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REVIEW ARTICLE

CAR T-Cell Therapy: Structural Variability, Clinical Challenges, and Future Advances

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ABSTRACT: As of 2024, approximately 2.0 million people in the United States are projected to be diagnosed with cancer, with over 600,000 cancer-related deaths expected, primarily due to relapse or advanced metastasis. Since 2017, CAR T-cell therapy, which involves genetically engineering a patient's T cells to target and destroy cancer cells, has revolutionized cancer treatment. This treatment has shown remarkable success, particularly in patients with hematological malignancies, achieving complete remission rates of up to 90% in some studies. As the therapy continues to advance, it holds promise for becoming a standard treatment for all cancer types, with tailored modifications for specific cancers. This review focuses on the mechanisms behind the effectiveness of CAR T-cell therapy in targeting cancer cells, highlighting both the clinical successes for various types of blood cancers and challenges, particularly for solid tumors. This paper also covers how the advancements in CAR T-cell technology could significantly reduce relapse rates, improve patient outcomes, and overall increase survival. By creating an overview of the current clinical achievements and struggles, this review aims to highlight the potential of CAR T-cell therapy to enhance cancer treatment widely.

KEYWORDS: Translational Medical Sciences, Disease Treatment and Therapies, Personalized Medicine, Blood Cancers, CAR T-cell Therapy.

Introduction

Immunology is the branch of medical science that studies the immune system, including its components and functions in protecting the body from foreign pathogens and harmful microorganisms. It involves the study of both innate and adaptive responses and includes the different immune cells, molecules, and different bodily defenses that could identify and attack the pathogens. The innate response provides an immediate, non-specific defense against pathogens, while an adaptive response is much slower but highly specific and capable of forming immunological memory. Gaining a deep understanding of how the immune system interfaces with autoimmune diseases, allergies, infectious diseases, and cancer can allow scientists to develop specific treatments.

Cancer is the uncontrolled growth of abnormal cells in the body. These cells form tumors and invade distant organs of the body through a process known as metastasis. Once metastasized, the cancer becomes much more life-threatening. These metastasizing tumor cells can surface on almost any organ or tissue, even if the tumor cells originated in a completely different region by migrating through the circulatory and lymphatic systems. However, in the bloodstream, various types of immune cells are circulating actively, trying to seek out and destroy foreign bacterial pathogens or native human cells.[1] Tumor cells have various mechanisms to evade these lymphocytes to achieve their metastatic potential, which refers to their ability to spread from the primary tumor area and form secondary lesions in other organs. [1,2]

Common defense techniques used by tumor cells include down-regulating the expression of tumor antigens on the cell's surface and developing resistance to apoptosis, even when targeted by cytotoxic immune cells. These evasion methods relate to the tumor's intra-tumoral niche, the microenvironments within a tumor that allow the growth and spread of cancer cells. These areas typically have low oxygen levels, unusual pH levels, and signals that weaken immune responses, all of which help tumor growth and make it easier for cancer cells to spread to other parts of the body.[3] The most commonly used methods of treatment are radiation therapy, chemotherapy, and surgery.[4] All of these techniques have been effective in reducing the symptoms or eradicating tumors across most cancer types, but disease mortality still poses a major challenge.

A recently discovered therapy, known as Chimeric Antigen Receptor (CAR) Therapy, has emerged as a groundbreaking advancement in cancer research. This form of therapy has only been FDA-approved since 2017. During its short lifespan, it has made radical changes in how cancer treatment helps patients.[5] CAR T-cell therapy is a form of immunotherapy engineered to treat particular types of cancer, specifically blood cancers like lymphoma and leukemia. The treatment uses the patient's own T cells. Unlike traditional forms of cancer therapy, CAR T-cell therapy is designed to primarily target cancer cells by recognizing the antigens on the surface of the cancer cells, enabling a more precise attack on the tumors while not impacting the healthy cells.[6] This allows the CAR T cells to overcome evasion strategies found in cancer, leading to impressive remission rates in many types of cancer, particularly in hematological cancers.

While many cancers exist today, this review primarily focuses on hematological cancers such as Diffuse Large B-cell Lymphoma (DLBCL), Multiple Myeloma, and B-cell Acute Lymphoblastic Leukemia (B-ALL). These cancers are well-

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suited for CAR T-cell therapy because they originate from immune cells like B cells, which effectively express well-defined surface markers like CD19 or B cell maturation antigen (BCMA). These markers make the cancers an ideal target for CAR T-cells to recognize and destroy.[7] However, solid tumors often have challenges like a lack of specific markers, a denser tumor microenvironment, and immune evasion methods, which overall limit the efficacy of CAR T-cell therapy.[8]

Even though the testing and clinical trials of this treatment are still in their early stages, CAR-T cell therapy shows promise as a new method of cancer treatment. This paper will discuss the current state of CAR T-cell therapy, new advances on the horizon, the complications, the structure and shape of the CAR T-cell, and the clinical successes regarding this form of therapy. By reviewing the advancements, challenges, and successes of CAR T-cell therapy, we can better understand the significant potential it has in revolutionizing cancer treatment and paving the way for more targeted and effective therapies in the future.

