CAR T Cell Therapy Application Toward B Cell Lymphoma and Future Enhancement

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Abstract: Chimeric Antigen Receptors (CAR) T cell therapy is a new emerging technique that mainly target B cell lymphoma. It isolates the patients' own immune cells and uses them to eliminate cancer after receptor engineering. To date, remarkable results have shown its strength and 5 drugs have been approved by U.S. Food and Drug Administration in treating Diffuse Large B-cell lymphoma (DLBCL) and some other subtypes of B cell cancer. Through CAR-T cells are undergoing significantly fast innovation, a series of side effects have occurred. For example, the cytokine releasing syndrome. In addition, as the quick development of CAR, scientists have met some obstacles in the past years and some of them remain troublesome. This review paper focused on the treatment direction of three FDA approved drugs, side effects, current barriers and potential solutions.

1. Introduction

Lymphoma is an aggressive disease that could potentially affects all types of lymphocytes. From infants to elderly, this disease could attack people in any age range and 4% of all cancer cases are made up of lymphoma in the U.S. Despite slow annual decrease in death rate by approximately 2%, the overall 5-year survival rate remains only 73%. As the association between tumor growth and immune system becomes way clearer than ever before, many oncologists have begun to appeal to mobilizing patients' own immune system to eliminate the blood cancer cells. CAR-T cell therapy, an outstanding branch in Adoptive Cell Therapy (ACT), has harnessed great achievement in treating B cell lymphoma.

CAR-T cell therapy refers to a specific type of immunotherapy in which artificially designed receptors (Chimeric Antigen Receptor, abbreviated as CAR later in the paper) are installed on lymphocytes to bind target antigen and exert attack. The resources of the therapeutic cells could be all effect lymphocytes in patients, either autologous or allogenic [1]. As a cell surface receptor, CAR is composed of an extracellular antigen recognition domain, a transmembrane domain, a spacer (also known as hinge region) that joins antigen recognition domain and transmembrane domain together to enhance the receptor flexibility, and an intracellular signal domain. After antigen binding, the signals would travel across the cell surface and stimulate the phosphatidylinositol. Subsequently, tyrosine kinase signaling cascades would be activated and the stimulatory and co-stimulatory signals are going to activate the effector cell.

Starting from 2017, several brands (Kymriah, Yescarta, Tecartus, Breyanziz and Abecma) have been approved by FDA in US and have shown considerable effect in treating Diffuse Large B-cell lymphoma (DLBCL), B-cell acute lymphoblastic leukemia (ALL) and Multiple myeloma. The first four models aim CD19, a typical marker expressed by B lymphocytes, and Abecma aims BCMA as its target. Though CAR-T cell therapy is considered a powerful enemy of a series of cancers, many unsolved problems are limiting the strength of CAR-T cells. For example, the off-target cytotoxicity, cytokine releasing syndrome, immune inhibition from tumor and exhaustion [2][3]. These barriers, to a large extent, are due to lack of understanding of the complex tumor microenvironment (TME) and long-term clinical data. What's more, the inability to maintain immune homeostasis has made it harder to minimize the adverse effect while intervening the patients' immune system. To overcome these

obstacles and ensure better T cell function in vivo, scientists do have tried a variety of methods and some of them have shown their capability in enhancing the CAR-T cell power. Considering from different perspectives, with many of them well-known for a long time, these enhancement methods include combination with chemotherapy, stimulating Patter Recognition Receptors (PRR), inducing RN7SL1 and PD-1 knockout et al. Nonetheless, the unsolved problems are still more than those handled.

Herein, this paper focused on the current stages of development and achievement scientists have made so far. Furthermore, existing obstacles, as well as side effect management protocols, were discussed.

