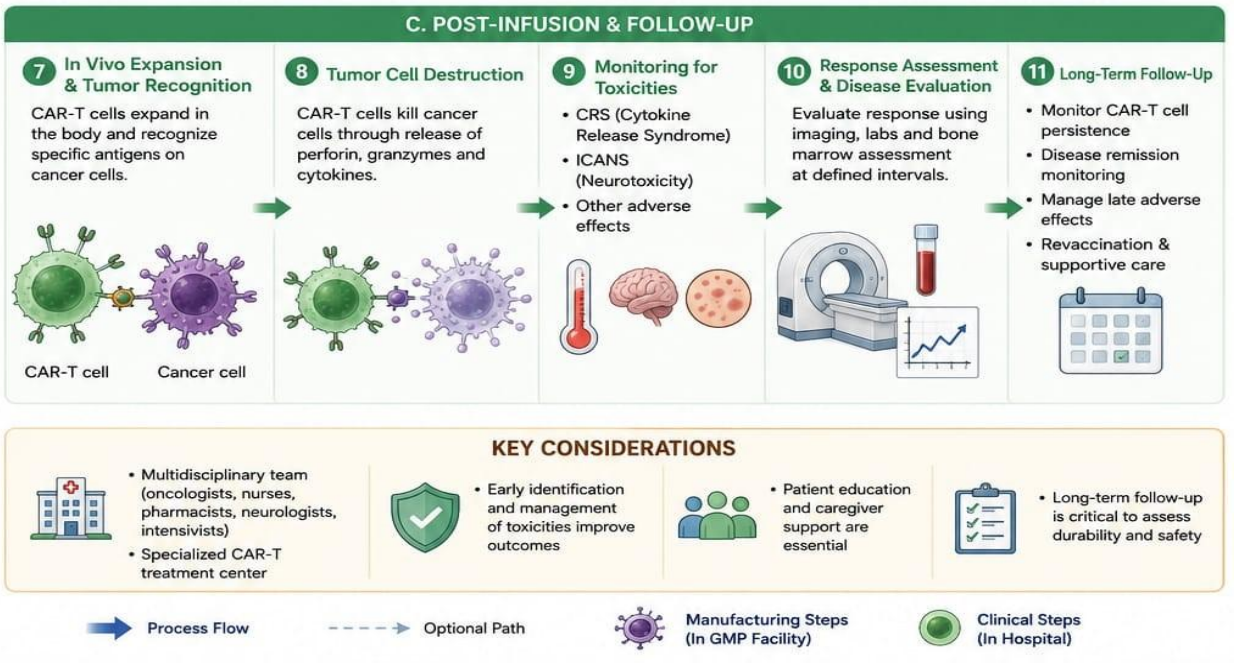


Figure.2. Clinical workflow of CAR-T cell treatment

8.3 Mantle Cell Lymphoma (MCL) and CAR-T Cell Therapy

Mantle Cell Lymphoma (MCL) is a rare and aggressive form of B-cell non-Hodgkin lymphoma that arises from mature B-cells found in the mantle zone of lymph node follicles. This cancer is often driven by a chromosomal translocation, t(11;14), which results in increased levels of the cyclin D1 protein, a cell cycle regulator that promotes proliferation. MCL is more common in older adults and tends to affect lymph nodes throughout the body, bone marrow, spleen, and the gastrointestinal system (Weber et al., 2024).

8.4 Multiple Myeloma and CAR-T Cell Therapy

Multiple Myeloma (MM) is a malignant proliferation of plasma cells (specialized B lymphocytes) that produce antibodies. In MM, cancerous plasma cells accumulate in the bone marrow, disrupt normal blood cell development, cause bone damage, kidney problems, anemia and increased risk of infection. Despite the advent of chemotherapy, immunomodulatory agents, proteasome inhibitors, monoclonal antibodies and stem cell transplantation, MM remains a largely incurable disease with many patients eventually developing relapsed and/or refractory disease. Most recently, CAR T-cell therapy, or T-cell immunotherapy engineered to express chimeric antigen receptors (CAR), has become a revolutionary new treatment for multiple myeloma in patients who are refractory to standard therapies (Maakaron & Hu, et al., 2022).

