

CAR-T cell Therapy: Existing Treatments and Improvements

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Abstract. Nowadays, researchers have investigated many treatments for cancer diseases besides the existing chemotherapy and surgical removal. Among the field of cancer immunotherapy, CAR-T cell therapy has become more and more popular because of its effectiveness. CAR-T cell therapy, also known as chimeric antigen receptor treatment, is a novel and powerful form of immunotherapy that employs genetically altered T cells to more precisely identify and combat cancer cells. Specific cells that make up the immune system can defend against infection and cancer in humans. However, occasionally, cancer cells manage to escape the immune system, and therefore it's crucial for the immune system to find a more effective method to target and attack cancer cells. CAR-T cell therapy is one of the innovative treatments that kill cancer cells [1]. Until now, the scientists have discovered four generations of CAR-T cell therapy. From 2017, CAR-T cell therapy started to be approved by FDA as the standard of care for some forms of relapsed or refractory blood cancers. But since CAR-T cell therapy is very new, it still has some limitations such as cytokine release syndrome, or called CRS, and neurological toxicity. Moreover, CAR-T cell therapy still have difficulties for attacking solid tumors. Since the T cells sample will be taken from the patients' bloods and the CAR-T cell indeed could target and recognize specific cancer cells, it is very significant for researchers to overcome these weaknesses and develop it into a therapy with more efficiency that could treat more types of cancers diseases. This review illustrates the existing FDA-approved CAR-T cell therapies, limitations, and future prospective of CAR-T cell therapy.

Keywords: cancer, cancer immunotherapy, CAR-T cell therapy.

1. Introduction

Cancer is a kind of disease when a few of the body's cells grow out of control and spread to other bodily regions. Cancer can appear almost anywhere in people's bodies. As usual, human cells would grow and multiply via a process called cell division to have new cells when people's bodies need them. When cells become damaged or get old, they will die, and the new cells take the place of the old cells. However, sometimes this process works in a wrong way. The abnormal or damaged cells would grow and multiply when they are not supposed to. In this way, these cells may form tumors, and tumors can be categorized into two types: cancerous tumor or benign tumor [2]. Since cancer is not extremely easy to be treated and the survival rate of patients is not very high, discovering an effective treatment for cancers is what researchers are putting importance on.

For decades, the fundamental methods of cancer treatment have been surgery, chemotherapy, and radiation therapy. These treatments continue to be the crucial main method. However, new categories of treatment have lately contributed to changing the way that persons with cancer are treated. [3]. With the improvement of medical technology, cancer immunotherapy has indeed become one of the useful, effective, and popular therapies which could treat cancers. Cancer immunotherapy is a kind of stimulation of the patients' immune system to treat their cancer. Cancer immunotherapy could enhance the human immune system's natural and innate abilities to fight cancer diseases. Furthermore, cancer immunotherapy can be categorized into two types: active or passive. The active immunotherapy could precisely target the tumor cells via the immune system directly. For example, cancer vaccines or treatment vaccines are designed to improve the patients' immune system to kill cancer cells. On contrary, passive immunotherapy does not directly target the tumor cells. It will boost the capability of the immune system to attack the cancer cells. The checkpoint inhibitors and cytokines are all instances of passive immunotherapy. Among active immunotherapies, there is an extremely "popular" immunotherapy that is related to immune cells: T cells and has been recognized

by researchers and doctors and started to be applied to plenty of real applications currently. This immunotherapy is called CAT-T cell therapy. Chimeric antigen receptor T cells, often referred as CAR-T cells, are T cells that have undergone genetic modification to form an artificial T cell receptor for use in cancer immunotherapy. Chimeric antigen receptors (CARs) are proteins that act as receptors and have undergone genetic engineering to enable T lymphocytes to target a particular antigen that would be present on cancer cells. The main reason why CAR-T cell therapy is extremely effective and significant is that after modifying the T cells to gain CAR, these CAR-T cells now can recognize and target the cancer cells to kill them precisely since without the CAR, the cancer cells have ways to hide from the T cells and continue growing inside patients' bodies. From 2017 to the present, scientists have developed six FDA-approved CAR T-cell therapies, and these treatments could help doctors to treat five types of cancers. Fortunately, these advanced CAR-T cell therapies could and have saved a lot of patients who got cancers. As of 2021, more than 500 clinical trials examining CAR-T cells for the treatment of cancer will be ongoing globally. East Asia is where the majority of these clinical trials are being conducted (269 trials), followed by the US (225 trials), and Europe, where 62 studies are now being conducted. [4]. Many of those clinical trials focus on targeting blood cancers, but clinical trials for solid tumors are less dominated by CAR T-cell therapy. These six FDA-approved CAR T-cell therapies are all for the treatment of blood cancers, including lymphomas, leukemia, and multiple myeloma [3]. Furthermore, from these FDA-approved therapies, also show that CD19 is indeed the most popular antigen target since four out of six therapies use CD19 as their antigen target, and BCMA has also become a "helpful" antigen target that is used for the therapies that treat multiple myeloma.

