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The brief introduction of CAR T-cell: From mechanism to application

Lin Yuna Q

Mercer Island High School, Mercer Island, 98040, US

yuna.lin@misd400.org

Abstract. Chimeric antigen receptor T-cell (CAR-T) gene therapy is a newly developed solution to fight tumor cells. The therapy develops from the combination of the immunity and cell bioengineering, taking advantage of the cell killing function of T-cells, scientists modify them with a bioengineering method to target specific cells. In recent days, this therapy shows great results in several trials especially in hematological malignancies. However, there are still many obstacles, such as toxicities, that need to be overcome. Taking the promising potentials of CAR T-cell gene therapy, it is still a revolutionary step in the improvement of immunotherapy. In this paper, we focus on the structure and target antigens of CAR T-cell, summarize the generations of CAR T-cell gene therapies, as well as list out the FDA approved treatments using CAR. Most importantly, we will discuss the past advancements, recent innovations, and the existing factors that still prevent CAR T-cells from becoming the first line treatment for cancers.

Keywords: CAR T-cells, immunotherapy, toxicities, tumor therapy.

1. Introduction

In 1986, scientists at the Surgery Branch of the National Cancer Institute, one of them being Steven Rosenberg, started using tumor-infiltrating lymphocytes as a treatment for cancers [1]. Tumor-infiltrating lymphocytes naturally occur in a patient's cancer tumor and function to kill cancer cells. Scientists remove these lymphocytes from the patient and take them to a lab where it is multiplied in numbers before it is placed back into the bloodstream, strengthening the body's ability to fight cancer. In 1992, a student of the Whitehead Institute at the Massachusetts Institute of Technology, Michel Sadelain, started the use of retroviral vectors, the RNA of a virus, to bring new segments of DNA to the T-cells in order to strengthen their attacks on cancer cells.

Chimeric antigen receptors were first created in 1993 [1]. At the Weizmann Institute, immunologist Zelig Eshhar developed the first CARs, though due to its simple components, it showed limited efficiency. CAR-T is a newly developed immunotherapy that has been created in recent decades. It is a promising treatment for many blood cancers: leukemia, lymphoma, and multiple myeloma [2]. T-cells are looking for a protein that surrounds the surface of cells, but on their own sometimes will not recognize a specific antigen and therefore cannot fight the specific disease [2, 3]. When CAR receptors are added to the T-cells, the cells will be able to fight and destroy antigens on cancer cells [3].

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2. CAR T-cell gene therapy

2.1. Structure of CAR

There are four main parts (Figure 1.) in CAR, the antigen binding domain, the hinge region, the intracellular signaling domain, and the transmembrane domain [4].

The antigen binding domain or antigen recognition region recognizes antigen receptors and is found on the surface of a cell [4]. Some of the antigen binding molecules are antibodies, cytokines, growth factors, innate immune receptors, structure protein, and tumor necrosis factor receptors. This region is made by single-chain variable fragments, light and heavy immunoglobulin chains, linked by serine-glycines or glutamate-lysines. Between the antigen binding domain and the cell membrane is the hinge region or spacer which provides more flexibility to the chimeric antigen receptors. Shorter spacers provide easier contact between antigen binding molecules and the distal membrane of the T-cell, while longer spacers provide easier contact between antigen binding molecules and the proximal membrane. Spacers from Immunoglobulin G or IgG have the two domains, CH2 and CH3. When the CH2 domains are deleted from this type of spacer, the issue of CAR T-cell off-target activation can be solved. The intracellular signaling domain is located inside of the chimeric antigen receptor and transmits the outer signals of antigen binding to the inside of the T-cell. This requires costimulatory domains or molecules like CD-27, CD-28, CD134, and CD-137 that signals for an immune response after a target antigen is discovered on a cell. The transmembrane domain is located between the hinge region and the intercellular signaling domain, with its alpha-helix structure that is water resistant, stabilizing the surface chimeric antigen receptors.