2. The development of CAR-T cell therapy

As a newcomer in immunotherapy, though only came out for few decades, chimeric antigen receptor has gone through several rounds of revolution. In 1990s, Arthur Weiss et al. created the first generation of CAR by using CD3ζ intracellular domains [4] and, several years later, the U.S. company Cell Genesys put the first-generation CAR into clinical trials. Unfortunately, along with some other trials around those years, the outcome was quite disappointing as these engineered cells were poor at persistence in vivo and showed low efficacy [5]. This failure results from the fact that though CD3ζ could give the cell a essential activation push, it is inadequate in fully activating the cell without any co-stimulatory partner [6]. In order to improve the effectiveness of CAR, CD28 or 4-1BB (CD137) were added onto the intracellular domain in the 2nd and 3rd generation of CAR respectively, therefore enhanced its viability and achieved longer persistence [7]. Up to date, CAR has brought remarkable clinical benefit to public view, especially their specialty at targeting non-solid tumors (e.g., blood cancers) [6] and the rapid effectiveness has given many scientists an impression of promising. Compared to other subtypes in ACT, CAR-T cell therapy has exhibited its unique advantages. The most intriguing one is that the CAR could be designed as scientists' wishes and be installed on the cell surface of all types of immune cells (NK cells, T cells et al.). This means that CAR could recognize antigens that are not normal tumor associated antigens (TAA), such as CD19 while treating of B cell lymphoma. Another noticeable aspect is that CAR could recognize antigens independent of MHC complexes since the antigen binding region is derived from the Fab region of antibodies. CAR has showed excellent outcome for treating B cell lymphoma after infusion into patients. Nonetheless, up to date, no CAR therapies has entered clinical practices partially due to the poor penetration into the tumor tissue and altered chemokine signals [8].

3. FDA-Approved CAR T cells application for specific cancer type

3.1 Kymriah: B-cell acute lymphoblastic leukemia

Tisagenlecleucel, using Kymriah as its brand name, was approved by FDA in August 2017 and was the first FDA-approved therapy ever in history. Clinically, Tisagenlecleucel is intended to treat B-cell precursor acute lymphoblastic leukemia (ALL) during refractory or relapsing phase. Since it is a B cell lymphoma targeting therapy, T cells isolated from the patients are edited to be installed with CD-19 specific CAR. In a single-cohort global study carried out by Maude SL et al. in 2018, 75 adult patients with relapsed or refractory B-cell ALL are treated with Tisagenlecleucel based therapy and the remission levels are measured. The outcome was quite satisfying. In the first 3 months after CAR-T cell infusion, the remission rate achieved 81%. At 6 months, the event-free survival was 73% and overall survival rate was 90%. At 1 year, the event-free survival and overall survival were 50% and 76% respectively. However, what concerns scientists are that 77% patients showed cytokine release syndrome and 40% patients reported neurologic problem. Though after proper care, the side effect can be counteracted, but in other clinical trials of Tisagenlecleucel, cytokine release syndrome always exists.

3.2 Breyanzi: Diffuse Large B-cell lymphoma

Diffuse Large B-cell Lymphoma (DLBCL) refers to the cases where systemic B symptoms occurs with massive growth and infiltration of abnormal B cells [9]. Different from other subtypes of B cell lymphoma, DLBCL usually affects elderly or those immunodeficient individuals. Therefore, although aging is a nonnegligible factor for the disease, infections of immunodeficiency related virus (e.g., HIV, EBV) are still the major significant risk for the disease. Lisocabtagene maraleucel (LM), brand name Breyanzi, is a CAR-T cell therapy that target exclusively the Diffuse Large B-cell Lymphoma by aiming at CD-19. LM was approved by U.S. Food and Drug Administration and became the third FDA approved CAR-T cell therapy. Headed by Cytokine release syndrome, LM often raise some side effects. However, most of them are restricted in mild symptoms and no reported death so far.