However, there are still plenty of issues that the CAR-T cell therapy has. The resistances include cytokine release syndrome (CRS), neurological toxicity, on-target/off-tumor recognition. In addition, it could cause some symptoms such as fever, headaches, dizziness due to low blood pressure, and difficulty breathing. Moreover, the CAR T-cell therapy is not very effective as a treatment for solid tumors. This is due to the difficulty in finding effective antigens, which must be primarily absent from normal tissues and substantially expressed in the majority of cancer cells [5]. Additionally, CAR-T cells are unable to penetrate solid tumors effectively, and the microenvironment of tumors actually inhibits T cell function [6]. Even though discovering CAR T-cell therapy for solid tumors is very challenging, scientists are still trying to find out if this immunotherapy might work against solid tumors such as ovarian cancer, breast cancer [7].

This review introduced three out of six FDA-approved CAR-T cell therapies (covering all five targeted cancers) in detail, illustrated the issues such as side effects and resistances of CAR-T cell therapy, and demonstrated some potential improvements of CAR-T therapy, especially explaining the new fifth generation of CAR-T cell and the improvements for treating humans' solid tumors.

2. Current FDA-approved CAT-T cell therapy

From 2017 to 2022, the U.S. Food and Drug Administration (FDA) has approved six CAR-T cell therapies that could treat blood cancers. These six FDA-approved therapies are: tisagenlecleucel, axicabtagene ciloleucel, brexucabtagene autoleucel, lisocabtagene maraleucel, idecabtagene vicleucel, and ciltacabtagene autoleucel. Four of them target the antigen CD19 and two of them target the antigen BCMA. The antigen recognition domain is all scFV. Additionally, the intracellular signaling domain of four of them is 41BB - CD3 ζ , and the intracellular signaling domain of the rest of them is CD28 - CD3 ζ . These six FDA-approved therapies can treat five different types of blood cancers, including B-cell acute lymphoblastic leukemia (ALL), Diffuse large B-cell lymphoma (DLBCL), Follicular lymphoma (FL), Mantle cell lymphoma (MCL), and the multiple myeloma. The fundamental mechanism of CAR-T cell therapy is to modify T cells to recognize and target the patients' cancer cells to destroy the cancer cells more precisely and effectively. The main step of making process of CAT-T cell therapy can be divided into five steps. Firstly, doctors need to collect blood from patients. Then, during one process named leukapheresis, the white blood cells including

T cells will be separated from patients' blood. In this way, doctors can collect the patients' T cells and the rest of the blood will be returned to patients' bodies. Secondly, patients' T cells will be genetically modified to become CAR-T cells that contain the chimeric antigen receptor (CAR) in the lab. Thirdly, these CAR-T cells are then grown and multiplied into millions of CAR-T cells in the lab. Lastly, patients could receive the CAR-T cell infusion. Eventually, the CAR-T cells will recognize and then bind to the patients' cancer cells and kill those cells to treat patients well. The following essay presented the background, making process and the usage of three out of six FDA-approved CAR-T cell therapies (covering all five targeted cancers).

