

Future Trends in CAR-T Cellular Immunotherapy and the Pros and Cons in Cancer Treatment

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Abstract. In recent years, the basic research of tumor immunology and the research of biotechnology have made rapid progress, which provides a profound knowledge base and technical reserve for the research and development of tumor immune drugs. The move helped make the topic of tumor cell immunotherapy a household word. As of May 2019, there were a total of 1011 cell therapies in the world, of which 568 CAR-T therapies, accounting for 56.2%, especially CAR-T cells for the treatment of blood tumors, which has achieved world-renowned efficacy. However, in the treatment of solid tumors, CAR-T cells have not yet shown extremely accurate inhibition of solid tumors due to the presence of immunosuppressive microenvironment, most of the features (signals) of abnormal antigens are expressed in normal tissues, and other complex factors. At present, domestic and foreign research on CAR-T cell therapy of solid tumor has been carried out in various directions and made some progress.

Keywords: CAR-T, Immunotherapy, Cancer Treatment.

1. Introduction

Some patients demonstrate a benign trend in their tumors after receiving CAR-T cellular immunotherapy, as evidenced by decreased tumor mobility[1]. According to clinical trials, it has been observed that once a tumor exhibits a benign trend, its tendency to move is reduced. This inhibition of mobility leads to a decrease in the probability of metastasis of the cancer cell population, which confirms the results of clinical observations. Sometimes it is not the initial cancerous condition that is fatal, but rather when the cancer cells have metastasized to important areas (e.g., brain, spinal cord, etc., or to hormone-producing glands, resulting in abnormal secretion of important hormones, etc.)[2], which can be very fatal. Reduced mobility also allows doctors and researchers to better visualize the cancer cells to determine their type. After making a detailed judgment, the next step in the treatment plan can be prescribed. An appropriate and effective treatment plan is very important. This will cost the patient as little time and money as possible and will have as few negative effects on the patient as possible if the treatment is successful. The tumor establishes fewer blood vessels in the surrounding area. It is well known that when cancer cells metastasize, they build up a lot of blood vessels around them to absorb enough nutrients and oxygen to divide more and support their further activities. The diminishing rate of cardiovascular establishment means that they no longer or rarely metastasize. It also means that these cancer cell clusters can create more distinct boundaries with other blood vessels and organs in the neighborhood (much less vascular connections to surrounding tissues). This will make the subsequent physical cutting (surgery) much easier and will also have as little impact on the patient as possible at the time of surgery[3]. This is important for the patient's post-operative recovery. Fewer and less extensive wounds are easy for the body's platelets to repair, even though there will be many minimally invasive procedures performed on blood vessels (including capillaries, veins and arteries). The frequency of bleeding is significantly reduced. The erosion of the tumor on the surrounding blood vessels will lead to the ulceration of the surrounding tissues or organs, and the rupture of the blood vessels caused by the ulceration will give rise to the symptom of bleeding. After the observation of each surgery, the conclusion is probably: each organ bleeding even in a small area will cause difficulty in hemostasis (high pressure inside the organ, resulting in high blood pressure, which is verified by the spattering hemorrhage rather than the flowing hemorrhage). A single organ bleed means multiple surgeries have to be performed. Each surgery, even if minimally invasive, has

a greater impact on the patient (not taking into account mishandling during the surgery, such as bacterial infection or additional damage to nerves or blood vessels). As an example, these effects include: muscle contraction around the scar can lead to tugging on the surrounding tissues and tingling nerves, loss of iron from the body after heavy bleeding can lead to iron-deficiency anemia (symptoms of which are pallor, dizziness, and fatigue); and low blood oxygen concentration due to loss of blood after the surgery (symptoms of which are shortness of breath and the inability to support more strenuous exercise such as running).

2. Pros and cons about the CAR-T cell therapy

2.1. Pros

Its recognition of tumor antigen does not depend on human leukocyte antigen, which can prevent tumor cells from evading the recognition and attack of the body's immune system through a variety of mechanisms, and will not be limited by the patient's human leukocyte antigen typing. Moreover, CAR-T immunotherapy can target all cell surface antigens to recognize a wider range of targets. It can also regulate most of the T-cells and improve the ability of T-cells to proliferate, generate and lyse target cells, which can enhance the anti-tumor effect in the tumor microenvironment.