Discussion

Background of CAR T-cell therapy:

CAR T-cell therapy became a groundbreaking cancer treatment that has revolutionized immunotherapy treatment. The person responsible for this breakthrough is Dr. Carl June. He is regarded as the "Father of CAR T-cell therapy" and serves in leadership positions at both the Center for Cellular Immunotherapies and the Parker Institute for Cancer Immunotherapy at the University of Pennsylvania. His work on genetically engineered T cells for treating acute lymphoblastic leukemia led to the FDA approval of tisagenlecleucel for ALL in 2017, specifically for younger patients.[5] He is a lead researcher on lymphoblastic leukemia activation mechanisms related to immune tolerance and cancer immunotherapy. As mentioned above, Dr. Carl June contributed to developing the first FDA-approved therapy for children and young adults with acute lymphoblastic leukemia (ALL). Even though his first successful therapy was in a teenager in 2012, the pioneers of CAR T cell did not receive FDA approval until 2017.[12]

The CTL019 trial, tested by Dr. Carl June, overcame the risk of Cytokine Release Syndrome and became the first chimeric antigen that was FDA-approved in 2017. This treatment used tisagenlecleucel as the medication for patients up to 25 years old who had B-cell Acute lymphoblastic leukemia. These patients didn't respond to the standard treatment, like chemotherapy and radiation, and, if improved, relapsed at least twice during their cancer journey. In the results of this clinical trial, 83% of the young adults and children being treated had a complete remission.[24]

Dr. Carl June hypothesized that tocilizumab, a monoclonal antibody-based drug commonly used to treat rheumatoid arthritis, could mitigate the life-threatening side effects of cytokine release syndrome (CRS) that endangered many patients, a hypothesis later confirmed. Tocilizumab reduced CRS within a short time frame for 69% of the patients involved.[25] Dr. Carl June significantly advanced the path to FDA approval, developing a solution that not only secured acceptance for CAR T-cell therapy but also paved the way for its application in treating other related diseases.

Difference between T-Cells and CAR T-Cells:

The difference between T cells and CAR T-cells lies in how they target foreign and abnormal material in the body, despite both having this capability. Normal T cells target a wider variety of threats because they are integral to the body's natural immune system. In contrast, CAR T-cells are engineered specifically to target antigens on the surface of tumor cells. T cells can only recognize certain antigens based on the Major Histocompatibility Complex (MHC) molecules, which ensure the cells only respond to specific antigens and avoid an immune response against the body's own healthy cells, also known as self-cells, by recognizing markers that identify them as part of the body. CAR T-cells completely avoid the need for MHC molecules because they are only programmed to target the molecules on a cancer cell's surface. However, one of the differences in T cells is that they can adapt to new threats over time and can change their function based on the invasion of the body. In contrast, CAR T-cells only have a fixed target solely dependent on the CAR's design. T cells develop and mature in the thymus, where they generate a diverse repertoire of T cell receptors (TCRs), enabling them to recognize a wide range of antigens and adapt to various targets. CAR T-cells have a fixed receptor engineered to recognize specific antigens, such as CD19, which is expressed on the surface of B-cell malignancies.[4] CAR T-cell therapy has emerged as a ground-breaking form of cancer therapy that enhances the immune system's ability to recognize and destroy cancer cells, particularly in blood-related cancers.

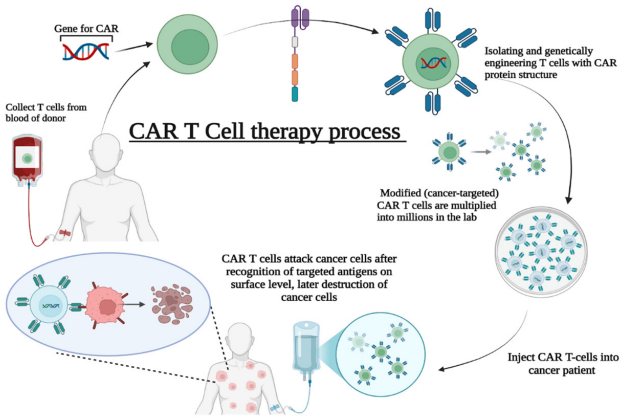

Figure 1: A diagram illustrating the steps involved in CAR T-cell therapy. The diagram details the main steps: Blood is drawn from the patient, extracting the T cells; T cells are isolated and genetically engineered to express CARs; Engineered T cells are multiplied in the lab; and Modified T cells are infused back into the patient to destroy cancer cells. This figure was created through BioRender.