2.1. Tisagenlecleucel

The first one is Tisagenlecleucel that targets the antigen CD19, sold under the brand name Kymriah, and the intracellular signaling domain of it is 41BB - CD3 ζ . On August 30, 2017, the U.S. Food and Drug Administration (FDA) approved the Tisagenlecleucel. This is the first chimeric antigen receptor (CAR) T-cell immunotherapy approved by the FDA. Based on a single-arm trial involving 63 patients with pediatric precursor B-cell ALL who had relapsed or been unresponsive to treatment, Tisagenlecleucel was approved. The proven overall remission rate, as determined by independent central review, was 82.5% for the 63 patients. 19% of patients had complete remission with incomplete hematological recovery, while 63% of patients had complete remission [8]. Tisagenlecleucel can treat three different types of cancers: B-cell acute lymphoblastic leukemia (ALL), diffuse large B-cell lymphoma (DLBCL), and follicular lymphoma (FL). Kymriah is licensed for use in children and young adults with B-cell ALL who are up to 25 years old and whose cancer was diagnosed as refractory disease if it did not respond to two or more prior treatments or relapsed if it did and did not respond to at least one prior therapy. Also, Kymriah is authorized for adult patients with DLBCL and FL whose cancer recurred (relapsed) or did not respond (refractory) to two or more prior treatments. In addition, kids with acute lymphoblastic leukemia under the age of two have not been investigated with Kymriah. Moreover, it is unknown whether Kymriah is safe and effective for use in DLBCL patients under the age of 18 and those above the age of 65 [9].

The process of making Kymriah indeed needs to take a while (Figure 1). The initial step is to draw T cells from the patient's blood. Blood must be drawn from a vein in the patient, a procedure that typically takes three to six hours and this step may need to be processed again. The remaining blood will then be returned to the patients' bodies after the leukapheresis procedure, which separates the patients' white blood cells, which contain T cells, from the patients' collected blood. The genetically altered white blood cells, including T cells, are shipped to be added a chimeric antigen receptor protein to their surface (CAR). The transformed T cells, also known as CAR-T cells, are then multiplied in the lab to produce millions of CAR T cells in the labs. This entire process can take 3 to 4 weeks to complete this process. Later, CAR T cells will be "injected" into the patients' blood and "delivered" back to them. T lymphocytes will eventually recognize the cancer cells and eradicate them when the CAR binds to the antigen CD19. Patients are typically given a brief course of chemotherapy two to fourteen days before to taking Kymriah (Figure 2) to lower the amount of white blood cells. The decrease in white blood cells gives Kymriah's CAR T cells adequate room to grow and enables them to stay in the patient's body for a longer period of time. In order to lessen the possibility of adverse responses to the infusion, patients must also get two medications called acetaminophen and antihistamine 30 to 60 minutes prior to receiving the Kymriah. Finally, Kymriah will be administered to patients via an intravenous (IV) infusion, and this procedure typically takes an hour [9].

