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# *The Advantages, Disadvantages, and Improvement Method of the CAR-M Technology*

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***Abstract:*** Chimeric Antigen Receptor Macrophages (CAR-M) is the latest generation of chimeric antigen receptor technology. Its main principle involves introducing the CAR gene into macrophages extracted from a patient's body, activating these macrophages, and then re-infusing them back into the patient. Compared to the previous generation of Chimeric Antigen Receptor T-cells (CAR-T) technology, CAR-M has significant advantages as it can circumvent the challenge of effectively penetrating the tumor microenvironment. CAR-M is capable of engulfing the corresponding tumor cells, and at the same time, CAR-M cells can present antigens and produce cytokines to enhance the killing ability of T cells against tumor cells. However, since macrophages are highly differentiated cells that cannot divide and proliferate, multiple injections are required. This article analyzes the technology by combining the strengths and weaknesses of CAR-M. By altering the inherent defects of macrophages and changing them to monocytes (the precursor cells of macrophages), and further improving or enhancing some of the defects or advantages of monocytes themselves, the effectiveness of the technology can be enhanced. Now, the integration of CAR-M technology with the CRISPR/Cas system can enhance the efficiency of the technology in disease treatment. Currently, Cas9 is relatively popular and has a broader range of applications. However, due to the significant off-target effects of CAS9, the CAS12a system, which has a lower off-target rate, can be selected.

***Keywords:*** CAR-M, CAR-T, tumor microenvironment, macrophages, monocytes.

## 1. Introduction

CAR-M technology is a kind of emerging technology, and the background of this technology is closely related to the development of CAR-T technology. CAR-T technology has developed for many years, it is mature now. However, CAR-T technology also faces a variety of challenges. For example, continuous stimulation or the expression of the inhibitory receptor can lead to the phenomenon of T-cell exhaustion. The tumor microenvironment (TME) consists of some complex structures such as capillaries, tissue cells, lymph, and nerve cells. Because of those reasons, CAR-T cells cannot get into TME effectively. With the development of the technology, CAR-M cells are the better solution to this thing. It can avoid the problem of the low infiltration of the TME. Also, CAR-M cells have more benefits than CAR-T cells.

Macrophages can differentiate into two types of cells, M1 and M2. M1 Macrophages can secrete various inflammatory factors such as TNF-α, ILs, and so on. They play an important role in the

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immune system [1]. So M1 macrophages can be the first choice of the vector of the CAR receptor. However, the macrophages are possible to polarize into the M2 macrophages [2]. Also, the M2 macrophages can produce some immunosuppressive molecules leading to the efficiency of the M1 macrophages being lower than before. Fortunately, one research found that the PPARs and the Krüppel-like factors (KLFs) play an important role in the polarization of the macrophages [3]. Now the CAR-M technology has been already applied in some clinical areas. One research shows that a kind of new macrophage can pass through the CD147, which can help the CAR-M cells be easier to get to the place where the cancer cells are [4]. And also. The research shows that CAR-M technology can be a type of treatment for cancer in the human body. Until 2020, the FDA has authorized two clinical experiments. Another research shows that the suppression of the SIRPa can stimulate the inflammatory pathways, and also the cGAS-STING signal transduction cascade ways can lead to a larger yield of cytokine which can increase the efficiency of cancer cell killing [4]. Also, one research shows that using CRISPR-Cas9 to reduce or cut off the expression of the SIRPa, and the macrophages that have low-level expression of the SIRPa have stronger tumor cell nuclear engulfment. Also, this experiment has been conducted on mice [5]. In conclusion, they proved that the knockout of the SIRPa can be a great help in cancer treatment.

This article seeks to explore the principles, advantages, and disadvantages of the CAR-M technology and put forward a suggestion that changing the macrophages into the monocytes, to improves the performance of this technology. Apart from that, this article deeply discussed the combination between the CRISPR system and the CAR-M technology. It also method the CRISPR-Cas12a, a kind of more effective system of CRISPR, especially, since it has a lower off-target rate than Cas9, this advantage can increase the speed of gene editing of some special DNA sequences in CAR-M cells. CAR-M technology means the improvement of CAR-T technology, CAR-M cells can overcome some questions that happen in the CAR-T cells for example, CAR-T cannot get into the TME, effectively, and they have more serious toxicity than the CAR-M cells. This research discussed the solution to the questions of the CAR-M technology by way of its DNA or some important enzymes and receptors by increasing or decreasing their expression. Also, this article will discuss another way to make the CAR-M cells more effective in cancer treatment, which is to change the macrophages into monocytes. In this way, it is possible to the solution of the disadvantages of the CAR-M cells. All in all, CAR-M technology not only can push the development of the area of immune and medicine but also can be edited by the CRISPR system, to be a personal and accurate medicine tool for patients. With the development of the technology and deeper research, CAR-M technology is expected to be a better way to treat cancer play a more important role, and bring more hope to patients in the future.