CAR T-Cell Therapy and Structures Involved:

The process of CAR T-cell therapy involves modifying and genetically engineering a patient's T-cells to target and attack the cancer cells found in the body. Before the T-cells can be reprogrammed, the patient's white blood cells are collected

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through leukapheresis. (Figure 1)4 This is a process where a machine separates the T-cells from a person's blood while returning the red blood cells, platelets, and plasma back to the body.

After the T-cells are isolated from the white blood cells, they are sent to a lab to be re-engineered with a gene that provides instructions for making proteins that can bind to the surface of cancer cells, known as chimeric antigen receptors (CAR). (Figure 1)[4] To introduce the proteins, scientists use a method called gene transfer where a vector, commonly a lentivirus (a type of modified virus), is used to safely deliver the genetic material into the T-cells. Lentiviral vectors are often used in CAR T-cell therapy because they can stably integrate the transgene into the "host genome", allowing them to transduce both dividing and non-dividing cells, and offer long-term expression of the CAR receptor. Once successfully integrated, the modified T cells now express a CAR receptor. However, before patients are infused with these modified CAR T-cells, the efficacy of the receptor needs to be tested.

The most effective method for validating the therapy involves *in vitro* testing. In these experiments, modified T-cells are evaluated to determine if they can recognize and bind to cancer cells in a controlled environment. Researchers also confirm that the CAR T-cells retain their ability to express the CAR protein and effectively target and destroy cancer cells.[9] To identify what CAR proteins need to be configured for the T-cell, researchers first identify the surface proteins that are expressed more frequently on a cancer cell's surface compared to normal, healthy cells, like CD19 and HER2. For example, CD19 is commonly found on B cells, including malignant ones in leukemia and lymphoma, while HER2 is overexpressed in certain breast and gastric cancers, but also is present at lower levels in normal tissues. Once the specific CAR protein required for targeting cancer cells is identified, they can stimulate and culture the modified T cells with cytokines to promote proliferation, increasing the number of CAR T cells used for the therapy.[10] After this protein is configured to the cell and efficacy is tested, these T-cells can be infused back into the patient and actively destroy cancer cells once returned to the bloodstream. (Figure 1)

Prior to the introduction of CAR T-cells into the bloodstream, the patient must undergo chemotherapy rounds to reduce the number of T cells and create a more favorable environment for the infused cells. Now, when an antigen is bound, the CAR T-cell is activated and can destroy the cancer cells that have this marker.[4] However, this process requires the involvement of four key structures within the CAR T-cell: an antigen-binding domain (scFv), a hinge region for flexibility, a transmembrane domain, and intracellular signaling domains like CD3ζ and co-stimulatory molecules.

Antigen-Binding Domain Recognizes Antigen:

To begin with, the Antigen Binding Domain is the part of the CAR that determines what the cell targets. It's made from the parts of antibodies called the VH (variable heavy) and VL (variable light) chains. These chains are essential because they allow the CAR to bind specifically to a target antigen (Figure 2) They connect to form a single-chain fragment called scFv, which maintains the specificity and flexibility required for antigen recognition.[11,12] The chain can target the proteins present on the surface of the cancer cells and effectively attack these cells.[13] However, how the VH and VL chains interact with one another impacts the CAR's ability to bind to its intended target. For instance, the strength of interaction between the VH and VL chains affects the binding affinity between the CAR and the antigens on the cancer cell.[11] A high-affinity interaction can improve the CAR T-cell's ability to bind to low levels of antigens on the tumor cells, making the T-cell much more effective in destroying cancer cells. (Figure 2) To affect how CAR binds to its target, scientists also need to consider factors like how much of its surface is present and where CAR binds to the target.[11]

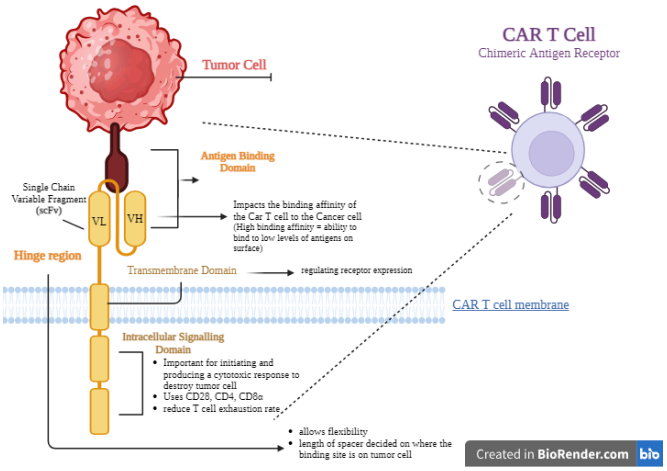

Figure 2: In this figure, the antigen receptors on the CAR T cells are focused on to give a deeper understanding of how each part of the receptor works together to bind to the CAR T cell and induce Apoptosis in the tumor cell. Without each of these structures, CAR T cells cannot play their part in targeting and attacking the tumor cells. This figure was created through BioRender.

