Proceedings of the 3rd International Conference on Biological Engineering and Medical Science
DOI: 10.54254/2753-8818/22/20230978

CAR T cell therapy: Current limitations and advancements in treating solid tumors

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Abstract. An emerging technology that tackles cancer via immunotherapy known as CAR T cell therapy has been gaining great popularity after its success in treating leukemia and other forms of blood cancer. However, major advancement to treat other forms of cancer have not been attained as this treatment is still facing major obstacles in addressing solid tumors due to tumor heterogeneity, hostile microenvironment, on-target/off-tumor toxicities, antigen specificity, and difficult infiltration. Moreover, this treatment has also been associated with threatening side-effects like mass dieoff of antibody producing B cells, CRS, and ICANS. Thus, because of simplified manipulation yet lack of knowledge of the physiology behind the mechanisms, significant progress has not yet been achieved-though on its way. Scientists explored multiple ways of addressing these major challenges by researching on feasible techniques, antigens, and mechanisms that can increase T cell reproduction, decrease toxicity, overcome and infiltrate into the hostile microenvironment, and target multiple antigens via TAA. It is a technology that is not yet fully developed; therefore, physicians still opt to target cancer via other forms of immunotherapies, adjuvant and neoadjuvant therapies including chemotherapy, drug consumption, and surgery. As clinical results for CAR T cell therapy have not presented prosperous results in targeting solid tumors, the future for research on solid tumors is still unclear. Scientists might either opt for other methods like microwave ablation, the use of TT fields, different combinations of current therapies to maximize efficiency, and ADCs.

Keywords: CAR T cell therapy, side-effects, determinants, solid tumors, technology.

1. Introduction

CAR T cell therapy is composed of a core component, an extracellular hinge domain, a transmembrane domain, and an intracellular signal domain. Its is known as a living drug because its chimeric antigen receptor allows a patient's own T cells to recognize and bind to tumor antigens in an HLA-independent manner and beyond their natural surface receptor [1]. It is personally customized to each patient in ex vivo, meaning that the T cells are collected and engineered in laboratories to produce chimeric antigen receptors (CARs), reproduced, and then injected into the patient's blood [2]. Under ideal circumstances, CAR T cells should continue its reproduction after being reinjected, allowing the T cells to target the cancerous cells.

In 2017, the United States Food and Drug Administration (FDA) authorized six CAR T cell-based therapies and two antigens, CD19 and BCMA, to treat B cell acute lymphoblastic leukemia (ALL) and

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diffuse large B-cell lymphomas (DLBCL) [2]. This treatment has achieved great success particularly in treating B-ALL with anti-CD19 CAR T cells with high complete remission rates of 70% to 94% [2]. Moreover, according to the NCI, around 60% of the children who received this treatment stayed alive without any cancer resurgence and the development of disease-related problems.

There have been three variations of T cell activation. The first generation involves the regulation of IL-2 secretion and activation of cytolysis and of tyrosine motif on CD3ζ chain or FcεRIγ, providing the "signal I" [3]. Its anti-tumor activity is limited only to in vivo, and is associated to apoptosis due to decreased reproduction of T cells. The second generation carries a "signal II" as it has an additional costimulatory signal constituted of CD28, CD137 or even NK cell receptor CD244 to encourage consistent activation of CAR T cells. Therefore, this generation was able to attain advantages like increased T cell proliferation, enhanced cytokine and anti-apoptotic proteins secretion, and delayed cell death. As per the third generation, scientists are combining two costimulatory signals with the goal of improving CAR designs. A study conducted in New York combined CD28 and 4-1BB costimulatory signaling domains to build a prostate specific membrane antigen (PSMA) which was successful in inducing "strongest PI3K/Akt activation and Bcl-XL expression in vitro, and the least apoptosis in transduced peripheral blood CD8+ T cells" [4]. However, comparisons between the second and third have shown no clear advantages to one specific side, and its clinical efficiency is still unclear. Exploring CAR T cell therapy, this paper has a main purpose to summarize the discovered key factors that help to determine the success of this treatment, the mechanisms of resistance and the ways to address them, current advancements on treatment for solid tumors, and future prospective [5].