3.3 Abecma: Multiple myeloma

Multiple myeloma is a special subtype of B cell cancer that affects the plasma cells. While getting multiple myeloma, the antibody quality and quantity would be altered, and the abnormal plasma cells would uncontrollably accumulate in the bone marrow [10]. The abnormal production and quality of antibodies alter the plasms characteristics and could lead to kidney failure and other blood-related disease [11]. Meanwhile, patients are more prone to infected by other pathogens and raise complications (e.g., amyloidosis). Abecma (Idecabtagene vicleucel) is the latest drug of CAR-T cell therapy that was approved by FDA in March 2021. Unlike four other types of approved therapy, ide-cel targets B-cell maturation antigen (BCMA) as its target. In phase II trial (KARMMA), the efficacy of the drug is confirmed. During the trial, 110 tumor samples were evaluated and $\geqslant$50% of the tumors shows positive level of BCMA expression. After treatment, the overall response rate was 73% and Complete response to treatment was 33%. However, similar to other types of CAR-T cell therapy, cytokine release syndrome is an inevitable problem that clinicians must deal with.

4. Challenges for CAR-T immunotherapies and solutions

4.1 Off-target Cytotoxicity

Off-target toxicity has been troubling since the publication of immunotherapy, especially in adoptive cell transfer. The off-target toxicity refers to the relatively indiscriminate attack between target cancer cells and healthy tissues. In other words, the CAR recognizes the correct antigens, but these antigens are still presented on healthy tissues at certain level. Up to date, over 90% of carcer drugs are repudiated by U.S. Food and Drug Administration [12]. Although most of them have shown their ability to cause tumor remission, the off-target effect results in serious dose limitation and ultimately leads to lack of efficacy. In the case of treatment towards B cell lymphoma, the conventional target antigen is CD19 expressed by B cells exclusively. However, since CD19 are expressed throughout all developmental stages of B cells, the drug could also aim at those non-cancerous B cell and kill the healthy ones. In severe cases, the off-target toxicity for CD19 can leads to extensive effector B cell death and cause B cell aplasia [13]. On the other hand, cross-reactivity is also responsible for the off-target effect. In this matter, the cytotoxic cells will respond to different but related antigens expressed in other organs. The “similar” here is that the type of peptides that bind to a receptor is determined only by some key anchor amino acids or motif, which means that though some healthy organs express their own unique proteins, these proteins happen to be identical to those tumors associated antigen with respect to the anchor region. Therefore, the effector cell receptor will recognize the healthy organ peptide as their target and kill the healthy cells. Interestingly, in the latest finding by Ann Lin et al, they proposed a discovery that most drugs undergoing clinical trials exert their function independent from their designed target. For example, the drug OTS964 was designed to inhibit the protein kinase B

in signaling cascade, but Ann’s results have demonstrated that it functions by blocking Cyclin-dependent kinases 11 (CDK11). Worth noticing, there was no previous data recording the inhibitor of CDK11, though several other CDK inhibitors have been approved by FDA or are undergoing clinical

trials for final rounds [14]. This gives the previously non-druggable CDK11 to be investigated regard to potential tumor inhibitor. If considering CAR-T cells themselves, in rare circumstances, they could be transformed into cancerous cells. Unlike the natural development and occurrence of tumor cells in human body, the oncogenic process of CAR-T cell happens within the engineered process. Due to the viral vector infection in editing process, there is inevitable chances that the vectors are inserted into tumor suppressor or oncogenes and therefore result in CAR-T cell oncogenicity [15]. Unfortunately, these problems or maloperations are mostly due to lack of genetic data or misidentification of essential genes. In other words, falsely deciphering the optimized target of tumors can cause failure.