Hinge Region & Transmembrane Domain:

For the antigen-binding domain to reach the targeted epitope on the antigen molecule and connect the binding unit to the transmembrane domain, the CAR T-cell needs to use the hinge region. The shape and length of these spacers influence the CAR's functionality, including its flexibility to navigate physical barriers, its expression efficiency, and its ability to accurately identify the region where the antibody binds to the antigen. (Figure 2) [4,11]

The length of the hinge region depends on where the target is located on the cancer cell, long spacers are more suited to targets situated closer to the cell membrane (membrane-proximal; more useful for antigens with sugar molecules attached), while short spacers are better used for targets farther away from it (membrane-distal).[11,13] While scientists should test different hinge lengths to choose the best one for each CAR structure, donor sequences from proteins like CD8, CD28, IgG1, and IgG4 are commonly included in viral builds to create synthetic hinge regions. Some spacers, like ones from IgG, can create problems when interacting with immune system components, so while making the chimeric antigen receptors, scientists

must be careful to avoid creating even more problems for the patient's immune system.[11]

The next structure needed is the transmembrane domain, which is used to anchor the CAR to the cell membrane and influence the function of the CAR T-cells. These domains are commonly found in CD3ζ, CD4, CD8α, or CD28 T-cells.[13] CD4 and CD28 are integrated into the receptor as activation switches, enhancing the T-cell's capacity to recognize and attack cancer cells. This leads to stronger and more sustained anti-tumor immune responses compared to using only the primary signaling domain. CD4 can be used to target specific tumor antigens, while CD28 can enhance T-cell proliferation and cytokine production when bound to the ligand.[14] The transmembrane domain is one of the least studied components, but without the transmembrane domain, CAR T-cells wouldn't be able to link the scFv to the intracellular signaling domain.[11,13]

Mechanism of Action:

The intracellular signaling domain initiates activation inside the T cell after contact with the antigen. This signaling is crucial for T cell activation and producing a cytotoxic response that causes the destruction of the tumor cell using, as seen in CD19-targeted CAR T-cell approaches. (Figure 2, Figure 3) This cytotoxic response releases granzymes and perforins that induce the tumor cell to undergo apoptosis.[15]

Initially, perforins form a pore at the junction between the cancer cell and the immune cell membrane, allowing cytotoxic proteins like granzymes to enter. These granzymes can then damage the tumor cell's DNA, causing irreversible harm.[15] Some of the major cytokines released due to this cytotoxic response are IFN-y and Il-2.[16] As for IFN-y, this cytokine can induce an increase in MHC I molecules so that the immune system can better identify this invader and help the CAR T cells as a whole recognize and destroy cancer cells. (Figure 3)[16] IL-2 can stimulate T cells to attack the cancer cells so that the memory T cells can remember what the specific tumor cell type looks like, and if there is a cancer relapse, IL-2 would enable a faster and effective immune response. (Figure 3)

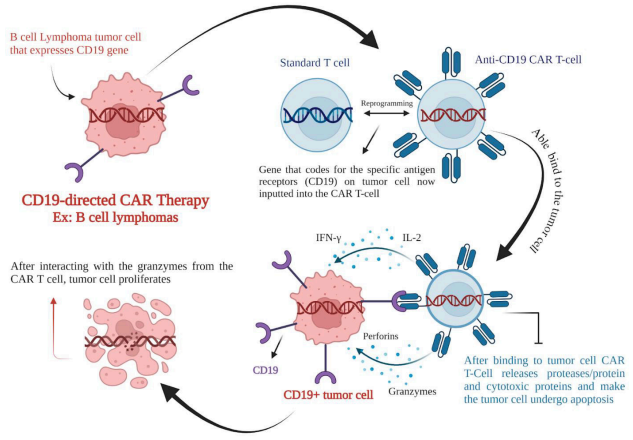

Figure 3: This figure depicts how CAR T-cell therapy works for B-cell lymphomas. First, the T cell needs to be embedded with the CD19 antigen receptor so the T cell can become an Anti-CD19 CAR T-cell. After, the T cell can bind to the cancer cell and release a cytotoxic reaction to cause the cell to proliferate. This figure was created through BioRender.