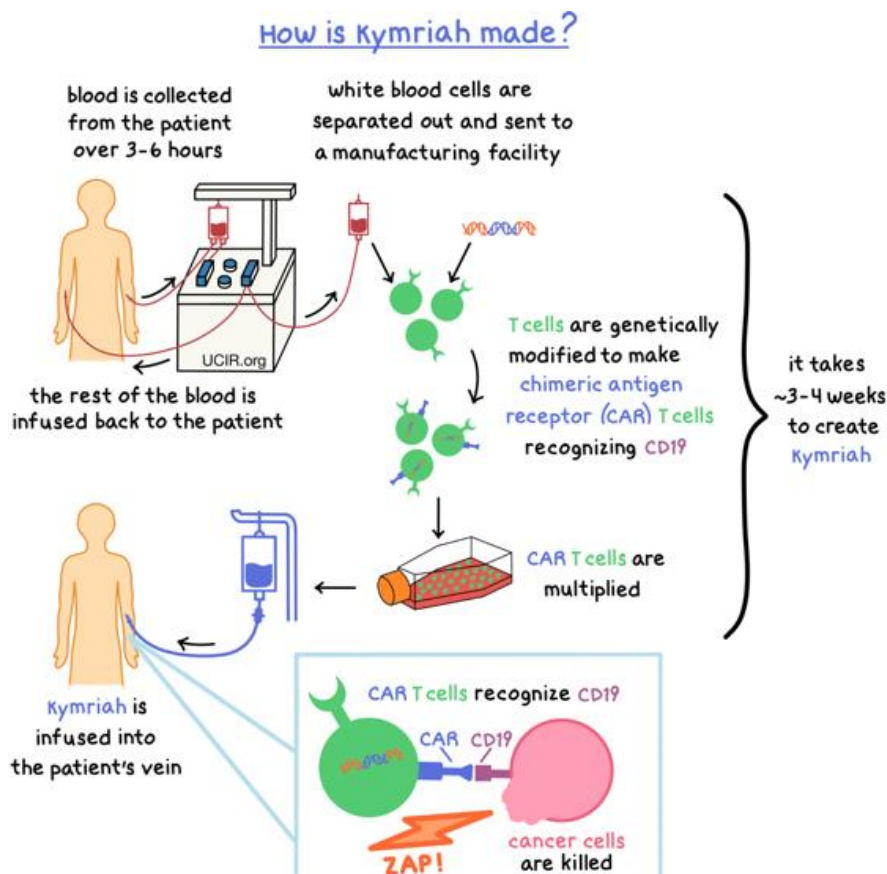


Figure 1. The Making Process of Kymriah [9]

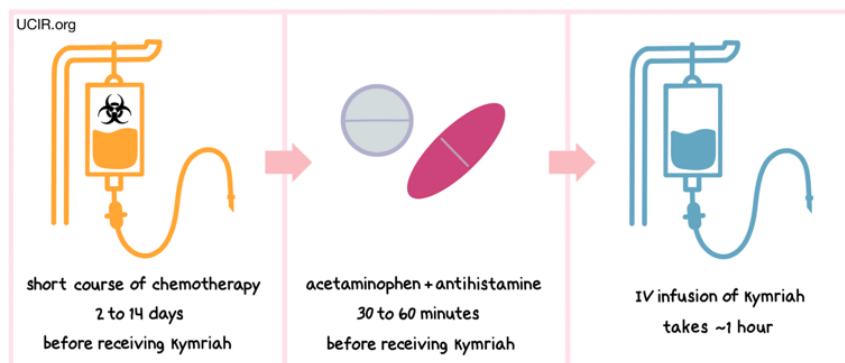


Figure 2. The Usage of Kymriah [9]

2.2. Brexucabtagene autoleucel

The second one is called Brexucabtagene autoleucel which also targets the antigen CD19, sold under the brand name Tecartus, and the intracellular signaling domain of it is CD28 - CD3 ζ . Brexucabtagene autoleucel, the first cell-based gene therapy approved by the FDA for the treatment of adult patients with mantle cell lymphoma, was given that approval on July 24, 2020. (MCL). In a multicenter clinical trial involving 60 adults with refractory or relapsed MCL who were monitored for at least six months following their initial objective disease response, the safety and effectiveness of Tecartus were determined. Following Tecartus therapy, 62% of patients experienced a full remission, and 87% of these patients showed an objective response [10]. Besides MCL, Tecartus also can be effective for treating B-cell acute lymphoblastic leukemia (ALL). Patients with MCL and B-cell ALL who have previously undergone treatment but whose cancer cells either did not respond to that treatment (refractory sickness) or responded but later came back (relapsed disease) are eligible for Tecartus. Additionally, Tecartus' effectiveness and safety in patients younger than 18 have not yet been determined [11].

It takes about the same amount of time to make Tecartus as it takes to make Kymriah (Figure 3). Like making Kymriah, the first step of making Tecartus is also to collect the patients' T cells from patients' bodies. Similar to when creating Kymriah, blood is drawn from a vein in the patient, and during a procedure known as leukapheresis, T cells and other white blood cells are removed from the bloodstream. The remaining blood will be then given back to the patients' bodies. It may be necessary to repeat this entire process, which normally takes 3 to 6 hours. Additionally, the gathered white blood cells are transported to a specialized manufacturing facility where they are genetically altered to acquire the chimeric antigen receptor protein on their surface. Then, to produce millions of CAR T cells in the lab, it is necessary to multiply the transformed T cells. It should take between two and four weeks to complete this process. CAR T cells will then be "implanted" into the blood of the patient and "given" back to the patient. Similarly, Tecartus also recognizes CD19 and kill the cancer cells like Kymriah. The usage of Tecartus is a little bit different from the usage of Kymriah (Figure 4). Same as receiving Kymriah, prior to taking Tecartus, patients have a brief course of chemotherapy to lower their white blood cell count. Mantle cell lymphoma patients get chemotherapy 5, 4, and 3 days prior to receiving Tecartus. Patients must additionally get acetaminophen and an antihistamine around 30 to 60 minutes before receiving Tecartus in order to lessen the possibility of adverse responses to the infusion. Patients will next get Tecartus via intravenous infusion, which typically takes 30 minutes to provide and requires less time than receiving Kymriah [11].