4.2 Cytokine release syndrome

The adverse effect concerned by most clinicians is the cytokine release syndrome (CRS). CRS, also known as systemic inflammatory response syndrome (SIRS), is a series of symptoms that shows abnormally high amount of inflammatory cytokines release into the blood stream [16]. After introducing an extra number of effector lymphocytes, the immune response would deviate towards inflammation by secreting an uncontrolled number of cytokines. Patients affected by CRS usually suffer from fever, low blood pressure, accelerated breathing and heartbeat and rash [17]. If left untreated, the activation of white blood cell could rapidly form a positive feedback loop, in which pro-inflammatory cytokines can attract more white blood cells and, once the recruited cells are activated, they further produce more cytokines. While most patients only experience mild symptoms, CRS can cause death in severe cases occasionally and at least seven patients have died from this syndrome. In clinical screening, serum analysis has supported the basic cause of the disease by showing remarkable increase in pro-inflammatory cytokines, such as IL-6, IFN-γ, and MCP-1 (CCL2) [18]. However, interestingly, the CRS caused by CAR-T cell therapy are barely caused by direct CAR-T cell infusion. Rather, they are indirectly caused by subsequently recruited cells mediated by T cell dependent activation. In 2016, experiments performed Barrett DM, et al. provided solid evidence that IL-6, MCP-1, and MIP-1 are produced by pro-inflammatory cells of myeloid lineage, instead of the induced CAR-T cells. Table 1 shows the other potential clinical factor that could affect CRS. To abrogate the unwanted increase in cytokine amount, many experiments have been carried out. To date, only one drug called Tocilizumab (anti-IL6 monoclonal antibody) has been approved by U.S. FDA [19]. Another monoclonal antibody Lenzilumab who targets GM-CSF also demonstrated its power in limiting the rapid activation of myeloid lineage cells by suppressing a wide range of cytokines but hasn't been granted by FDA yet.

Table 1 Clinical factors related to cytokine release syndrome

		Total Cohort	
	Total cohort	***Grade 1-3***	***Grade 4-5***
Clinical factor	***(N=48)***	***(N=34)***	***(N=14)***
Start	***1(0-10)***	***2.5(0-10)***	***1(0-6)***
Stop	***10(5-24)***	***9(5-24)***	***11.5(5-16)***
		Days to fever (N=41)	
Start	***1(0-10)***	***2(0-10)***	***1(0-6)***
Stop	***8(5-23)***	***8(5-23)***	***9(5-15)***
Total days febrile	***6(1-17)***	***6(1-17)***	***8(3-13)***
		Intubation	
Yes	***9(19%)***	***0(0%)***	***9(64%)***
No	***39(81%)***	***34(100%)*****	***5(36%)*****
		Days to intubation	
Start	***6(3-13)***		***6(3-13)***
Stop	***15(5-66)***		***15(5-66)***

		Vasoactives	
Yes	***20(42%)***	***6(18%)***	***14(100%)***
No	***28(58%)***	***28(82%)*****	***0(0%)*****
		Days to vasoactives	
Start	***5(1-13)***	***4.5(1-7)***	***5(2-13)***
Stop	***10(2-16)***	***8(2-10)***	***10.5(5-16)***
		Encephalopathy	
Yes	***13(27%)***	***5(15%)***	***8(57%)***
No	***34(71%)***	***29(85%)****	***5(36%)***
Unknown	***1(2%)***	***0(0%)***	***1(7%)***
		Infection	
Yes	***6(13%)***	***2(6%)***	***4(29%)***
No	***42(88%)***	***32(94%)***	***10(71%)***

4.3 Poor Tumor infiltration

Around hundreds of clinical trials of CAR-T cell in global range, most of them are still targeting non-solid tumors and CAR-T cell therapy accounts for more than 50% of all B cell malignancy cases [20]. The data above could be astonishing, but the ultimate reason for the inability of CAR-T cell to treat solid tumor is due to the poor infiltration. Put some other minor reasons aside, the TME that surrounds the tumor mass and the alteration in chemokine signals are already strong enough to keep the infused CAR-T cells from entering inside. In the case of blood cancers, the CAR-T cells have much more capacity to interact fully with the malignancies as almost all the infused T cells would return to the blood stream. In other words, the CAR-T cells have already reached their destiny upon entry. Besides the problem of destiny, the high endothelial venules (HEVs) also play an important role in mediating T cell migration. In healthy tissues, the arrangement and distribution of vessels are normal. However, the venules become terribly distorted when approaching to the inner core of the tumor mass [21]. On the other hand, the disoriented vessels, as well as the cellular matrix, would lead to the alteration in the chemokine pattern near the tumor mass. For example, the chemokine CCL11 that expresses normally in healthy tissues would be down regulated around tumor tissue, thereby further limiting the chance of effector T cells penetration through the venule into the tumor. To overcome these obstacles, scientists do have come up with some ideas. Firstly, the T cells will contact the tumor directly if the migration steps are jumped over. In other words, a local injection of therapeutic T cells is much effective than systemic injection. In this regard, intraventricular injection to treat glioblastoma recurrent brain or leptomeningeal metastases have been proposed effective and safe in preclinical models [22]. Similarly, intrapleural introduction for human pleural malignancy shows advantages as well compared to systemic injection [23]. Another recently emerging strategy is further editing the CAR-T cell itself, thereby adding special chemokine receptors that respond to tumor associated attracting signal [24]. In 2019, Whilding, LM. et al. discovered that αvβ6-targeting CAR-T cell would gain much more homing and migration ability if it express CXCR2 at high level. Some other innovative trafficking methods are still constantly being created. For example, CAR-T cell express heparinase to degrade the heparin sulfate proteoglycan (HSPG) in the extracellular matrix, thus crossing the migration barrier efficaciously.