However, like all other T cells, CAR-T cells have to undergo T-cell exhaustion, a progressive loss of function that occurs after prolonged exposure to antigens, such as those on cancer cells. In normal immune responses, T-cell exhaustion helps prevent overactivation and protects healthy tissue from immune damage. However, in CAR T-cell therapy, this exhaustion limits the cell's ability to continue attacking tumors, reducing long-term efficacy. Exhausted CAR T-cells have reduced cytotoxic capabilities.[17] This is one of the main challenges regarding CAR T-cell therapy, and a sustained reduction in tumor growth and stability. Only by understanding the complex process and structure of the CAR T cells can one advance their design, enhance their efficacy, and avoid issues like T-cell exhaustion.

Structural Milestones: Future Generations of CAR T-Cells:

The concept of infusing cells into patients with hematologic cancers started coming into focus in the 1950s. It was not until the early 2000s that doctors were exploring the idea of genetically advancing T-cells by embedding genes to affect certain factors. The development of CAR T cell therapy since the 1990s has been divided into different generations to show how it has advanced over time. (Figure 4)

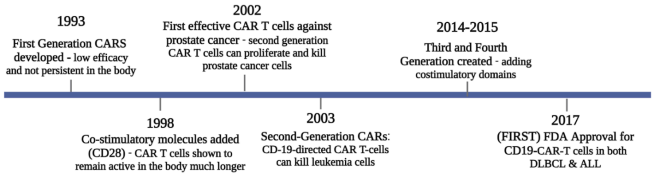

Figure 4: Timeline of the progression of CAR T cell therapy and its generations from 1993 to 2017 and further on as generations improve further to better accommodate different illnesses beyond cancer. This figure was created through BioRender.

The first generation (1993) included the two key structures in a CAR T cell, the antigen-binding domain region and the intracellular domain. The former contains a single-chain variable fragment that helps the cell recognize and bind to target cancer cells, while the intracellular domain contains a CD3ζ signaling domain to help coordinate signals through the CAR T cell. The early design of these CAR cells allowed them to bind to the cancer cell and release cytokines like IL-2 to activate other immune cells at the tumor site and induce apoptosis.[18] But without co-signaling domains like in the present design, the first-generation CAR T cells had reduced proliferation, meaning there would be a smaller number of active CAR T cells, limited cytokine release that created a weakened immune response, and reduced persistence in the body. (Figure 4)[19] Due to these limitations, the first generation of CAR T cells was far less effective at destroying large numbers of tumor cells than was considered the primitive design.

By identifying these limitations, the new design of second-generation CAR T cells included both the intracellular domain and co-stimulatory molecules like CD28 or 4-1BB. As shown in clinical studies, 4-1BB-based CARs had a longer persistence and strong response but a weaker activation compared to CD-28-based CARs, which had a quicker response, greater T cell expansion and survival, and quicker signal

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ing.[20] This created the conclusion that CD-28 CARs would be more efficient in targeting cancer cells, especially with CD-19 antigens. This additional signaling domain enhanced T cell activation, increased proliferation, and cytokine production.[21]

The CAR T-cells can now target CD19 on B-cell malignancies and are being implemented in clinical practices.[18] All current FDA-approved CAR T-cell therapies use this generation's designs as of 2023.[22] However, there were still issues regarding the persistence of the CAR T-cells and cancer relapsing in single co-stimulatory signaling domains, which is what scientists decided to improve in the third generation. (Figure 4)[23]

CAR T-Cell Therapy for Blood Cancers and Solid Tumors:

Individual cancers have varying responses to CAR T-cells due to different tumor structures, antigen expressions for other types, and the tumor microenvironment. Hematologic malignancies like certain leukemias and lymphomas often respond well to CAR T cell therapy, which can target specific antigens that reside on the surface.[7] However, solid tumors present additional challenges due to the expression of multiple antigens, immune suppression created by the tumors, and the barriers within the tumor microenvironment. These solid tumors form a hostile microenvironment with physical barriers like dense extracellular matrix and angiogenesis, which can prevent the CAR T-cells from binding to tumor cells.[8]

We performed a survey of clinical trials on clinicaltrials.gov using search terms "CAR T" or specifically "multiple myeloma" to obtain these studies. This data is not an exhaustive list of all ongoing or completed trials, of which there were 166 as of January 2021.[26] While CAR T-cell therapy has shown achievements in treating blood cancers such as Diffuse Large B-cell lymphoma, Multiple Myeloma, and B-cell Acute Lymphoblastic Leukemia, ongoing research is necessary to overcome the limitations and improve clinical trial results even with solid cancer types.

Table 1: A brief survey of published clinical trial data was conducted on clinicaltrials.gov. (Table 1) Here, we have outlined the posted results. This table was created through BioRender.