8.5 Chronic Lymphocytic Leukemia (CLL) and CAR-T Cell Therapy

Chronic Lymphocytic Leukemia (CLL) is a blood cancer that occurs in adults in which mature, but abnormal, B lymphocytes (a type of white blood cell) accumulate in the blood, bone marrow, lymph nodes, and spleen. This type of leukemia affects mainly older adults and develops gradually. Fortunately, Chimeric Antigen Receptor (CAR)-T cell therapy has become a valuable immunotherapy for patients with refractory/relapsed CLL and can often produce prolonged clinical responses (Maakaron & Hu, et al., 2022).

9 FDA-Approved CAR-T Cell Products

The U.S. Food and Drug Administration (FDA) approval of Chimeric Antigen Receptor (CAR)-T cell therapies represents a significant leap in cancer immunotherapy. This novel approach to treating patients with relapsed or refractory hematological malignancies involves genetically modifying patients' T lymphocytes to recognize and attack their cancer cells. Since the initial FDA approval of the first CAR-T therapy in 2017, a growing number of products targeting antigens like CD19 and BCMA have been approved for clinical use, delivering remarkable efficacy in patients whose conditions have not responded to conventional therapies such as chemotherapy, targeted therapy, and stem cell transplantation (June & Sadelain, et al., 2018).(Table.2)

Table.2. Key FDA-Approved CAR-T Cell Products

CAR-T Product	Target Antigen	Approved Indications
Tisagenlecleucel (Kymriah)	CD19	B-cell Acute Lymphoblastic Leukemia (B-ALL), Diffuse Large B-Cell Lymphoma (DLBCL), Follicular Lymphoma
Axicabtageneciloleucel (Yescarta)	CD19	DLBCL, Primary Mediastinal B-Cell Lymphoma, Follicular Lymphoma
Brexucabtageneautoleucel (Tecartus)	CD19	Mantle Cell Lymphoma (MCL), B-cell Acute Lymphoblastic Leukemia
Lisocabtagenemaraleucel (Breyanzi	CD19	Large B-Cell Lymphoma, Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma
Idecabtagenevicleucel (Abecma)	BCMA	Relapsed or Refractory Multiple Myeloma
Ciltacabtageneautoleucel (Carvykti)	BCMA	Relapsed or Refractory Multiple Myeloma
Obecabtageneautoleucel	CD19	Relapsed or Refractory B-cell Acute Lymphoblastic Leukemia (recent approvals)

9.1 Tisagenlecleucel (Kymriah) in CAR-T Cell Therapy

Tisagenlecleucel (Kymriah) is a Chimeric Antigen Receptor (CAR)-T cell therapy and was the first CAR-T product approved by the U.S. Food and Drug Administration in 2017. It represents a major breakthrough in cancer immunotherapy and is used for the treatment of certain relapsed or refractory B-cell malignancies (Maude et al., 2018).

9.2 Axicabtageneciloleucel (Yescarta) for CAR-T Cell Therapy

Axicabtagene ciloleucel (Yescarta) is a genetically modified autologous T-cell product known as CAR-T cell therapy, approved for the treatment of various relapsed or refractory B-cell malignancies. It's a second-generation product designed to recognize the CD19 antigen, a protein expressed on the surface of most B-cells, including both healthy and cancerous ones. Developed as a highly personalized form of immunotherapy, axicabtagene ciloleucel has achieved remarkable success in patients suffering from aggressive B-cell lymphomas who have failed standard treatments like chemotherapy, immunotherapy, and stem cell transplant. Its approval marked a major step forward in cancer immunotherapy (Locke et al., 2019).

9.3 Brexucabtagene Autoleucel (Tecartus) in CAR-T Cell Therapy

Brexucabtagene autoleucel (Tecartus) is a CD19-directed autologous Chimeric Antigen Receptor (CAR)-T cell therapy created for treating certain relapsed or refractory B-cell cancers. It is a personalized cancer immunotherapy where a patient's own T cells are genetically modified to recognize and destroy cancer cells with the CD19 antigen. Brexucabtagene autoleucel became the first CAR-T cell therapy approved for *Mantle Cell Lymphoma (MCL)* and is also approved for relapsed or refractory *B-cell Acute Lymphoblastic Leukemia (B-ALL)*. This development has given patients a valuable treatment option when their disease has continued to worsen despite multiple prior therapies (Wang et al., 2020).

9.4 CAR-T Cell Therapy WithLisocabtageneMaraleucel (Breyanzi)

Lisocabtagenemaraleucel (Breyanzi) is a type of CD19-directed autologous Chimeric Antigen Receptor (CAR)-T cell therapy approved for patients who have relapsed or refractory B-cell cancers. It's a custom-made immunotherapy designed to treat patients with blood cancers, particularly lymphomas that express a target protein on cancer cells called CD19. This involves using the patient's own immune cells, specifically T-cells, that are genetically engineered to fight the cancer (Abramson et al., 2020).