2.2. Cons

Although it can play an auxiliary anti-tumor role in the treatment, but at the same time, it will also cause damage to the organism, triggering a systemic toxic reaction, central toxic reaction. In addition, pro-inflammatory cytokines act on the endothelium of blood vessels, and the increase in vascular permeability will lead to the occurrence of vascular leakage, causing skin flushing, edema, and alveolar effusion, which may lead to symptoms such as coughing, chest tightness, and dyspnea, and it may also lead to the reduction of blood volume, resulting in a drop in blood pressure, and even cause renal function damage. Though there are some drawbacks to CAR-T cell immunotherapy, the presences of those side-effects are inevitable. Compared to the huge benefits it brings, the negative influences can be ignored. So, the CAR-T cell immunotherapy is an overall dependable technique to use in treating cancers).

3. New Trends

3.1. The first new trend (fourth generation CAR T cells)

It now extends to the fourth generation of CAR-T. The cytokine gene is added to the structure of CAR-T, and the cytokines that enhance the activity of T cells can be highly expressed at the same time after the activation of CAR-T cells, so as to improve the anti-tumor activity of CAR-T.

3.2. The second new trend (SynNotch technology)

Professor Lim has developed a SynNotch technology. SynNotch is A synthetic Notch receptor that can be permanently expressed on the surface of T cells through genetic engineering, and its extracellular structure is also an antibody fragment that recognizes and binds to antigen A. The binding of antigen A causes the peptide chains within the SynNotch membrane to be cleaved, releasing the linked transcription factors. The transcription factor enters the nucleus and specifically initiates the transcription of the second CAR. After the second CAR expression is inserted into the cell membrane, if the second antigen B is encountered, it will further activate a special signal to kill tumor cells. The SynNotch technique allows CAR-T cells to be activated to kill tumor cells only if the tumor cells express two antigens A and B. The results of animal experiments showed that inoculation of single antigen tumor on the left side and double antigen tumor on the right side, intravenous injection of human SynNotch CAR-T cells had curative effect only on double antigen tumor. Due to the heterogeneity of solid tumors, few solid tumors can be distinguished by a single

surface antigen. The advantage of SynNotch CAR-T cells is that they are able to kill tumor cells expressing 2 antigens more precisely, without harming healthy cells or tissues expressing only 1 antigen in the patient. Therefore, the identification of dual-target SynNotch CAR-T cells can not only reduce adverse reactions, but also expand the targeting space of CAR-T cells.

3.3. The third new trend (CAR-X CELLS) :

3.3.1 CAR-NK cells

They are a type of immune cells inherent in the human body, referred to as NK cells, with powerful immune killing and immune memory capabilities. With the advent of chimeric antigen receptor T cell (CAR-T) technology, scientists have turned their attention to NK cells, which are also immune cells, to explore their potential for CAR modification. Thanks to their apparent lethality against foreign invaders, NK cells made scientists feel that this would be more effective than T cells in treating cancer. The development of CAR-NK cells follows the classic CAR-T cell development methodology, but differs from CAR-T cells due to the different structure and defensive methods of natural killer cells. CAR-NK cells have several distinct advantages over CAR-T cells. First, in terms of cell source, NK cells can be derived from pre-existing cell lines or allogeneic NK cells from the major histocompatibility complex (MHC)[4]. On the other hand, CAR-NK cells can kill cancer cells through both CAR-dependent and CAR-independent pathways. Cytokine release syndrome and neurotoxicity are less frequent and less detrimental when CAR-NK cells are working[5].

International and Chinese trends in the development of CAR-NK cell immunotherapy: In response to the innovative nature of immunotherapy, top international pharmaceutical companies have been laying the groundwork to enhance their core competitiveness based on R&D capabilities and innovative technologies. Sanofi spent more than \$1 billion to introduce CRISPR genome editing technology to develop CAR-NK cell therapy for treating tumors. Although Chinese companies started a little later, they are also rising rapidly and the competition is fierce. iPSC-CAR NK cell field has a number of pharmaceutical companies such as Star Pharmaceuticals, Bosengi Pharmaceuticals, Xinchuan Biologicals, Conch Xintu, and Pumice Biologicals[6]. The impact of the epidemic has basically disappeared, and domestic companies that have been dormant for three years will show their strengths, and soon come out with a development path that suits China's clinical needs.

3.3.2 CAR-M macrophages

The primary function of macrophages is to engulf (i.e., phagocytose and digest) cellular debris and pathogens in body fluids and tissues and to activate lymphocytes or other immune cells to respond to pathogens through antigen presentation. Macrophages have a major source, which is the precursor cells in the embryo. Those macrophage cells can easily develop in various tissues and organs and are transported towards damaged or ill tissues to perform repairing functions such as tissue-reconstruction or inflammatory response[7].