Cancer Type	Patients Enrolled	CR/CRi%	Phase	Generation of CAR T	Name and ID of Trial
Diffuse Large B-Cell lymphoma	93	40 / 12 %	Phase II	Second	JULIET; NCT02445248
Multiple Myeloma	113	67 / 30 %	Phase II / I b	Second	CARTITUDE-1; NCT03548207
B-Cell Acute Lymphoblastic Leukemia	36	81% achieved CR, only 26% stayed in CR	Phase II / I b & Phase I	Third	Pooled Analysis from ALLCAR19 and FELIX Phase Ib; NCT02935257 & NCT04404660
Solid Tumors	≈ 12	Still Recruiting	Phase 1	Fourth	MAGNETO; NCT05990751

Diffuse Large B-cell Lymphoma:

CAR T-cell therapy is particularly effective for hematologic cancers, including Diffuse Large B-cell lymphoma (DLBCL). Especially for patients whose DLBCL hasn't responded to other treatments, CAR T-cell therapy is a promising option since the therapy is incredibly personalized. The treatment directly takes the patient's T cells and modifies them to target antigens on cancer cells. This is much more effective than treatments like chemotherapy, where healthy cells may also be negatively impacted. CAR T-cells can target CD19, an antigen commonly expressed on malignant B cells in DLBCL but not on most normal cells. This targeted approach directs the CAR T-cells specifically to the cancer cells.[27] (Figure 3). This provides a precise and potent immune attack on the tumor antigens and avoids all other cells.

This therapy has shown successful results, especially in the JULIET trial using the CAR T-cell therapy, tisagenlecleucel (also known as CT019). This study involved 93 patients who received CAR T cell therapy and were evaluated after 14 months. In this trial, 52% of patients responded to the treatment, experiencing a reduction in the disease. Among them, 40% achieved complete remission, with no cancer detected upon re-assessment, while 12% showed only partial responses after receiving tisagenlecleucel.[28]

At 12 months post-treatment, 65% of the patients who first responded to the treatment were relapse-free, and the ones who reached complete remission had a relapse-free rate of 79%. The results suggest that a substantial portion of the patients maintained their response throughout the trial. However, this does not mean there were no adverse side effects during the trial; over 20% of patients experienced severe CRS, and 12% faced significant neurological issues, primarily immune effector cell-associated neurotoxicity syndrome (ICANS), which can include symptoms like confusion, seizures, and speech complications.[28] The trial was very effective, with overall response rates around 50% or higher in the trial. CAR T-cell therapy shows promising responses after relapses, with notable remission rates, even when using second-generation CAR T-cells.

Multiple Myeloma:

Another excellent candidate for CAR T cell therapy is Multiple Myeloma (MM). This type of blood cancer affects plasma and, consequently, can affect the production of antibodies created to protect against harmful invaders of the body. In Multiple Myeloma, CAR T-cells target B-cell maturation antigens (BCMA); just like in Diffuse Large B-cell Lymphoma, the protein is found on the surface of harmful B cells (plasma) and makes an easy target for the CAR.[29,27] To distinguish between normal cells and multiple myeloma cells, it's important to note that, compared to other cancers, MM expresses very high levels of BCMA, while normal tissues show little to no expression of this antigen. Engineered CAR T cells can be designed to recognize the B-cell maturation antigens on myeloma cells, enabling them to activate and kill the malignant cells.[29]

One of the key trials showing positive results for CAR T-cell therapy in treating MM is CARTITUDE-1, which uses JNJ-4528 to target BCMA antigens. The trial consisted of 113 patients, of whom only 97 were given CAR T cell therapy. A year post-treatment, results showed that 97% of patients responded to the treatment, and 67% of patients had a complete response where cancer could not be detected after receiving treatment.[30]

Positive results started even in the first month after treatment, with heavy responses increasing over time, and the survival rate was fortunately stable at 89% at the end of the

experiment. While the positive responses produced by this clinical trial were high and made up the majority of the patients, there was also a significant risk of adverse effects like DLBCL. 95% of the patients experienced CRS after receiving treatment. While the cases were resolved only 24 days after seeing symptoms, this seemed to be the most common adverse event. Only 4% experienced severe grades of CRS.[30]

B-cell Acute Lymphoblastic Leukemia:

A third promising application of CAR T cell therapy is in B-cell Acute Lymphoblastic Leukemia (B-ALL), also due to its high expression of CD19 antigen. B-ALL is an acute leukemia, so the disease progresses fast, and diagnosis and treatment need to follow closely with each other to reduce the mortality rate of the patients.[7] CAR T-cell therapy can be a much more reliable form of treatment since the consequences affecting healthy self-cells will not be as much of an issue.[18]

In the trials ALLCAR19 and FELIX Ib, the use of the CAR T cell therapy (obe-cell) had a significant positive impact on the remission rates of the patients. The trial states that there were 36 patients in total from the two trials, and 81% of these patients achieved complete remission with CR or CR with an incomplete hematologic recovery after receiving the obe-cell transfusions. After a follow-up of 43 months, 26% of patients remained in remission, with continued observation in 13 patients to check for signs of remission. Notably, 91% of the patients who continued to respond well to the treatment still had CAR T-cells in their bodies and did not show signs of remaining cancer, and the overall survival rates were 39% after 4 years.[31]