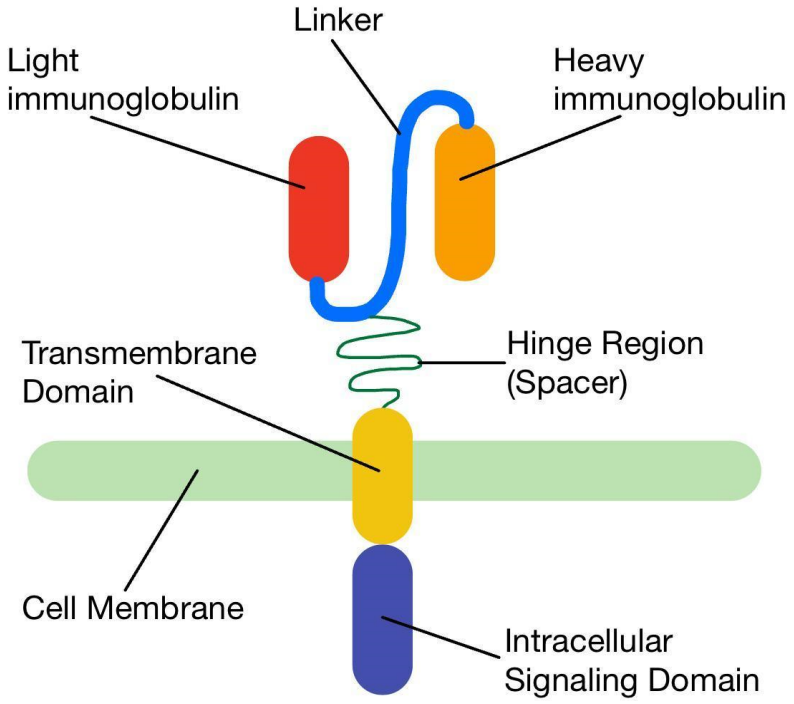

Figure 1. The structure of CAR.

2.2. CAR T-cell generations

The first generation of CAR T-cells contains only the CD3 intracellular domain and an external single-chain variable fragment [5]. The issue with this generation came to be the insufficient signaling pathways and lack of cytokine production, which is essential to the functions of T-cells. The second generation of CAR T-cells added one costimulatory domain with the CD3 intracellular domain which boosts cytokine production, T-cell life span in the patient's body, and response to fight tumors. The third generation of CAR T-cells put together multiple costimulatory domains which improves the production

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of cytokines, T-cell life span, and efficacy against tumors. The fourth generation of CAR T-cells, TRUCKS, had artificially modified proteins added to the third generation CAR which helps T-cells resist the immunosuppressive microenvironment around the tumor and brings the immune system into the fight against them. The fifth generation of CAR T-cells binds transcription factors to the protein, which stimulates the immune system, makes sure the T-cell continues its functions, and creates memory T-cells.

2.3. CAR target antigens

The target cancer antigens that researchers look for are those that are located on the surface of cancer tumor cells, produced by genes that are mutated, and different from the cells of vital organs in the human body [6]. The three types of CAR target antigens are tumor specific antigens, tumor associated antigens, and embryonic cancer germaine antigens.

Tumor specific antigens are located on malignant cells only [6]. It is unlikely that it will cause on target off tumor toxicity, but these antigens are hard to find and can be shared with other essential organs. Tumor associated antigens are mostly found on malignant cells, though some are also found on healthy cells. These antigens are easier to find, but like tumor specific antigens are likely to cause on target off tumor toxicity. These antigens also require a lot of preclinical validation for its safety in use. Embryonic cancer germaine antigens are mostly found on healthy cells, especially the cells of testes and ovaries, though some are also found on malignant cells. They also mainly appear on embryonic cells and not adult cells. These antigens control cell functions and can be safer to use in treatments, though it has a limited number of antigens to work with and can possibly destroy healthy reproductive cells.

2.4. The process of CAR T-cell therapy

The procedure starts with leukapheresis. A catheter, a thin, flexible tube, is inserted into a blood vessel either at the back of the patient's neck or below their collarbone [3]. This catheter is connected to a machine that pulls out the patient's blood and separates out the T-cells before returning the rest of the blood back into the bloodstream. In the lab, an inactive virus is placed into the T-cells, adding additional genetic material into the cell, causing the cell to produce CAR receptors on the outside of the cell, and molecules that carry the activation signals. The T-cells are then allowed to replicate and multiply in number until there is a sufficient amount to place back into the patient. The cells are then frozen until they can be injected back into the patient's bloodstream. Typically, one more round of chemotherapy is performed on patients before the new T-cells are reintroduced to the body, in order to make sure the patient's body will not resist the cells.