2. Determinants and Mechanisms of Resistance

A study conducted by the University of Pennsylvania performed genomic, phenotypic and functional evaluations to identify a series of determinants of the success and failure of CAR T cell therapy in patients. They reached significant conclusions that the composition of the cellular product, intrinsic T cell fitness, and differentiation state of the cells after ex vivo expansion directly influenced the response of the therapy [6]. For example, for complete remission (CR) patients, the success of CD19 CAR T cells demanded in vivo cell expansion, showing decreased tumor progression, thus tumor morbidity, while partially (PR) and nonresponding (NR) patients were characterized by high levels of key regulators of late memory and T cell. Moreover, pharmaceutical inhibition of glycolysis and the activation of STAT3 in this treatment could have promoted the formation, expansion and maintenance of T cell memory. However, the activation of STAT3 in CAR T cells was also associated to decreased reproduction of T cells due to reasons not yet understood [2].

A major by-product of CAR T cell therapy is the mass die-off of antibody producing B cells, infections, and cytokine release syndrome (CRS). CRS, considered as a highly life-threatening type of on-target/off-tumor toxicity, occurs when CAR T cells release extensive amounts of inflammatory cytokines after antigens bind with target tumor cells as shown in Figure 1. It can lead to symptoms like fever, fatigue, multiorgan dysfunction in severe cases, and even harm healthy cells and organs within the patient's body [7]. CRS is mainly treated with steroids or by blocking IL-6 with its IL-6R inhibitor, a drug known as tocilizumab [8]. Studies have shown a close relationship between the severity of CRS and the extent of cancerous cells present in the patient's body, and the amount of T cells working. Therefore, these discoveries emphasize the importance of finding safe antigens that reduce toxicity and prevent the complete abolishment of T cell function. A feasible approach is to manipulate CAR expression or by introducing a switch triggered when under severe immune-related adverse events (irAE), an inhibitory CAR (iCAR) capable of recognizing antigens present only in normal cells, or suicide genes that may help to control unwanted toxicity [2].

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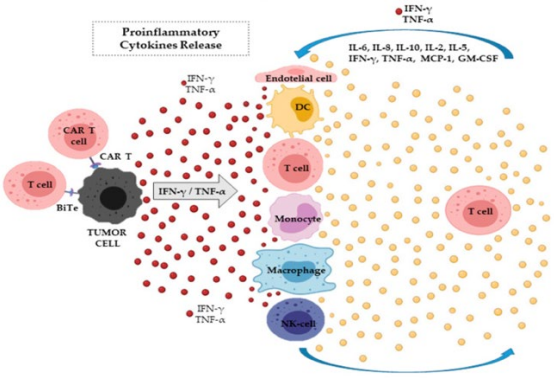

Figure 1. Sequence of events that lead to CRS [1].

Another endangering consequence is immune effector cell-associated neurotoxicity syndrome (ICANS), a neurologic side effect that often occurs either during or after CRS. Its causes are still unclear, and its symptoms include severe confusion, seizure-like activity, impaired speech, toxic encephalopathy, motor weakness, and cerebral edema. Similarly, studies have noticed an association between the degree of severity to CAR T cells expansion, which tend to be found in patients' cerebrospinal fluid. Some suggest that increased T cell traffic to the central nervous system happens because of the endothelial activation and blood-brain barrier disruption, while others believe it to be a consequence of myeloid cell action [9]. As there is little understanding behind this neurotoxicity, ICANS is mostly dealt with corticosteroids and with supportive care for low-grade toxicity.

Most importantly, recognition of toxicities at an early stage is crucial to prevent severe consequences of CAR T cell therapy. Thus, CARTOX group has proposed different grade levels of CRS and neurologic toxicity, suggesting the appropriate and specific methods of control and treatments for each side-effect [10].

3. Treatment for solid tumors

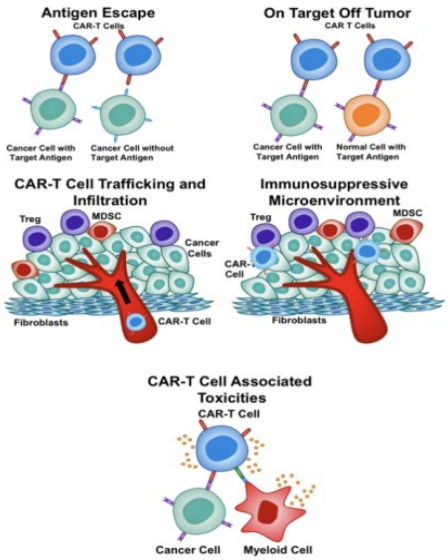

Figure 2. Current struggles in addressing solid tumors via CAR T cell therapy [1].