## 2. The principal and advantages of the CAR-M technology

### 2.1. Principle

Macrophages are a kind of immune cell, that plays an important role immune system. These immune cells can phagocytize a variety of pathogens and can also secrete cytokine and chemokine that the T cells and NK cells gather at the places where the tumor cells in. Because of this, this technology can bring more security and efficiency to cancer treatment. Apart from these functions, they can present antigens and have a strong power of penetrating dense tissue [6]. Because of these characteristics, macrophages can be better vectors of the CAR receptor. Researchers used the mice to do research that CAR-M has a good result for the treatment of HER2+ during the research of mice [7]. After a single injection of CAR-M cells into HER2+ immune-deficient mice, 50% of mice survived 40 days longer than the control group. Compared with the normal mice, CAR-M cells reduced the CR of the mice by 75%. This shows the efficiency of the technology.

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## 2.2. Advantages of the CAR-M technology

### 2.2.1. CAR-M technology has a lower rate of off-target

CAR-T cells have a larger off-target rate than CAR-M cells, off-targeting can result in higher toxicity, which can cause cellular injury. In the research of the treatment of cardiac fibrosis, CAR-M technology has been used in is research. The experiments targeting cardiac fibrosis show that the CAR-M cells have extremely high efficiency in the treatment of cardiac fibrosis [8]. At the same time, these CAR-M cells have an extremely low off-target rate. The result of the experiments shows that the CAR-M cell which is used to cure cardiac fibrosis has very low levels in other organs besides the heart. Also, the content of the cytokine in mice treated with this kind of CAR-M cells is not so significantly different from the content of the cytokine in the mice that have not been treated with these CAR-M. This research can prove the security and the low off-target rate of this technology.

### 2.2.2. CAR-M cells can phagocytosis the cancer cells and also have the function of presentation.

Research shows that CAR-M cells have the function of adsorbing, and also, they can also present this kind of antigen to the T cells. CAR-M has shown that the function of absorbing is activated by the specific antigen named (TAA). This function can make it easier to kill the cancer cells. One research is about the evaluation of the toxicity and infiltration of the CAR-M cells shows that the CAR-M cells have a higher power of direct tumor killing in the 3D tumor. Also, the CAR-M technology has the durable activity to eliminate the tumor.

## 2.3. Applications of the CAR-M technology

SIRPa (signal-regulatory-protein-alpha) is a kind of surface protein of the cells, which is expressed in the immune cells such as macrophages, dendritic cells, and so on. It belongs to the immunoglobulin Superfamily. This kind of immunoglobulin plays an important role in the immune system. However, in some cancer cells, the expression of the CD47 is higher, so that can combine with the SIRPa which are on the macrophages, this will cause the phenomenon that the function of absorbing will be reduced which cuts down the efficiency of the tumor-killing rate. Because of this, one research shows a way to reduce the expression of the SIRPa. They combine the FcyRlla with the humanized single-chain and also the hairpin RNA to silence the SIRPa, to reduce the rate of the combination of the SIRPa and the CD47.

# 3. Disadvantages of the CAR-M technology

## 3.1. Patients must inject the CAR-M cells repetitively.

Because macrophages are a kind of highly differentiated cells, they cannot ongoing division, and also the content of these kinds of cells is lower, so to get the target of the treatment, the doctor must inject the CAR-M cells one by one, this drawback will lead the increasing of the cost and the period of the tumor treatment. To avoid the negative influence of this advantage, there exists a way to solve this problem to replace the Macrophages with the monocytes, there is a possible way to conjugate the CAR receptors to the monocytes, and inject the monocytes which are added to the CAR receptors into the patient's body. Nowadays, one research shows that the monocytes can proliferate locally under certain conditions. For example, the LyC6+ monocytes can fission before they differentiate into interstitial macrophages (IM) by the way of relying on the Csf1 receptor [9]. Changing the macrophages into monocytes can avoid the question that the CAR-M cells should be injected into

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patients' bodies repetitively. Monocytes are the precursor of the macrophages, they can differentiate into the macrophages and can produce the efficiency which is the same as the CAR-M cells.

### 3.2. Macrophageshages will be the "traitor" of the immune system.

Tumor-associated macrophages (TAMs) are a kind of most of the immune cells in the TME. They play an important role in the tumor treatment. However, in some cases, TAMs can promote the growth of tumor cells, and this phenomenon can cause a reduction in the efficiency of tumor treatment, for example, the research which is about brain tumors shows that TAMs absorb the myelin debris and then become the LLMs [9] (a kind of rich-lipids macrophages). And then, LLMs take this kind of lipids to the tumor cells. This progress promotes the increase of tumor cells. Fortunately, the research shows that the CD36 can reduce the myelin phagocytosis. And also, the Abca1 can control the lipid transportation [10].

## 4. Solutions for the drawbacks and the improvement methods of the CAR-M technology.

### 4.1. Increasing the chemotaxis towards the area of the tumor by reducing the bad influence of the IFN-g

One research shows the application of a kind of chip to test the chemotaxis of the monocytes, and the results of the experiment indicated that the monocytes can migrate toward the tonsure of the CCL2 preferentially, however, the IFN-g can reduce the chemotaxis of the monocyte which toward the tonsure of the CCL2, and also reduce the expression of the CCR2 [11], so it is possible to adopt some ways to reduce the bad influence of the IFN-g. For example, it is possible to increase the expression of the CCR2 or reduce the sensitivity of the CCR2 to the IFN-g to increase the chemotaxis of the monocytes.