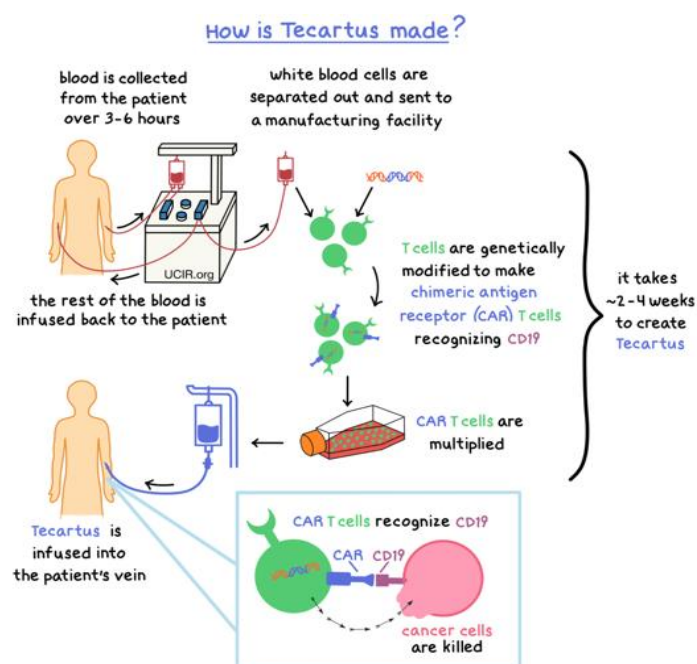


Figure 3. The Making Process of Tecartus [11]

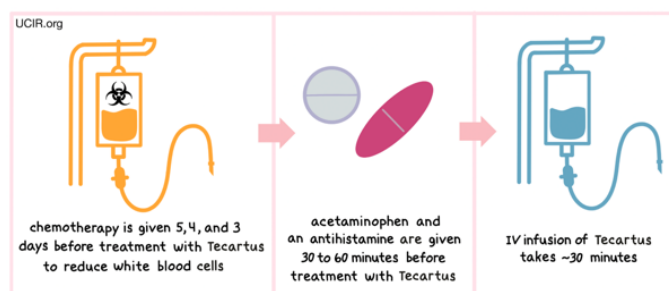


Figure 4. The Usage of Tecartus [11]

2.3. Idecabtagene vicleucel

The last one is Idecabtagene vicleucel which targets the antigen BCMA, sold under the brand name Abecma, and the intracellular signaling domain of it also is 41BB - CD3 ζ . The FDA granted

permission for idecabtagene vicleucel on March 26, 2021, to treat multiple myeloma in adult patients who had received four or more prior lines of therapy, such as immunomodulatory medicines, proteasome inhibitors, and anti-CD38 monoclonal antibodies. Abecma is the name of the first cell-based gene therapy for multiple myeloma to earn FDA approval. The safety and efficacy were assessed in a multicenter study involving 127 participants with relapsed and refractory multiple myeloma. 88% of the patients had previously undergone four or more lines of treatment. 72% of respondents overall and 28% of respondents completely completed the survey. Additionally, it is believed that 65% of patients who reached CR stayed in CR for at least a year [12]. Abecma only works for multiple myeloma. This CAR T-cell therapy may be helpful for patients whose cancer was either resistant (not responsive to treatment) or relapsed after treatment. The fundamental making process of these CAR T-cell therapies is quite similar. However, different from the making process of Kymriah and Tecartus, making Abecma needs to take a longer time: this whole process needs to take around one month to create Abecma (Figure 5) [13]. In addition, instead of adhering to CD19 on the surface of multiple myeloma cells, the transformed T cells, or CAR-T cells, will adhere to a protein called BCMA. The T lymphocytes will then identify and eliminate the cancer cells. It is very similar to how Tecartus is administered in that patients undergo a brief course of chemotherapy to reduce the amount of white blood cells initially and roughly 30 to 60 minutes before receiving Abecma. Furthermore, the delivery of Abecma may take up to an hour, which is similar to how Kymriah is administered.