9.5CiltacabtageneAutoleucel (Carvykti) in CAR-T Cell Therapy

Ciltacabtageneautoleucel (Carvykti) is an autologous Chimeric Antigen Receptor (CAR)-T cell therapy developed for the treatment of relapsed or refractory multiple myeloma, a cancer of plasma cells found in the bone marrow. It is a highly personalized form of immunotherapy in which a patient's own T lymphocytes are genetically modified to recognize and destroy malignant plasma cells. Ciltacabtagene autoleucel specifically targets B-cell maturation antigen (BCMA), a cell-surface protein highly expressed on myeloma cells but minimally present on most normal tissues (Berdeja et al., 2021).

10 Advantages of Chimeric Antigen Receptor (CAR)-T Cell Therapy

Chimeric Antigen Receptor (CAR)-T cell therapy is one of the most significant advances in modern cancer immunotherapy. It involves the genetic modification of a patient's own T lymphocytes to recognize and destroy cancer cells expressing specific target antigens. Since its introduction, CAR-T cell therapy has demonstrated remarkable success in treating various hematological malignancies, particularly in patients who have not responded to conventional therapies. The therapy offers several advantages over traditional cancer treatments, making it a promising approach in precision medicine and personalized oncology (Rejeski, K et al., 2023).

Key Advantages at a Glance

1. Highly specific targeting of cancer cells.

2. Long-term persistence and immune surveillance.

3. Personalized therapy using the patient's own T cells.

4. Effective in relapsed and refractory cancers.

5. MHC-independent tumor recognition.

6. Strong cytotoxic and anti-tumor activity.

7. Potential for durable remission and cure in some patients.

8. Reduced damage to normal tissues compared with chemotherapy.

9. Expanding applications in multiple cancer types.

10. Platform for future cellular and gene-based therapies.

10.1 Target Specificity in Chimeric Antigen Receptor (CAR)-T Cell Therapy

Target specificity is a cornerstone of Chimeric Antigen Receptor (CAR)-T cell therapy and the very essence of its power as a targeted cancer immunotherapy. Simply put, target specificity is the ability of the engineered CAR-T cells to specifically identify and attack cancer cells expressing a defined target antigen, without significant damage

to healthy tissues. This selective targeting is accomplished by the structure of the CAR itself, which is an engineered T cell receptor that has been designed to recognize a specific target antigen present on the surface of cancer cells. When the CAR binds to its target, intracellular signaling events are triggered, activating the T cell to proliferate, release signaling (Maus, M. V et al., 2023).

10.2 Individualized Therapy With Chimeric Antigen Receptor (CAR)-T Cell Treatment

Individualized therapy is one of the primary benefits of chimeric antigen receptor (CAR)-T cell therapy. Contrary to general cancer therapies that use standardized medicines or treatment regimens for large numbers of individuals, CAR-T therapy is designed specifically for an individual affected by cancer, utilizing their own immune cells. This strategy makes it possible to customize treatment in accordance with the specific biological profile and condition of the affected person, which in turn enhances the treatment effectiveness and reduces the chances of immune system rejection. Thus, CAR-T cell therapy marks an essential advancement in precision medicine and personalized cancer care (Rejeski, K et al., 2023).

10.3 Long-term immune memory of Chimeric Antigen Receptor (CAR)-T Cell Therapy

One of the greatest strengths of Chimeric Antigen Receptor (CAR)-T cell therapy is long-term immune memory, which contributes significantly to its ability to achieve enduring clinical responses in cancer patients. While treatments like chemotherapy and radiotherapy only act for as long as the medicine is delivered, CAR-T cells can survive in a patient's body for an extended period, offering continued defense against cancer recurrence. This is because a subset of these cells differentiates into memory T cells, which retain their capacity to identify and destroy cancer cells that express the target antigen. This memory system can not only eliminate extant tumor cells but also lay the foundation for ongoing immune surveillance that may guard against a return of disease Rejeski, K et al., 2023).