3. Limitations

CAR T-cell gene therapy is still fairly new and lots of challenges are still being combated by researchers in order to improve this therapy including life threatening toxicities, use in solid tumor cancers, and inaccessibility to much of the population [7, 8].

3.1. Toxicities

Toxicities are a huge problem with CAR T-cell therapy, sometimes being life threatening [9]. This is one of the reasons that this therapy cannot become the first choice treatment for patients with blood cancer.

The most common toxicity is Cytokine Release Syndrome (CRS), when cytokines are quickly flowing into the bloodstream, caused by CAR T-cell activation from infections or immunotherapy [9]. Symptoms being fever, fatigue, headaches, rashes, and organ failure. The second most common toxicity is neurological toxicity, altering the normal functions of the nervous system [7]. One recent discovery is the immune effector cell-associated neurotoxicity syndrome (ICANS) coming from a blood brain barrier dysfunction resulting in memory loss, seizures, and headaches.

There are also other toxicity risks. The Tumor Lysis Syndrome (TLS) is a metabolic abnormality due to the breakdown of dying cancer cells, causing organ damage such as irregular heartbeats and kidney

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failure [7]. The Macrophage Activation Syndrome (MAS) is the uncontrolled activation of Macrophages or T-cells and can cause organ failure [9]. Anaphylaxis is an extreme, possibly life threatening allergic reaction to the treatment, hematologist toxicity is a blood toxicity that causes decreasing amounts of bone marrow or blood, and on-target-off-tumor toxicity leads to damage on cells without cancers resulting in dysfunction and deaths on those cells [7].

3.2. Inaccessibility

CAR T-cell gene therapy is difficult for patients to receive even if they reach all standards of the treatment. Only around 25% of patients who are eligible receive this treatment, because of the long wait times, a median of 6 months, caused by the delays of manufacturing [8]. Even with the opportunity to receive this treatment, cooperation between the manufacturers, patients, and medical centers are key to success.

CAR T-cell therapy is a personalized cellular therapy, using autologous, or patient cells [8]. The manufacturers are unable to take on many people at a time and have a limited amount of slots to fill up. For each patient, manufacturers only have between 3 to 5 weeks to modify and assess the cells, for their short shelf life, before they have to be injected back into the patient.

Through the Risk and Mitigation Strategy programs, it is stated that patients need to have a caregiver or some other family member or friend present with them at all times to monitor their conditions [8]. They also need to secure reliable ways of transportations or even temporary living spaces, as not many medical centers offer this therapy and may be far from where they live.

CAR T-cell therapy requires high levels of resources and an incredibly well trained staff [8]. Because of this, only some certified medical centers are able to offer this treatment. The already limited resources can strain medical centers as more cell and gene therapies are available.

This therapy also has really high costs, around 1.5 million dollars can be spent throughout the entire process [8]. And this high cost also causes around two thirds of US healthcare plans to not provide insurance coverage for this particular treatment. This excludes those who are not able to pay the huge expense, especially in the African American community where Multiple Myeloma is more common among the population. In the study of individuals being treated by CAR T-cell therapy in the US from 2016 to 2021 conducted by the Center for International Blood and Marrow Transplant Research, 79% of patients were White, while only 6% were Black.

3.3. The limited efficiency of solid tumors

Solid tumors have been a challenge for CAR T-cell therapy which have not made this treatment for patients of lung cancers or breast cancers, some of the most common types of cancers. One trouble is with tumor antigen escaping off of the cancer cell, causing the cancer cell to lose partially or all of its targeted antigen expression, making it hard for CAR T-cells to locate and destroy and causing the cancer to relapse in patients [7]. Another trouble is with difficult penetration into the tumor. The extremely dense fibers around the tumor become a barrier, blocking drugs from entering the tumor. Other issues in the application of solid tumors include:

1. A natural immunosuppressive microenvironment around tumor cells that causes CAR T-cells to lose their functions [7].

2. Tumor heterogeneity, common among tumor cells and healthy cells [7]. Its effects that are directed to tumor cells sometimes end up harming or killing healthy cells in the patient's body. It is very difficult to find antigen targets that only exist in damaged cancer cells.

