

Trends for CRISPR and CAR-T in the future medical field

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Abstract. CRISPR as a protein editor that identifies specific genes and then sending off transcriptions to later be used for the new strand of DNA that is altered so that the problems within the previous codes would be fixed. In addition, the other method, CAR-T, this is a newer method that edits cells directly and treats diseases such as cancer and tumors. Using human autologous, allogeneic, or heterogeneic (non-human) cells that have been altered in vitro and then injected (or implanted) back into the body is referred to as cell therapy. CAR-T cell therapy, one of them, is an adoptive cell immunotherapy that has emerged recently. It works primarily by combining the specificity of chimeric antigen receptor with the immune effect of T cells, and then killing malignant tumor cells through specific recognition. The technology is also being enhanced and expanded to other disease areas given its promise and benefits. There are many generations of this, and each has been improved. For now, the current technology still has posed limitations on CAR-T, but it is progressing at a steady pace. Therefore, there are positive futures for the technology of gene engineering. From the successful cases that has been proceeded, this also proved the development of this technology in the future. This review systematically summarizes CRISPR and CART, these are two ways that we use as cell and gene engineering.

Keywords: CRISPR, CAR-T, immune therapy.

1. Introduction

CRISPR-Cas9 gene editing technology was first discovered in the late 1980s and in 2011, a molecule called Cas9, which is part of the bacterial immune system, was demonstrated could be used to cut DNA at specific locations within a genome, allowing for precise editing of the genetic code [1]. The following year, in 2012, Feng Zhang and his team at an area in the MIT and Harvard has proved that the CRISPR-Cas9 system had the potential be used to edit the genes of human cells [2]. This breakthrough opened up the possibility of using CRISPR to treat genetic diseases and revolutionized the medical field. Since then, the application of CRISPR-Cas9 continues to expand, with new applications and improvements to the technology being developed every year [3].

The groundbreaking cancer treatment CAR-T cell therapy uses the immune system's ability to find and kill cancer cells. CAR-T cell therapy functions by taking a patient's own T cells and editing them to express CARs which are able to recognize and bind to specific cancer cell markers. Once infused back into the patient's body, these CAR-T cells can seek out and destroy cancer cells, leading to potentially long-lasting remissions in patients with certain types of blood cancers. A significant advancement in the field of cancer immunotherapy is CAR-T cell therapy, and has shown promising results in clinical trials, making it a highly anticipated therapy for patients with advanced cancers. While CAR-T cell therapy is still a relatively new treatment, its success in clinical trials has led to its approval for certain types of blood cancers, and ongoing research is exploring its potential for use in other types of cancers. CD19, a protein seen on the surface of B-cells. This kind of therapy is mainly used in the latest cases of tumors and cancer treatment. This has been shown a majority of recognition, as there has already been lots of successive cases in the uses of this therapy. Also, for this technology it had shown little side effects in the patients after treatment. By editing intercellular genomes, it rarely makes any mistakes, also demonstrating how safe this kind of therapeutic is. The use of CAR-T cells to treat hematologic malignant malignancies has become increasingly advantageous as CAR-T technology has advanced. The negative effects of CAR-T therapy have, however, gradually come to light and have raised concerns as research into the treatment continues to advance and be used more widely.

2. CRISPR/Cas9 system

2.1. The components of CRISPR/Cas9 system

Due to the structural differences of Cas 9, the now-famous CRISPR/Cas system is divided into 2 broad classes (Class I and II), six kinds, and numerous subtypes. Each type has a unique Cas protein [4]. Class II systems work by using a single cas protein, in contrast to Class I systems, which are composed of multi-subunit cas protein complexes [5]. The molecular tool for CRISPR-Cas9 consists of the guide RNA and the Cas9 protein and belong to the type II CRISPR-Cas system, which is the oldest and most developed CRISPR system studied. The natural RNA module consists of crRNA, which locates the target DNA site, and tracrRNA, which helps the crRNA to mature. These two components were then artificially engineered to combine into a single guide RNA, making it easier to target the target sequence. To achieve gene editing, the sgRNA-Cas9 protein complex typically binds to a specific protospacer-adjacent motif (PAM) sequence to cause a flat-ended DNA double-strand break. This triggers intracellular homologous recombination (HDR) or non-terminal homologous junction (NHEJ), which then repairs the DNA [4, 5]. Despite its complexity, *E. coli*'s INTEGRAL mechanism targets 10 kb of foreign DNA fragments into the bacterial genome with about 100% efficiency and without any selective pressure. With the use of this technology, site-specific insertion of particular bacteria in intricate microbial communities has been made possible, in addition to the targeted insertion of *E. coli*, *Klebsiella oxytoca*, and *Pseudomonas putida* [6]. Possibly marking particular sequences to make them easier to find and correct later.

2.2. Function mechanism of CRISPR/Cas9 system

There are two main parts to this genetic process. The NHEJ and HDR pathways are two ways to repair problems like DSB that are brought on by Cas-9 protein. By uniting genetic pieces by an enzyme (key to lock) process without the involvement of external homologous DNA, NHEJ facilitates the repair of DSBs. Figure 1 illustrates the market for CRISPR in the medical industry. Another sign of the technology's increasing acceptance is the fact that more and more patients are putting their trust in it, which has increased the market for CRISPR in the medical industry.