10.4 High Remission Rates in Chimeric Antigen Receptor (CAR)-T Cell Therapy

High remission rates in patients with certain blood cancers are one of the most important benefits of Chimeric Antigen Receptor (CAR)-T cell therapy. A remission means that signs and symptoms of cancer are reduced or have disappeared after treatment. In many cases of relapsed or refractory cancers that did not respond to chemotherapy, radiation therapy, targeted drugs, or stem cell transplants, CAR-T cell therapy has achieved unprecedented remission rates, giving these patients the hope of a cure or long-lasting control of their disease (Rejeski, K et al., 2023).

11 Limitations and Challenges of Chimeric Antigen Receptor (CAR)-T Cell Therapy

While Chimeric Antigen Receptor (CAR)-T cell therapy has been a breakthrough in cancer treatment and has demonstrated extraordinary success in patients with blood cancers, numerous limitations and challenges still impede its broader application. These include treatment-related toxicities, antigen escape, insufficient efficacy in solid tumors, manufacturing hurdles, prohibitively high costs, and persistent long-term safety issues. Efforts to overcome these obstacles are the subject of current research, and are critical for the expansion of CAR-T cell therapy (Neelapu, S. S., et al., 2018).

11.1 Cytokine Release Syndrome (CRS) in Chimeric Antigen Receptor (CAR)-T Cell Therapy

Cytokine Release Syndrome (CRS) is one of the most common and serious side effects of Chimeric Antigen Receptor (CAR)-T cell therapy. CRS is a systemic inflammatory response that occurs when CAR-T cells are activated by encountering their target antigen on cancer cells. This rapid proliferation of CAR-T cells leads to a mass production of inflammatory cytokines, causing an overwhelming systemic immune response. Although CRS indicates the CAR-T cells are actively responding to the tumor cells, severe CRS can be life-threatening and require

immediate medical attention. As a result, understanding the mechanism, clinical symptoms, and management of CRS is critical for the safe and effective use of CAR-T cell therapy (Locke, F. L., et al., 2019).

11.2 Neurotoxicity (ICANS) In Chimeric Antigen Receptor (CAR)-T Cell Therapy

One of the most crucial and potentially dangerous side effects of Chimeric Antigen Receptor (CAR)-T cell therapy is Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS). ICANS is a neurological adverse effect occurring after the CAR-T cells are activated and proliferate within the patient's body. The syndrome is most commonly associated with or occurs after Cytokine Release Syndrome (CRS), and it is characterized by diverse neurological manifestations primarily affecting the central nervous system (CNS). Although the majority of patients recover with prompt treatment, severe neurotoxicity can be life-threatening (Lee, D. W et al., 2019).

11.3 Tumor antigen escape in CAR-T cell therapy

Tumor antigen escape is the phenomenon in which cancer cells lose, alter, or reduce the expression of their target antigens on the cell surface, and therefore can avoid detection and destruction by CAR-T cells. Because the effectiveness of CAR-T cell therapy relies on the ability of CAR-T cells to recognize antigens on the surface of tumor cells, a change in these antigens allows cancer cells to evade CAR-T cell killing. Consequently, tumor antigen escape is the main cause of recurrence after successful CAR-T cell treatment (Neelapu, S. S., et al., 2018).

11.4 Relapse in CAR T-cell Therapy

Relapse after chimeric antigen receptor (CAR)-T cell therapy is a persistent problem in an era of remarkably promising clinical results in many patients with hematologic malignancies. Relapse is defined as the return of cancer after a complete or partial remission has been achieved as a result of treatment. Even though CAR-T cell therapy has resulted in unprecedented remission rates in such diseases as B-cell acute lymphoblastic leukemia (ALL), diffuse large B-cell lymphoma (DLBCL), chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), and multiple myeloma, a portion of patients still experience recurrence of disease. The mechanisms of relapse in these individuals should be understood in order to improve the long-term outcome of patients treated with CAR T cells (Locke, F. L., et al., 2019).

11.5 Manufacturing Complexity In Chimeric Antigen Receptor (CAR)-T Cell Therapy

Manufacturing complexity is one of the greatest obstacles to the widespread implementation of Chimeric Antigen Receptor (CAR)-T cell therapy. In stark contrast to traditional cancer therapies, which are standardized and can be produced in massive batches for distribution to a large population of patients, CAR-T therapy is highly personalized. It involves a series of highly intricate steps: the collection of a patient’s own T lymphocytes, their genetic engineering to express the chimeric antigen receptor, proliferation of these cells to adequate numbers, comprehensive quality control and testing, and finally their re-infusion back into the patient. The bespoke nature of this process requires specialized facilities, strict quality-control protocols, and highly skilled personnel, which inevitably contributes to the significant expense, time, and technical difficulty associated with CAR-T production (Locke, F. L., et al. 2019).