4.4 Immune exhaustion and suppression

Another puzzled situation that scientists face is the immune-suppressive nature of the TME. Myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and regulatory T cells (Tregs) are the three dominant types of immune suppressive cells presented in the TME [25]. Different from non-solid tumors who have little capacity for immune suppression, the suppressive cells in solid tumors could reside within the tumor firmly and secrete cytokines to promote tumorigenesis, such as IL-4, IL-10, and TGFβ. In addition to the cytokines, the inhibitory check points are utilized by all the tumors in human body. Through the up regulation of PD-1 or CTLA-4

upon exposure to T cells, with the addition of poor lymphocytes infiltration, nearly all the effector CAR-T cells would be blocked from functioning normally. Under the complicated TME and the interior immune-suppressive agents, CAT-T cells can hardly achieve long enough persistence and satisfying efficacy. In current clinical development stages, due to lack of sufficient understanding of TME partially, the focus point lies on the combination with immune check point blockade (ICB). In a study performed by Li, A. M. et al. in 2018, 14 children with B cell Acute Lymphoblastic Leukemia are treated with PD-1 blockade together with CAR-T cells specific to CD-19. The results were quite exciting as the persistence of CAR-T cells are improved largely. Also, while using EGFRvIII-CAR-T Cells to treat glioma, the knockout of PD-1 in CAR-T cells greatly increases the anti-tumor activity [26]. To further improve the universal application of CAR-T cells in solid tumors, scientists are investigating combination of different immunotherapies together. Nonetheless, a more comprehensive understanding for the TME is extremely necessary as well.

4.5 Antigen Lost

Antigen lost refers to the ability of tumor cells to evolve overtime, in order to escape from the elimination of killer cells. In the clinical trials of B-cell acute lymphoblastic leukemia (B-ALL), in which CD-19 specific CAR-T cells were used, nearly a quarter of the patients have relapsed later in the therapy with a significant reduction in CD-19 expression, though 70% to 90% of them showed excellent remission at the beginning of the therapy (Figure 1) [27]. In other cases, similar patterns of relapsing were also observed. In 2016, some studies carried out an experiment regard to regression of glioblastoma and they found that once the tumor showed relapsing, L13Ra2 expression are down regulated in response to the L13Ra2 targeting therapy. To minimize the possibility of tumor recurrence, the most prevalent strategy at present is to install several types of CARs onto a single T cell [28]. For example, the combination of CD19 and CD20 specific CAR co-expressing on a single T cell. In other clinical cases, combination of CD19 and BCMA bi-specific CAR-T cells also have shown excellent results [29] (Fig 1). In general, multiple targeting CAR-T cells have given scientists a promising outlook, but still require more information towards the different antigen target at genetic level and practical data.