Researchers are facing major challenges identifying feasible proteins and antigens on solid tumors like breast, brain and kidney cancer for CAR T cells to aim. The main reasons that are preventing T cells

DOI: 10.54254/2753-8818/22/20230978

from mobilizing and invading tumor lesions are: hostile tumor and immunosuppressive microenvironment, on-target/off-tumor toxicity, antigen specificity, and secretion of chemokines – as depicted in Figure 2 [2].

As CAR T cells are inhibited from carrying out their function because of the physical barriers and the immunosuppressive molecules produced in the tumorous microenvironment, scientists have addressed this limitation by modifying T cells to express chemokine receptors of the same released by the solid tumors. A successful study in demonstrating how T cells could migrate towards melanoma cells via chemokines secreted by tumor cells was conducted by Kershaw on CXCL1 receptor [2], with its underlying mechanisms being illustrated in Figure 3. They were able to conclude how CD28 costimulatory molecules assisted and enhanced T cells in overcoming the hostile microenvironment by resisting to Treg cells and overcoming TGF-β-mediated repression [2]. Another solution is to directly deliver CAR T cells to the desired location via intraperitoneal and intertumoral injections to eliminate the physical barriers [2]. However, this strategy only becomes feasible for tumor cells found in easily accessible areas like the scenarios in ovarian cancer.

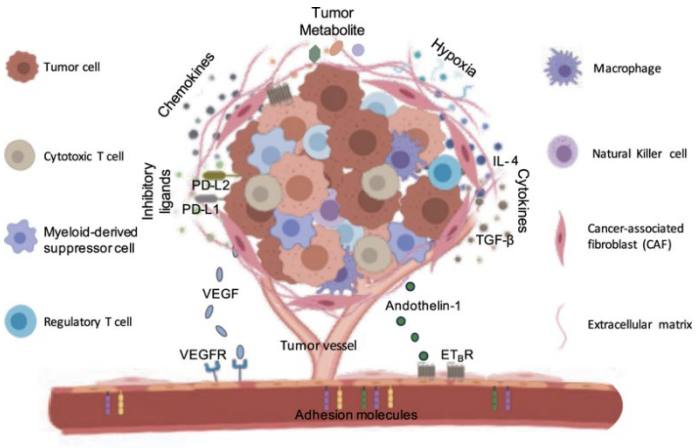

Figure 3. Chemokine receptors as an approach to target tumor cells [1].

As solid tumors are placed in hostile microenvironment known for its complexity due to histopathological features like concentrated blood vessels, tumor-associated fibroblasts, extracellular matrix, hypoxia, immune suppressor cells, and tumor-derived cytokines, infiltration continues to be a major challenge that has shown little development as it involves rolling, adhesion, extravasation and chemotaxis [2]. However, researchers have proven that IL-12 was successful in altering the tumor microenvironment, in eliminating antigen-negative tumor cells, and in prolonging survivability of T cells through recruiting and activating existing macrophages in one's body. Moreover, dominant-negative TGF receptors and armored CAR T without PD-1 expression have also helped inhibit immunosuppressive cytokines, enabling cells to navigate through this type of environment by secreting proinflammatory cytokines and other molecules, and to improve antitumor activity. Other groups have found that CAR T cell targeted in fibroblast protein activation protein enhanced not only cytotoxic functions, but also its infiltration. Plus, if the cell was expressed with HPSE, an enzyme that degrades HSPG, CAR T cells had improved capabilities to degrade the main component of stroma, ECM [2].

Tumor heterogeneity, in other words, the same cancer being molecularly different between individuals, or even within the same individual, is another significant burden as it largely decreases the population of tumor cells that can be targeted. Currently, EGFR variant III (EGFRvIII) is believed to be the solemn gene expressed only in human cancer cells and not in healthy cells, thus, the only authorized tumor-specific antigen (TSA) for CAR T cells [2]. However, as they have not shown promising results, most research is now opting for tumor-associated antigens (TAAs), which is said to be more effective in both eliminating a larger group of tumor cells and preventing on-target/off-tumor toxicities by tgargeting multiple antigens at once. The basic approach to TAA is to build a CAR pool formed by

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either two unispecific CAR T cell products, or by a single T cell platform that expresses biCARs, triCARs, and even tanCARs. An example of biCARs is the combination of HER2 and IL13Rα2, which has indicated greater efficiency together with less antigen escape variants than CAR T cells targeting only the first antigen [2]. As per tanCARs, they can recognize each antigen individually and strengthen their activity when both antigens interact. Another way that this can stimulate safer CAR T cell therapy with increased specificity is by setting a mechanism to only allow T cells to activate when both selected CARs are expressed in a cell or via an affinity-tuned CAR, with the latter showing more effective results in maintaining potent antitumor efficacy. However, TAA can in fact be a very damaging technique as the antigens are not exclusively found on human cancer cells. In view of this, it is crucial to check the level of expression of the target antigens on tumor cells via immunohistochemical (IHC), which in optimal conditions, the antibody should bind to the same epitopes as the CAR [2], and should in fact be 100% expressed in tumor cells but not in normal cells – hence the success of CD19 [1].