3. CAR T-cell Exhaustion, caused by consistent activities of the T-cell, tiring itself out, and an increase in inhibitory receptors that block signals of CAR T-cells [7].

4. Strategies to improve CAR T-cell therapy

To face the challenges in solid tumors, researchers have started making discoveries that are showing the potential of CAR cell therapy in some solid tumor cancers. In non-small cell lung cancer, trial results have shown that when CAR therapies are used with target agents directed towards drug resistant cancer

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cells' surfaces, it slows down the process of the cells to become completely immune to treatment much more than only the target agents [7]. In small cell lung cancer, researchers have found more effectiveness with many specialized treatments of CAR T-cell therapy directed towards the different proteins surrounding subtypes of the cancer. In kidney cancer, a new CAR T-cell therapy treatment using allergenic cells has shown good results in early trials.

To eliminate antigen heterogeneity, scientists have been researching many strategies to allow the target to expand to multiple antigens [7]. Strategies have been using avidin-conjugated CAR together with biotinylated antibodies, split universal and programmable CAR, and leucine-zipper motif CAR with free scFv motifs. Small Molecules-Based or Chemogenetic-Based Switchable CAR T-cells can be used as an on or off switch to further gain control over CAR T-cell activities. Combinations of heparanase with anti-GD2 CAR T-cells is explored to increase the ability to penetrate the dense fibers and the immunosuppressive microenvironment around the tumor. Methods to decrease the chance to toxicities are lowering the antigen-binding domain affinities to avoid targeting of healthy cells with less amounts of antigens, changing the hinge and transmembrane parts of the CARs, and modifying design of the CAR through the costimulatory domain [9].

5. FDA approved treatments

So far, there are six treatments approved by the US Food and Drug Administration.

Axicabtagene ciloleucel is made for two cancers [10]. Adults who have follicular lymphoma who have relapsed or have been resistant to 2 or more lines of therapy can also receive this treatment. Adults who have large B-cell lymphoma and have been resistant to chemoimmunotherapy for the cancer or those who have relapsed within 12 months of the therapy can receive the axicabtagene ciloleucel treatment. This treatment however cannot be received by patients with primary central nervous system (CNS) lymphoma.

Lisocabtagene maraleucel also treats large B-cell lymphoma in adult patients [10]. The patients who experience relapse within one year of treatment or resistance, especially those who cannot receive hematopoietic stem cell transplantation, and adult patients who have received 2 or more lines of treatment with relapse or resistance. Like Axicabtagene ciloleucel, patients with CNS lymphoma also cannot receive this treatment.

Brexucabtagene autoleucel is made for two cancers [10]. One of which is the patients with mantle cell lymphoma (MCL) who have been resistant to other treatments or have experienced relapse. Another would be B-cell precursor acute lymphoblastic leukemia (ALL) patients with relapse or resistance to treatments.

Both ciltacabtagene autoleucel and idecabtagene vicleucel treatments are targeted for adult patients with multiple myeloma who have received 4 or more lines of therapy or treatment [10].

Tisagenlecleucel is used to treat three types of cancers, B-cell precursor acute lymphoblastic leukemia (ALL) patients younger than 25 years old and experiencing resistance or relapse 2 times or more and large B-cell lymphoma or follicular lymphoma adult patients experiencing relapse or resistance after 2 or more lines of therapy [10].

6. Conclusion

CAR-T cell therapy is the newly developed tumor therapy with more advantages than traditional tumor therapies. However, there are still many shortages. With many limitations still existing in CAR T-cell gene therapy, scientists have been able to discover strategies that can overcome some of the current challenges. These potential solutions to the many limitations can continue the long term process of strengthening CAR T-cell gene therapy and expanding this treatment to become an option for more cancer patients.

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