CAR-modified macrophages (CAR-macrophages, or CAR-M) are considered a promising cell type. CAR-M is centered on macrophages, which can be extracted easily and safely from the patient's body, and the well-known 'CAR' will be introduced into the macrophage cells through genetic engineering methods to finally achieve the goal of eliminating tumor cells.

3.3.3 Advantages of CAR-M therapy

Macrophages may be more likely to infiltrate tumors in an immunosuppressive microenvironment than immune cells such as T-cells and NK-cells, providing new opportunities for tumor immunotherapy, an area that has received increasing attention from researchers and investors in recent years. T cells are unable to enter the tumor environment due to a specific physical barrier, which is formed by the stroma around the tumor cells. The stroma will make cells in immune system unable to perform their role after infiltration due to immunosuppression; macrophages, however, will easily invade into patient's bodies environment.

In 2016, Saar Gill and Michael Klichinsky, CAR-T cell therapists at the University of Pennsylvania, worked on developing CAR-M therapies to treat tumors. They have found that HER2-

CAR-M was able to transform M2 macrophages into M1 macrophages, induce an inflammatory tumor microenvironment, and enhance the anti-tumor cytotoxicity of T cells[8]. Thus, this reflects the major advantage of CAR-M in creating a pro-inflammatory environment within the tumor.

3.3.4 CAR-Treg cells

Regulatory T cells (Treg) are a small subset of immune cells whose primary function is to inhibit immune activation and protect immune homeostasis. Over the past few decades, the enhancement of chimeric antigen receptor (CAR) and gene editing technologies has facilitated the upgrading of T cell therapy. CAR technology could be used to improve the functionality of Treg cells. Thus, the Treg cell therapy is still rapidly evolving. Treg cells have the following five main functional mechanisms: secretion of inhibitory cytokines (including IL-10, TGF- β , and IL-35), killing of effector cells through granzymes and perforins, and affecting effector cell function, respectively; Treg inhibits the growth of effector T cells by competing with effector T cells for the depletion of IL-2; Treg inhibits the growth of effector T cells through the production of the extracellular enzymes CD39 and Treg promotes the production of adenosine in TME through the production of extracellular enzymes CD39 and CD73, which exerts inhibitory and antiproliferative effects on effector cell conductance; and transfers large amounts of cAMP to effector T cells through gap junctions, which interferes with their metabolism [9].

MDSCs and Treg can produce some factors, and those factors can form a positive feedback loop that promotes proliferation and enhances the suppressive environment. CAR-Treg is a strategy for the treatment of autoimmune diseases. CAR has unique advantages over TCR-Treg: activations of CAR-expressing T cells detours HLA restriction; increased specificity through co-receptor signaling activation; and targeting flexibility of CAR (any soluble or surface multivalent antigen can be targeted)[10]. CAR-Treg has unique advantages over TCR-Treg which bypassing HLA restriction upon activation of CAR-expressing T cells; increased specificity through co-receptor signaling activation; and CAR targeting flexibility.

4. Unique advantages of CAR-Treg cell therapy

4.1. Synthetic biology

The continuous developing of Treg cell medicines for the curing of autoimmune illness will not be limited into the application of TCRs and CARs; after few years of developing, synthetic immunology has already generated loads of artificial receptors and systems that had being tested in various types of Treg cells. It is undoubtful that cytokines have also multiple key effects in the immune system. What's more, through the modification of CAR-Treg cells, proinflammatory cytokines' signals can be changed into other specific signaling to improve the performance of suppressing the inflammation.

4.2. Edition of genes

Preclinical studies which used CRISPR-Cas9 to try to reprogram human T cell genes including cutting out the gene for CCR5 in CD4⁺ T cells to generate T cells resistant to HIV infection; moving out the gene for CD7 in CD7-CAR T cells, thereby preventing self-interaction; and remove the gene for CD19-CAR T gene for PD1 in CD19-CAR T cells, thereby enhancing the performance of tumor clearance in an artificial model.

4.3. Upgrading the delivery system

In recent days, CAR-T cells are being produced by using retroviral and lentiviral transduction to deliver and combine different genetic material into a new integrated T cells. Time-consuming, expensive and safe, will be a major nuisance for nowadays.

5. Future perspectives

On the limitations of CAR-T cell immunotherapy using viruses as vectors: Till now, most of the CAR-T cell therapies had been approved or researched in many clinical trials which have utilized especially viral vectors and both retroviral and lentiviral vectors. Viral vectors are standardized systems with effective gene transferring which have already demonstrated enough safety in the innovative T-cell therapy. With newer types of CAR-T technology, there is a need obviously to transduce more activating progenitors with other various specificities to achieve higher safety, efficiency and capability of application.