Another factor to consider is that solid tumors tend to suppress the function of the immune system by overexpressing inhibitory immune-checkpoint ligands [2]. Therefore, by blocking ligands like the PD-L1, which inhibit CAR T cell activation, with a specific antibody or dominant negative receptor, CD28 CAR T cells with constitutive anti-PD1 secretion, for example, can increase in functionality, expandability and efficiency when eradicating tumors specific to a human lung carcinoma xenograft mouse model. However, with the clear complexity behind all these mechanisms, it is extremely challenging and time-consuming to find exactly which antigens, receptors, and molecules that will maximize the benefits of this treatment.

4. Discussion

In view of all the information presented, it can be observed how CAR T cell therapy is highly versatile in the sense that it can be manipulated into different ways in order to accommodate and reach the desired result. Although it has a great potential, there has been a clear pattern to make this technology become even more narrowed and specific to certain diseases as a general one has not achieved promising results. CAR T cell therapy has an arguably uncertain future, as it can both further advance with increased discoveries on genetic engineering or be substituted by other developing technologies like microwave ablation and antibody-drug conjugates due to its complexity.

With artificial intelligence rendering the world even more technological, researchers might be looking for possible ways of combining AI and CAR T cell therapy and using AI to understand the mechanisms behind unknown phenomenon. This means that with increased knowledge, CAR T cell therapy will be closer to attaining desired results when combining with TALON and CRISPR, which are already being used to induce donated T cells to produce CARs [1]. Moreover, another way to perceive CAR T cell therapy is by allowing one's human body to produce its own CAR T cells via nanotechnology and mRNA-based approaches.

On the other hand, the major limitations of this treatment still close multiple doors. The treatment for solid tumors already perceives CAR T cell therapy as an eliminated option under current circumstances, meaning that CAR T cell therapy might head on a downwards slope with regards to clinical trials and research. This is specially aggravated as other technologies have shown to be more promising like microwave ablation, currently being developed by Johnson & Johnson on its Lung Cancer Initiative (LCI), and antibody-drug conjugates (ADC), a technology that is extremely similar to CAR T cell therapy but does not face the current limitations on antigen specificity and toxicity levels. Therefore, the current trend indicates how solid tumors will be treated via the combination of existing treatments like chemotherapy, surgery and traditional forms of immunotherapy in an enhanced manner. For example, ADCs are meant to further enhance chemotherapy delivery to tumor cells, and existing studies like CheckMate 816 has shown combinations that eradicate and target tumor cells more effectively.

Moreover, CAR T cell therapy is highly costly and generally only utilized when patients are under critical conditions that have not responded to other treatments. This indicates that patients and their families under this circumstance become highly vulnerable, further stipulating a decreased demand for

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this treatment, especially when compared to other more accessible and traditional treatments that have proven to be more effective. Nonetheless, cancer is a disease in which its treatment becomes highly unfavorable when discovered in later stages, thus, the development of technologies that help to increase tumor detection in earlier stages is crucial. Therefore, the approach should perhaps be to invest more in developing research on biomarkers, radiomics, detection of circulating tumor DNA (ctDNA) via liquid biopsy – all helping physicians to understand the current knowledge more in depth and to detect cancerous signs at an early stage.

5. Conclusion

With immunotherapy becoming increasingly more utilized to treat cancer after it was able to eradicate advanced tumors, chimeric antigen receptor-modified (CAR) T cells therapy has a great potential in the near future, especially in a decade in which technologies are advancing exponentially after each remarkable jump. Cancer treatments have relied on surgery, chemotherapy, radiation therapy, and immunotherapy for decades long. With the emergence of this treatment, it has the potential to in fact become a leading and standard treatment for lymphomas, especially after its success in treating blood cancers like leukemia. However, as it is not yet fully developed and there are still many limitations and gaps of knowledge, its utilization should be careful, and its consequences should be transparent. Nonetheless, CAR T cell therapy was a crucial mark on the revolution of cancer treatments, and a lever that will fuel further progress, helping researchers to further understand the mechanisms which rely beneath cancer activity, and enlightening different approaches to this long haunting disease.

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