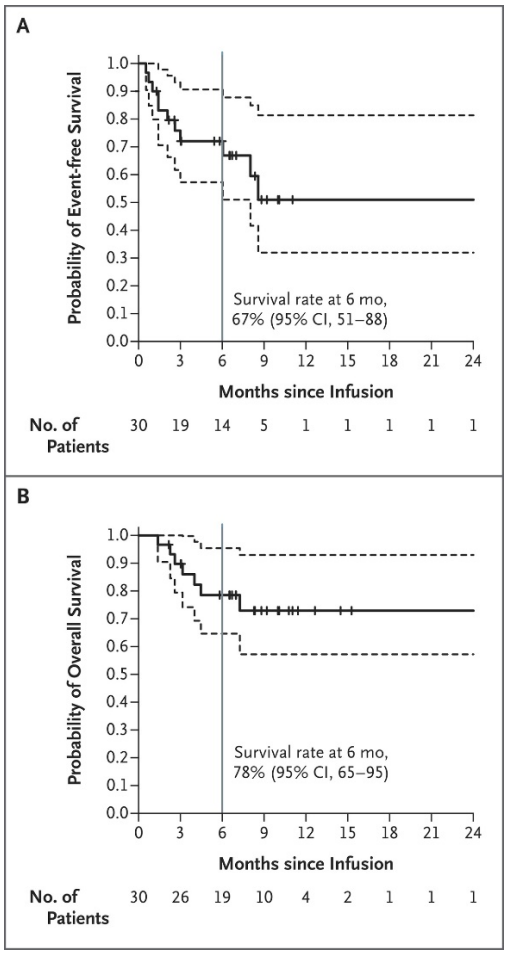

Fig 1. Probability of event-free and overall survival rate at 6 months. After treatment, 27 patients had a complete remission, within which 19 remained in remission: 15 patients received no further therapy and 4 patients withdrew from the study to receive other therapy. The data was recorded for 7 months, no treatment related death reported. At 6 months, the event-free survival rate was 67% (95% confidence interval [CI], 51 to 88) and the overall survival rate was 78% (95% CI, 65 to 95).

4.6 Other CAR-T cell enhancement: Induction of RN7SL1

Except some of the CAR-T cell refinement listed above, scientists recently began to set about motivating therapy from innate immune system. Basically, a normal innate immune system is necessary for human body to transfer into an effective adaptive immune system. Pattern Recognition Receptors are one of the most crucial mediators of the innate immune system (e.g., RIG-I and MDA5). Once activated, it could signal through MAVS signaling pathway and ultimately give rise to a variety of immune response, such as interferon production. Based on this, scientist found out that RNA *RN7SL1* could act as a agonist for PRR activation. In normal healthy tissues, the RN7SL1 is coated by RNA binding protein. However, in tumor tissues, the alteration in cellular activity downregulates the RNA binding protein level and RN7SL1s become exposed to PRR. After inducing RN7SL1 into CAR-T cells, the RIG-I/MDA5 signaling further stimulated the activation of T cells and, interestingly, promoted the expansion and memory formation.

5. Conclusion

CAR-T cell therapy have been an indispensable part in immunotherapy and its advances are obvious to all, especially in treating blood cancers. Furthermore, the next generation of CAR-cells are actively being investigated, several drugs have already been in clinical trials. However, regardless of the promising sign that CAR-T cells gave to public, barriers such as CRS or off-target cytotoxicity still need to be overcome. Besides, up to date, scientists still have trouble in targeting solid tumors through

CAR. To cope with these difficulties, several strategies are being adopted, such as synchronous immunosuppression to inhibit the adverse effect of CRS and local administration of CAR-T cells to be enriched around tumor tissues. Nonetheless, many problems are remained unsolved. The priority of research should emphasize a better characterizing of tumor specific protein, rather than tumor associated protein. A clearer view of discrimination between cancer cells and healthy cells is extremely necessary to avoid a series of off-target effect. Another aspect of development is the combination of different novel strategies, such as immune check point inhibitors, together to achieve a more comprehensive treatment effect. Additionally, the puzzle of TME isn't fully understood yet. How the different factors act together for immune suppression and how to counteract with the immune exhaustion are the main two questions that need addressing.

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