Furthermore, viral manufacturing for clinical purposes usually takes two to three weeks, takes place under GMP conditions and in Biosafety Level 2 (BSL2) facilities, and demands a skilled, well-trained crew. The sheer cost, sophistication, and need for customized therapies can eventually impact on the cost of CAR-T products, which can run into the hundreds of thousands of dollars per capita, making them prohibitively expensive for the average patient.

There is a hope to discover a non-viral approach to CAR-T cell production, referred to as the Sleeping Beauty System. The Sleeping Beauty System utilizes an electroporation method to transfer genes from a DNA plasmid into T cells. The DNA plasmid is more convenient and less expensive than the lentiviral vectors presently used for preparing CAR-T cells, and the Sleeping Beauty System enables the revision of T-cells on the fly, without the need to wait for the lentiviral vector-modified T-cells to grow in culture flasks.

After a long period of evolutionary "sleep", the SB gene identified in the fish genome has opened up new horizons for gene therapy by becoming the first transposon to demonstrate activity in vertebrate cells. A significant benefit of this strategy is the increased loading capacity compared to viral systems. The magnitude of the insert is negatively correlated with the efficiency of the transposition mechanism. The optimal loading size does not surpass 6 kb. Nevertheless, its upgraded version, which contains several completed transposon units, thus increases the loading capacity up to 11 kb and expanding the cloning capacity based on SB vectors. In addition, when incorporated into a bacterial artificial chromosome (BAC), the SB can deliver up to 100 kb of transgenes in human embryonic stem cells. The design of the next generation of CAR T is mainly focused on increasing the activity and persistence of T cells, overcoming the immunosuppression of the tumor microenvironment, preventing the immune escape of tumor cells, reducing the toxicity of therapy and improving the clinical accessibility of patients[11].

6. Conclusion

CAR-T cell therapy has been developed for more than 30 years, and has achieved good therapeutic effect in clinic. However, risks and opportunities coexist, and potential toxicity and drug resistance problems are also shown in CAR T cell therapy, which accelerates the pace of finding new tumor targets, studying signaling mechanisms and developing new technologies. Though there are some drawbacks for CAR-T cell immunotherapy, the presences of those side-effects are inevitable. Comparing to the huge benefits it brings, the negative influences can be ignored. Innovations in CAR molecular design, transduction methods, and selection of optimal cell types may lead to new breakthroughs that change the way tumors are treated in the future, and cell therapy is in the future. So, the CAR-T cell immunotherapy is a overall dependable technique to use in treating cancers..

References

- [1] Peijie Shi, Jianpei Fang. (2017). Chimeric antigen receptor t-cell (car-t) therapy in the therapeutic field of common pediatric tumors. Chinese Journal of Pediatric Hematology and Oncology (1).
- [2] Chris, & Morrison. (2015). Car-t field booms as next-generation platforms attract big players. Nature biotechnology.

- [3] M.Juan, & S.Rives. (2020). [car-t immunotherapy in pediatric hemato-oncology... present and future]. *Anales de pediatria*, 93(1), 1-3.
- [4] Heuvel, R. V. D. (2023). Next-generation cell therapies for cancer: car-nk cells. *Medicom Conference Report EBMT 2023*.
- [5] Pomeroy, E. J., Lahr, W. S., Chang, J. W., Krueger, J., Wick, B. J., Slipek, N. J., et al. (2021). Non-Viral Engineering of CAR-NK and CAR-T cells using the Tc Buster Transposon System. Cold Spring Harbor Laboratory.
- [6] Xin-Yang, L., University, S. N. (2019). Advances in research of car-nk in tumor immunotherapy. *Biological Chemical Engineering*.
- [7] Zhang, L., Tian, L., Dai, X., Yu, H., Wang, J., & Lei, A., et al. (2020). Pluripotent stem cell-derived car-macrophage cells with antigen-dependent anti-cancer cell functions. *Journal of hematology & oncology*, 13(1), 153.
- [8] Pengbo Ning. (2023). A Cold and Hot Tumor-Modulated CAR-Monocyte/Macrophage Cell and Its Preparation Method and Application. CN116240173A.
- [9] Cremoni, M., Massa, F., & Sicard, A. (2022). Overcoming barriers to widespread use of car-treg therapy in organ transplant recipients. *HLA*, 99(6), 565-572.
- [10] Beheshti, S., Shamsasenjan, K., Ahmadi, M., & Abbasi, B. (2021). Car treg: a new approach in the treatment of autoimmune diseases. *International immunopharmacology*, 108409.
- [11] Williams, M. E. (2014). Further Progress in CAR-T-Cell Therapy for Acute Lymphoblastic Leukemia.