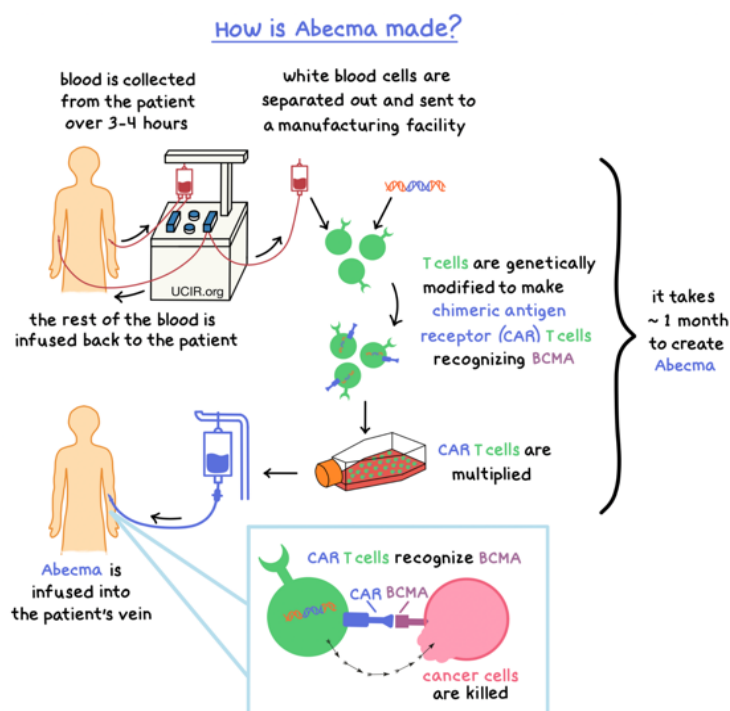


Figure 5. The Making Process of Abecma [13]

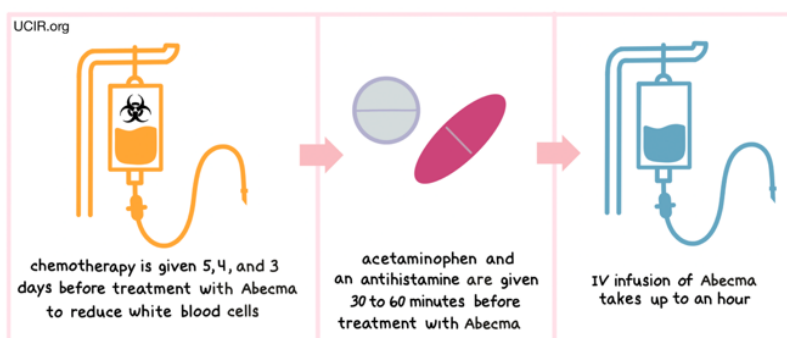


Figure 6. The Usage of Abecma [13]

3. Improvements

The difficulty in finding effective antigens because they must be substantially absent in normal tissues but significantly expressed on the majority of cancer cells is one of the reasons why CAR-T cell therapy is not very "useful" for treating solid tumors [5]. Furthermore, CAR-T cells cannot be properly transported into the center of solid tumors, in contrast to the treatment of blood cancers, which merely requires injecting the medications into patients' bodies. Additionally, the microenvironment of tumors does in fact limit T cell activity, making it impossible for T cells to function as effectively as expected by researchers and medical professionals [6].

In 2018, a group of researchers published an essay illustrating the potential treatments for solid tumor via CAR-T cell therapy. In that scholarly journal, the researchers indicated that one method to treat solid tumors is using CAR-T cell therapy with the chemotherapy since the chemotherapy can enhance the efficacy of CAR-T cell therapy. The chemotherapeutic agents are able to sensitize tumor cells to immunotherapy, and the chemotherapeutic agents also can improve the recognition and presentation of tumor antigen. In this way, the combination of CAR-T cell therapy and chemotherapy could be a potential method for treating solid tumors. Additionally, combining radiation with CAR-T cells is an effective strategy to treat cancer because it can make tumor cells more susceptible to cytotoxic lymphocytes that are specific to the tumor and foster the movement and infiltration of CAR-T cells. Another potential approach discussed in that study is the use of CAR-T cell therapy in combination with chemotherapy and radiation therapy for the treatment of solid tumors [14].