12 Recent Advances (2020-2026) in Chimeric Antigen Receptor (CAR)-T Cell Therapy

The period from 2020 to 2026 has witnessed remarkable progress in Chimeric Antigen Receptor (CAR)-T cell therapy, transforming it from a treatment primarily used for relapsed hematological malignancies into a rapidly evolving platform with applications in solid tumors, autoimmune diseases, and precision medicine. Advances in genetic engineering, synthetic biology, manufacturing technologies, and clinical trial design have significantly improved the efficacy, safety, and accessibility of CAR-T therapy. These multi-specific CAR-T cells reduce the risk

of tumor antigen escape and improve long-term remission rates by eliminating heterogeneous cancer cell populations (Olson et al., 2024).

12.1 AI Drives Design of CAR-T Therapy in the Fight Against Cancer

Artificial Intelligence (AI) is revolutionizing the field of Chimeric Antigen Receptor (CAR)-T cell therapy, offering unprecedented capabilities for the design, optimization, and deployment of these potent cancer-fighting agents. CAR-T cell therapy, a groundbreaking approach in immunotherapy, genetically modifies a patient's T cells to recognize and eliminate cancer cells, leading to remarkable success in blood cancers. However, challenges like tumor antigen loss, on-target but off-tumor toxicity, poor persistence, treatment resistance, and complex manufacturing continue to hinder its widespread application. AI is stepping in as a crucial ally, leveraging machine learning, deep learning, and computational modeling to overcome these obstacles and enhance the efficacy and safety of CAR-T therapies (Bera et al., 2023).

12.2 Nanotechnology-assisted CAR-T cell Therapy

Nanotechnology-assisted CAR-T cell therapy is a novel and innovative strategy that leverages the unique properties of nanotechnology to augment Chimeric Antigen Receptor (CAR)-T cell therapy to achieve greater efficacy, safety, and accessibility of cancer immunotherapy. Although CAR-T therapy has demonstrated exceptional clinical outcomes in the treatment of hematologic cancers, limitations such as complex manufacturing procedures, high costs, inadequate efficacy in solid tumors, CAR-T cell exhaustion, and treatment-related toxicities have hindered its broader use. Nanotechnology offers ingenious solutions for these constraints by enhancing CAR-T cell engineering, improving therapeutic delivery, modifying the tumor microenvironment, and promoting in vivo production of CAR-T cells (Smith et al., 2023).

12.3 Armored CAR-T cells in Chimeric Antigen Receptor (CAR)-T Cell Therapy

Armored CAR-T cells are a new generation of Chimeric Antigen Receptor (CAR)-T cells engineered to overcome the limitations of traditional CAR-T therapy especially in solid tumor treatments. While traditional CAR-T cells have shown an unprecedented success in treating hematological malignancies, their efficiency is often hampered by immunosuppressive tumor microenvironment (TME), decreased persistence, poor tumor infiltration and T-cell exhaustion. Armored CAR-T cells are designed to defy these restrictions through additional genetic engineering enabling enhanced survival, activation and anti-tumor activity of engineered T cells. Thus they are sometimes called fourth generation CAR-T cells or TRUCKs (T cells Redirected for Universal Cytokine-mediated Killing) (Chmielewski & Abken, et al., 2020).

12.4 CAR-NK Cells in Cancer Immunotherapy

CAR-NK (Chimeric Antigen Receptor Natural Killer) cells are an emerging cellular immunotherapy that fuses the tumor-targeting specificity of Chimeric Antigen Receptors (CARs) with the innate cytotoxic power of Natural Killer (NK) cells. Although CAR-T cell therapy has transformed the treatment of several hematological cancers, the extensive toxicity associated with this treatment, such as Cytokine Release Syndrome (CRS), neurotoxicity, the high costs of manufacture, and the prolonged production timelines, have all hindered widespread clinical utilization. CAR-NK cell therapy offers an encouraging alternative which could deliver similar anti-tumor benefits, yet with greater safety and improved accessibility (Liu et al., 2020).