### 4.2. Increasing the expression of the B7-H3 to reduce the apoptosis of the macrophages

B7-H3 is a kind of Type I transmembrane protein which belongs to the B7 superfamily. They do not express in the monocytes and the human normal tissue. However, they often express in some immune cells such as T cells, B cells, NK cells, and macrophages. And also, the B7-H3 expression in some tumor cells. B7-H3 can increase the anti-apoptotic ability of the macrophages by the activation of NF-κB upregulates HIF-1α [12]. They use the FCM to analyze the proportion of the apoptosis cells, the result shows that the high expression of the B7-H3 mainly influences the early apoptosis. According to this result, it is possible to reduce the bad influence of the first disadvantage is to increasing the expression of the B7-H3 in the CAR-M cells to reduce the apoptosis of these cells to ensure the count of the CAR-M cells and the efficiency of the tumor treatment.

### 4.3. The combination of the CRISPR/Cas system and the CAR-M or CAR monocytes technology

Nowadays, the CRISPR/Cas system has been applied in a variety of areas in biology. The most concerned one of the CRISPR/Cas system is CRISPR-Cas9. This system originated from a kind of archaea, it can prevent the archaea from the virus, so this system also be regarded as the immune system of the archaea. CRISPR-Cas9 consists of the Cas9 nuclease and the gRNA (guide RNA). The gRNA consists of the crRNA and the tracrRNA [13]. But now, these two kinds of RNA merge into one RNA called sgRNA. It is effective and has a variety of advantages. However, CRISPR-Cas9 could not avoid having some drawbacks, for example, because the compound which consists of Cas9 protein and the sgRNA needs to identify the PAM sequence to ensure the progress of cutting can go smoothly. If the sgRNA cannot connect with the PAM completely, there is a serious disadvantage the

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high off-target rate will be made, this phenomenon will reduce the efficiency of gene editing, so that make the CRISPR/Cas9 can only be used in small-scale gene editing like the group of some bacterium. And also, the gRNA of the former CRISPR/Cas9 system has the same phenomenon of off-targeting [14]. To avoid the influence of this phenomenon, there is a relatively effective way to change the Cas9 into the Cas12a. Compared with the Cas9, the Cas12a system has a variety of advantages. For example, the Cas12a system has more simpler structure than the structure of the Cas9 system, because it only consists of two parts, which are the Cas12a protein and the crRNA. This advantage makes the Cas12a can be easier to get into the cells. Also, the research shows that the Cas12a system has a more serious identification of the PAM sequence than that in the Cas9 system [15]. Because of these advantages, the CRISPR/Cas12a system will be an applicant in more and more areas of tumor treatment, and also it will take the place of the CRISPR/Cas12a. It is possible to use the Cas12a system to reprogram the CAR-M cells and improve the expression of the B7-H3 to reduce the early apoptosis rate thereby reducing the damage of the CAR-M cells. Also, the other available way to solve this problem is to reprogram the CAR monocyte so that the expression of the CCR2 is higher than the normal monocytes.

## 5. Conclusion

In conclusion, this article analyzes the applications of the CAR-M technology, from the advantages, disadvantages, and improvement plan of the CAR-M technology. And explored the complex situations, this technology has inestimable potential in the area of tumor treatment. CAR-M is at the forefront of the technology. CAR-M cells can get into the TME and also, and they can phagocytize the tumor cells, and can also present antigens to some other kinds of immune cells. Apart from these, the lower off-target rate leads to stronger security. These characteristics make it hopeful to replace the CAR-T technology to be applied in the field of medicine. However, macrophages cannot proliferate, and they sometimes be the “traitor” of the immune system. These drawbacks make the CAR-M technology face a giant challenge. To solve these problems, this article shows a way to solve the first problem is to replace the macrophages with monocytes and utilize the ability of proliferation of the monocytes. This article also introduces the monocyte chemotaxis to CCL2 and the effect of IFN-g on CCL2. Also, the CRISPR/Cas system is used to reprogram the CAR-M cells or the CAR monocytes to increase the efficiency of tumor treatment. Regarding the second problem, it is possible to use the CRISPR/Cas system to knock down the Abca1 gene to reduce the ability of the lipid transportation, to reduce the lipid output of the LLM. This can reduce the influence of the second drawback. Later, the article shows the principle and the structure of the Cas9 system and finds out the drawbacks of it. Cas12a system is a better tool to replace the Cas9 system to reprogram these CAR cells, because of its simpler structure and lower off-target rate than the Cas9 system. Although this article analyzes the CAR-M technology and provides solutions to its drawbacks, there are also a variety of limitations in this article. For example, the influence of knockdown of the Abca1 is unclear, and increasing the expression of the CCR2 whether influent the life of the monocytes or not. If the CAR-M technology can combine with AI models or the nanorobots, the CAR-M cells will get to the position of the tumor more controllable and accurate.

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