While the statistics from the trials were overall negative, and the number of people who remained in remission was quite low, B-ALL itself is an aggressive cancer that progresses quickly and has a high relapse rate.[7,31] Common treatments like chemotherapy and radiation often do not lead to significant remission, whereas CAR T cell therapy shows more promise. As research and technology continue to advance, CAR T cell therapy is expected to undergo significant improvements that could enhance its effectiveness in treating B-ALL.[7] Future developments include improvement in the design of CAR constructs to target additional antigens such as CD22 and BCMA, which is useful in overcoming antigen escape and resistance methods in leukemia and other cancers. Some strategies also involve dual targeting techniques, where CAR T-cells are engineered to recognize both CD19 and CD22 to increase treatment efficacy and reduce relapse rates.[32]

Solid Tumors:

While CAR T-cell therapy has shown significant progress in treating blood cancers, its application to solid tumors remains challenging, and most research in this area is still in the development phase.[8] Researchers have started to experiment with using CAR T cell therapy for neuroblastoma. Neuroblastoma overexpresses antigens like the ganglioside GD2, and CAR T cells can be designed to target these specific antigens while disregarding normal cells. Unlike other solid tumors, neuroblastoma has a more favorable immune microenvironment that can allow for better T-cell infiltration and activity. This solid tumor can produce pro-inflammatory cytokines that can attract immune cells and eventually induce an immune response, unlike some tumors that constantly suppress immune activity.[8] Because of this, multiple clinical trials regarding Neuroblastoma have been taking place with CAR T cell therapy. However, all of these trials still don't have results available to the public, indicating that even though steps have been made to start including solid tumors as a category for CAR T cell use, there is still a long way before this becomes a viable treatment.

Navigating the Obstacles of CAR T-Cell Therapy:

One of the biggest limitations that has hindered the progression of CAR T-cell therapy is the inability to penetrate solid tumors.8 One of the reasons for this obstacle is the difficulty in finding antigens on the surface of solid tumors. Solid tumors have a dense and complex microenvironment containing connective tissue, immune cells, and blood vessels, which creates physical restrictions for CAR T-cells penetrating to tumor tissue from the surface. Solid tumors also express heterogeneity in their cell surface antigens, meaning not all cancer cells express the same antigens. This makes it difficult for CAR T-cells to target all the cancer cells on a solid tumor's surface.[35,36]

Engineered T cells are designed to recognize a specific antigen, and if that antigen is not expressed throughout the tumor, some cancer cells will be able to evade destruction. Another immune evasion mechanism from solid tumors could be down-regulating the expression of harmful antigens or increasing the activity of inhibitory molecules that weaken T-cell function. This limits the effectiveness of CAR T-cells and makes it harder to identify harmful antigens in cancer cells.

The tumor's microenvironment can also be immunosuppressive, as cytokines can inhibit T cell activation and may not be able to maintain their activity after reaching the tumor.[36] Some immunosuppressive molecules in tumor cells worth mentioning are program death 1 (PD-1) and program death ligand 1 (PD-L1). Solid tumors express PD-L1, which binds to PD-1 on CAR T-cells; this interaction can reduce the efficacy of CAR T-cells and prompt T cell exhaustion within a tumor's microenvironment. Some trials are currently being developed to inhibit the binding of PD-L1 to PD-1 interactions, like combining CAR T-cells with checkpoint inhibitors to increase anti-tumor activity.[35]

Complications regarding Treatment:

One of the complications regarding CAR T-cell therapy is CRS. CRS is one of the major side effects that occurs when activated immune cells release large amounts of cytokines into the blood, leading to an extreme inflammatory response. Some causes of CRS are after T cell activation and high tumor load.[10] When CAR T-cells bind to their tumor antigens, they activate and signal the CAR T-cells to proliferate for an immune response against the cancer cells. After being activated, CAR T-cells can release cytokines like IL-2, TNF-alpha, and IL-6, creating even more immune activation in the form of inflammatory responses.[37]

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Patients with many tumor cells can have a more unstable immune response because more tumor cells can lead to greater CAR T cell activation, increasing cytokine release. Some of the symptoms of this side effect include mild or high-grade fever, nausea, severe migraines, respiratory distress, etc. This side effect can range from grade 1 severity (not requiring treatment) to grade 4 (with life-threatening symptoms needing emergency medical care). Still, regardless of the severity, CRS is a very common side effect after CAR T cell therapy.[4] 77-93% of patients with leukemia and 37-93% of patients with lymphoma who received CAR T cell therapy had any grade of CRS.[38]