Another updated existing method is called dual-targeting CAR-T cells. By independently activating CD28 and 4-1BB pathways and adjusting CD3-chain-mediated signaling, some researchers from the University of North Carolina created CAR-T cells that simultaneously target two antigens and provide optimal co-stimulation and T cell metabolic fitness. According to their research, CAR-T cells with low antigen density that simultaneously target two tumor-associated antigens and provide transacting CD28 and 4-1BB co-stimulation while sharing the same CD3-chain cause rapid antitumor effects in *in vivo* stress conditions, protection from tumor re-challenge, and prevention of tumor escape (Figure 7) [15].

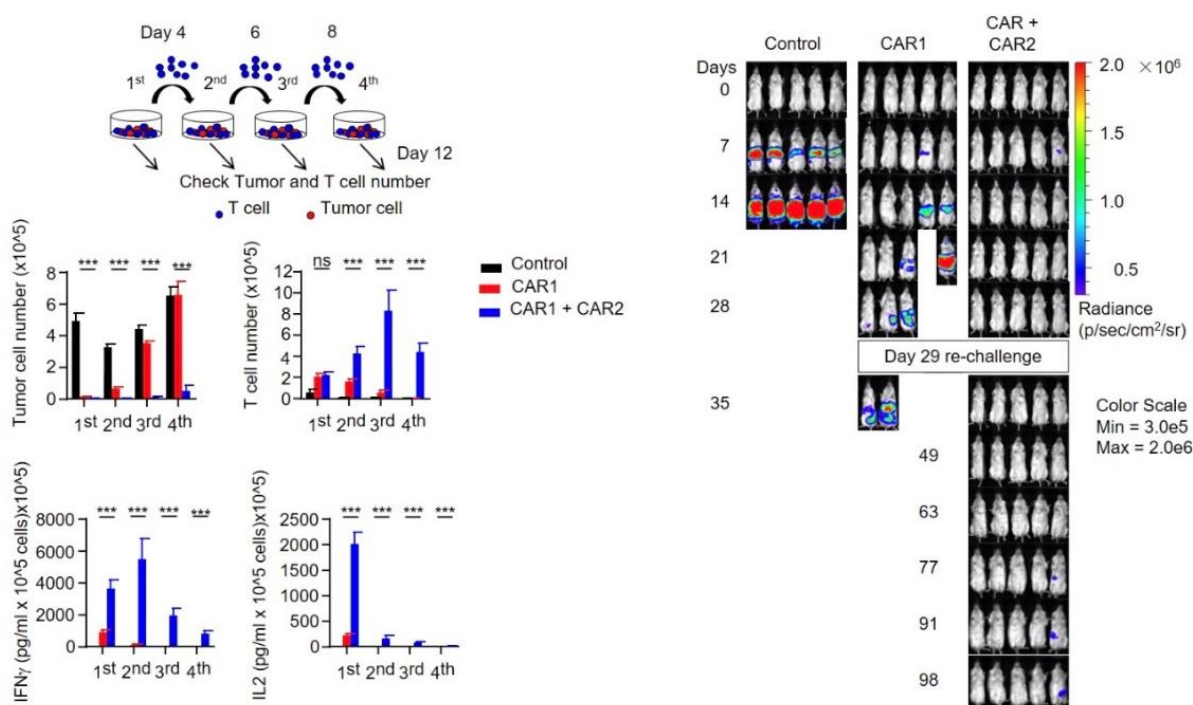


Figure 7. Dual targeting CAR-T cells provide sustained antitumor activity [15]

The durability, proliferation, safety, and efficacy of CAR-T cell treatments have all improved during the past few years. The off-target and off-tumor toxicity of CAR-T cells must still be addressed,

nevertheless. Nowadays, fifth generation has started to be investigated (Figure 8). The different point of the new generation is that they integrate an additional membrane receptor. The fifth-generation acts following a different principle. The inclusion of IL-2 receptors, which enables JAK/STAT pathway activation in an antigen-dependent manner, is the most promising strategy. The discovery of switch receptors was one of its most exciting developments. Recent studies have successfully integrated a drug-dependent OFF-switch that causes CAR depletion or an ON-switch that causes activation. With a better safety profile and larger therapeutic window, the current generation of CARs may be easier to manage than earlier versions [16].