12.5 Ready-to-use CAR Therapies in CAR-T Cell Therapy

Off-the-shelf CAR therapies are already available and manufactured cellular immunotherapies that can be infused to the patient, without any need to harvest cells individually from the patient, and then have it processed. Traditional CAR-T cell therapy is autologous, this means that T cells are taken from the patient, genetically modified to express

the Chimeric Antigen Receptor (CAR), expand it and then infuse it back to the patient. Although the treatment has proved very effective, it takes time (weeks), and is very expensive. Off-the-shelf CAR therapies are intended to eliminate these shortcomings, as they use donated healthy cells or renewable sources of cells and so they are immediately available to the patients (Depil et al., 2020).

13 Future Prospects of Chimeric Antigen Receptor (CAR)-T Cell Therapy

Chimeric Antigen Receptor (CAR)-T cell therapy has revolutionized the cancer immunotherapy field by providing highly personalized and targeted therapeutic options for patients suffering from hematological malignancies. Since the commercial approval of the first CAR-T cell products, remarkable advancements have been achieved in the design of CAR molecules, gene editing technologies, manufacturing platforms, and their clinical applications. Although the current CAR-T cell therapies have demonstrated remarkable clinical efficacy against leukemias, lymphomas, and multiple myeloma, there are still considerable challenges, including treatment-related toxicities, tumor antigen escape, limited efficacy in solid tumors, manufacturing complexity and high costs. Ongoing research and innovation are being driven towards overcoming these hurdles and widening the therapeutic application of CAR-T cells (Chong et al., 2024).

13.1 Personalized Immunotherapy in Chimeric Antigen Receptor (CAR)-T Cell Therapy

Personalized immunotherapy is one of the most compelling advantages offered by Chimeric Antigen Receptor (CAR)-T cell therapy. Unlike traditional cancer interventions like chemotherapy and radiation therapy, which broadly impact both cancerous and healthy cells, CAR-T therapy is uniquely tailored to the individual patient. The treatment involves drawing on the patient's own immune cells, modifying them genetically to specifically target and attack tumors, and then reintroducing them into the body for their targeted action. This individual-centric approach has transformed how cancer is treated, enabling highly precise, efficacious, and lasting anti-tumor effects (Maude et al., 2024).

13.2 Treating Solid Tumors With CAR-T Cell Therapy

One of the more active and promising frontiers in cancer immunotherapy is the treatment of solid tumors with Chimeric Antigen Receptor (CAR)-T cell therapy. While CAR-T cells have proven revolutionary in treating blood cancers like leukemia, lymphoma, and myeloma, they have had a more challenging journey with solid tumors due to several biological and structural hurdles such as limited infiltration, survival, and activity. However, researchers are making headway with modifications to CAR engineering, gene editing, nanotechnology, and combination treatments to harness the full potential of CAR-T cells in solid cancers (Ahmed et al., 2022).

13.3 Next-generation CAR Designs in Chimeric Antigen Receptor (CAR)-T

Cell TherapyNext-generation Chimeric Antigen Receptor (CAR) designs refer to innovative approaches in CAR-T cell engineering designed to overcome the limitations of first-generation and second-generation CAR-T therapies. Despite the remarkable success achieved in hematologic malignancies with early CAR-T cell designs, concerns regarding their persistence, T cell exhaustion, antigen escape, serious toxicities, and limited effectiveness against solid tumors have prompted the development of more sophisticated CAR constructs. These advanced designs utilize novel genetic, molecular, and synthetic biology strategies to enhance safety, specificity, persistence, and anti-tumor activity (Liu et al., 2020).

Conclusion

Chimeric Antigen Receptor (CAR)-T cell therapy stands as one of the most revolutionary advancements in cancer immunotherapy, having revolutionized the treatment of a growing number of hematological malignancies. By reprogramming the patient’s own T cells to recognize and destroy tumor cells, CAR-T therapy provides a

personalized and highly targeted approach that has delivered unprecedented clinical outcomes for patients with acute lymphoblastic leukemia (ALL), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma (MCL), chronic lymphocytic leukemia (CLL), and multiple myeloma (MM). The unique capacity of CAR-T cells to identify tumor-specific antigens, expand in vivo, and establish long-lasting anti-tumor immunity has led to long-term remission and improved survival for many patients who had no other treatment options.

In conclusion, CAR-T cell therapy represents a groundbreaking milestone in the field of cancer immunotherapy, offering highly specific, individualized, and potentially curative options for a number of devastating cancers. Although challenges remain, ongoing innovation in both technology and clinical practice is steadily overcoming these barriers. With sustained effort, global collaboration, and improved accessibility, CAR-T cell therapy has the potential to transform cancer treatment and bring hope to patients with diseases once considered intractable.

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