Mild CRS can be managed with IV fluids and antipyretics, while more severe cases of CRS might need corticosteroids to hinder the immune response and reduce inflammation; however, there is no definitive cure for CRS.[4]

In addition to CRS, immune effector cell-associated neurotoxicity syndrome (iCANS) is also a significant toxicity that can be seen during the CAR T-cell treatment. After the infusion of CAR T-cells, the engineered immune cells become activated after recognizing and attacking tumor cells, leading to the release of inflammatory cytokines. Some symptoms of iCANS are confusion, seizures, and other cognitive defects, showing the impact of an activated immune system on the central nervous system. The occurrence and severity of iCANS are correlated to the severity of CRS that may occur at the same time, and just like CRS, corticosteroids with the most efficient way to reduce the inflammatory response affecting the body.[10] This occurrence of toxicity highlights the balance between the efficacy of CAR T cell therapy in targeting cancer cells and the potential for neurological side effects.

One of the causes of these forms of toxicity is on-target, off-tumor toxicity, where CAR T-cells may attack healthy self-cells that express low levels of the same target antigen present on the surface of the tumor cells.[39] Since CAR T cells are programmed to recognize these specific antigens, the cells might inadvertently bind to healthy tissues and destroy them in the process of targeting cancer cells.

For example, in some therapies targeting CD19 antigens found in blood cancers, CAR T-cells sometimes attack self-B cells, which typically also express CD19. This leads to a decrease in healthy B cells, known as B cell aplasia, and results in an increased risk of infections since B cells are necessary for antibody production. Specifically in cases of acute lymphoblastic leukemia/Lymphoma (expressing the CD19 antigen), when patients are treated with CAR T-cell therapy, nearly 100% of patients have some moderate toxicity symptoms in terms of CRS and iCANS.[7]

Some methods to combat this off-tumor toxicity are using dual-targeting CARs and safety switches. Dual-targeting CARs require CAR T-cells to recognize two antigens at once, reducing the chance of attacking normal cells that express only one of the two target antigens.[39] However, if toxicity occurs, scientists could engineer CAR T cells with "suicide genes" that allow them to deactivate the T cells before the toxicity spreads further to the organ systems.

Future Innovations: Car T-cell Therapy for Infectious Diseases:

On top of cancer-type malignancies, scientists have been trying to implement CAR T cell therapy into infectious diseases, specifically HIV. However, the progress isn't substantial, as CAR T-cells still have many flaws in incorporating CAR T-cells in HIV, such as viral escape, CAR T cell infectivity, and access to hidden viral reservoirs. Doctors first believed that, compared to vaccines and immune checkpoint inhibitors, chimeric antigen receptors might have more success in enhancing immune responses against HIV-infected cells.[33] The first clinical application, in 2022, tried to treat HIV by changing the cytolytic properties of CD8+ T cells to now express chimeric antigen receptors with a CD4 extracellular domain that can recognize and target specific antigens on HIV-infected cells.[34] This creates a specific immune response that is separate from the MHC, so even if the HIV cells reduce their expression of MHC I to evade detection from immune cells, the CAR T cells can target and destroy them.

Regardless of the laboratory studies showing that the therapy was safe and that using CAR T cells had a good survival rate in patients, it couldn't prevent the relapse of HIV infection. On top of this, compared to blood malignancies, there were much lower levels of the targeted antigen since not all the infected cells didn't express the HIV envelope glycoprotein (Env).[34] This prevented the full effectiveness of the CAR T-cell therapy. The issues above carried over throughout most of the clinical trials conducted with HIV patients, and HIV-infected cells might continue to evade the immune system's detection through viral escape, but CAR T Cell seems to be a feasible treatment for the patients compared to antiretroviral therapy or entry inhibitors that don't account for HIV reservoirs.[33] Overall, despite these challenges, some clinical trials in the future seem to show a promising future down the line for CAR T cells in HIV therapy.

Conclusion

Chimeric Antigen Receptor therapy has emerged as a groundbreaking advancement in the field of immunotherapy, progressing from oncology studies and providing significant survival opportunities for patients with blood cancer. This review paper has highlighted the current stage of CAR T-cell therapy, including the structure of the technology, clinical success, side effects, and future advancements in the field. Despite its current success in the oncology field, specifically in hematological malignancies, CAR T-cell therapy faces challenges such as the ineffectiveness against solid tumors and chronic side effects like CRS and iCANS that persist after treatment. Currently, ongoing research into multitargeted CAR T cells and combined therapies seems to be the most efficient ways to address these limitations. Although the treatment is still in its early stages, CAR T-cell therapy is paving the path for personalized cancer treatments, specific to the target antigens on cancer cells, and as our understanding of the anatomy of cancer and immune responses increases, its potential in cancer care will continue to expand and become a catalyst for future advancements in the battle against cancer.

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