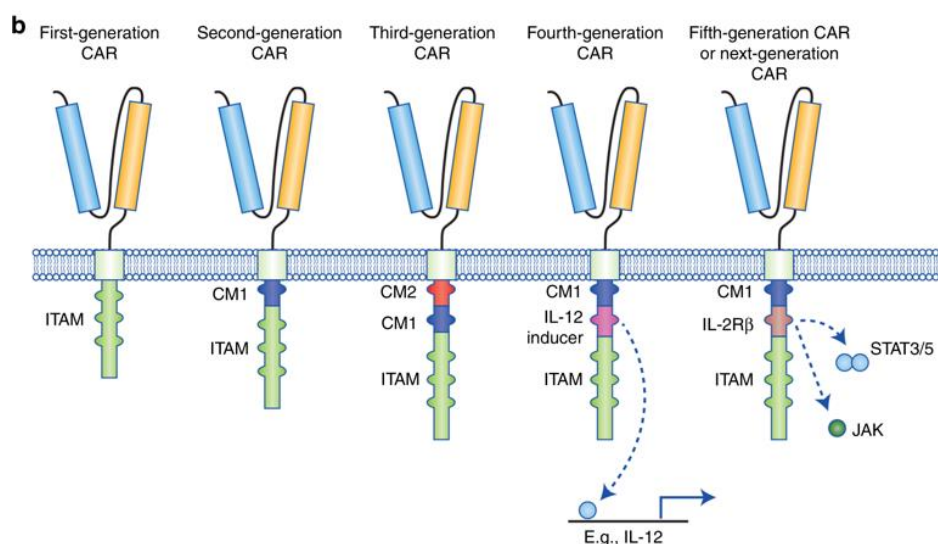


Figure 8. The Five Generations of CAT-T Cell Structure [17]

4. Conclusions

Renier J. Brentjens from Memorial Sloan Kettering Cancer Center in New York commented that “CAR T cells are the equivalent of “giving patients a living drug.” Since cancer cells grow and spread in human bodies extremely fast and it’s very hard to control the growing rate of cancer cells by our immune system itself, discovering an effective treatment like CAT-T cell therapy to enhance patients’ immune systems to recognize and attack cancer cells more efficiently could be a great milestone for the treatments of cancer diseases. From 2017 to present, the FDA has approved six CAR-T cell therapies that could treat five types of blood cancers. These six FDA-approved therapies are: tisagenlecleucel, axicabtagene ciloleucel, brexucabtagene autoleucel, lisocabtagene maraleucel, idecabtagene vicleucel, and ciltacabtagene autoleucel. Four of them target the antigen CD19 and two of them target the antigen BCMA. Additionally, the intracellular signaling domain of four of them is 41BB - CD3ζ, and the intracellular signaling domain of the rest of them is CD28 - CD3ζ. The five different types of blood cancers include B-cell acute lymphoblastic leukemia (ALL), Diffuse large B-cell lymphoma (DLBCL), Follicular lymphoma (FL), Mantle cell lymphoma (MCL), and the multiple myeloma. However, the CAR-T cell therapy also contain some common side effects such as CRS, neurological toxicity, on-target/off-tumor recognition. Furthermore, discovering an updated CAR-T cell therapy for treating solid tumors is a crucial limitation needed to be addressed as well. For the improvements, the combination with CAR-T cell therapy and other kinds of treatments could be a new effective way of attacking solid tumors. Moreover, the dual-targeting CAT-T cell under investigation also can be a helpful method for solving the problems that current CAT-T cell therapy has for treating solid tumors. In addition, the fifth generation of the CAT-T cell would improve its efficiency and provide a better treatment for patients. With more and more researchers’ efforts and these new developments, CAR-T cell therapy would become the one that could save more patients with cancer diseases efficiently.

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