Figure 1. The market for CRISPR in the medical industry

The cellular repair process that is most active in cells is the earliest and finest one, but it is also most prone to mistake. This might lead to a chance insertion or deletion at the cleavage site, which would produce a mutation or an early stop codon. Contrary to what was previously indicated, HDR is quite accurate and calls for the usage of a homologous DNA template [7]. A significant number of diner DNA templates with an interesting sequence are needed for HDR (which has been programmed

into the original gene), it operates similarly to the earlier approaches. Normally, cells repair damaged DNA by effective NHEJ. The target gene is disabled and gene knockout is made possible by frameshift mutations that come from base mismatches of insertions or deletions that frequently happen during repair. If a DNA repair template enters the cell after the DNA double-strand is broken, the genome break section will undergo HDR in accordance with the repair template, leading to gene knock-in. The size of the altered sequence determines the length and position of the homologous arm, which is made up of the homology sequence (homologous arm) upstream and downstream of the target gene and the target sequence to be imported.

3. CAR-T therapy

The main process of CAR-T therapy includes isolation of the patient's T cells, culturing and expansion in vitro, followed by gene editing to express CARs on the surface of the T cells, and finally infusion of the engineered CAR-T cells back into the patient. CAR-T specifically recognises binding tumours and activates T-cells via the CAR structure to secrete cytokines, perforin and granzyme to exert anti-tumour effects. With the development of various anti-tumour drugs, CAR-T has made unprecedented progress in the field of anti-tumour therapy: Small molecule targeted drugs can provide signal transduction to help CAR-T cells improve their killing ability and persistence at the epigenetic level; monoclonal antibodies can directly prevent immunosuppression of CAR-T cells at the cellular level; Tumour vaccines pave the way for the high recognition ability of CAR-T in advance; Tumour-targeted drugs increase the attackable area of CAR-T by sensitising tumours; Oncolytic viruses not only carry antibodies, but also help CAR-T infiltrate by lysing tumour cells while producing cytokines to fight with CAR-T.

3.1. Procedure of constructing CAR-T gene therapy

The building blocks of CAR-T gene therapy include choosing the right target antigen, developing the CAR, transducing T-cells, expanding and purifying the CAR-T cells, and finally injecting the CAR-T cells into the patient.

The first step in the development of CAR-T gene therapy is the selection of the target antigen. A protein that is expressed on the surface of cancer cells and distinguishes them from healthy ones is the target antigen. It is crucial to select an antigen that is unique to the type of cancer and is absent from healthy cells. Clinical trials have identified and investigated a number of target antigens, such as CD19, CD20, and BCMA. The chimeric antigen receptor is created in the following phase. The CAR is a man-made receptor that is designed to detect the target antigen on the surface of cancer cells. It comprises three basic components: an intracellular domain that can activate the T-cell in response to an antigen binding, a transmembrane domain that can anchor the CAR to the T-cell membrane, and an extracellular domain that can recognize an antigen [8]. Typically, a monoclonal antibody that binds the target antigen produces the extracellular domain.

T-cell transduction is the third phase. Blood is drawn from the patient, and T-cells are typically altered to express the CAR. The CAR gene is often delivered into the T-cells using a viral vector, such as a lentivirus or a retrovirus. The transduced T-cells are subsequently multiplied and purified to boost their quantity and get rid of any non-CAR-T cells.

The fourth step involves CAR-T cell expansion and purification. The patient's body receives the enlarged CAR-T cells once more. To guarantee the CAR-T cells' security and effectiveness, this stage calls for stringent quality control. The patient is attentively watched for any negative side effects and indications that the medication is working [9]. The high expense and complexity of producing the CAR-T cells, and the potential side effects are some of the difficulties that come with the therapy.

3.2. Different generations when coming to CAR gene editing

1st: To activate the T-cell, the initial generation of CAR T-cells utilized a single signaling domain, often the CD3 chain. In clinical trials, these CAR T-cells displayed modest effectiveness and

durability. Clinical studies have shown that first-generation CAR-T cells have a constrained ability for growth *in vivo*, nonetheless.

2nd: To improve the activation and proliferation of T-cell, the second generation of CAR T-cells added an extra costimulatory domain. According to certain research, the co-stimulation signaling domain can raise the cytokine production of CAR-T cells, encourage T cell replication, lower the possibility of CAR-T cells failing, boost T cell antitumor activity, and increase CAR-T cells' survival rates in patients. In clinical trials, these CAR T-cells shown increased efficacy and durability, and their use in the treatment of various blood cancers has been authorized.

3rd: To increase the CAR T-cells' potency and specificity, the third generation of CAR T-cells made additional modifications to the costimulatory domains. While, Whether the third-generation CAR-T is superior to the second-generation CAR-T is still controversial. Clinical trials, though, have not yet clearly shown why first-generation CAR T-cells are superior.

4th: The "armored" CAR T-cells of the fourth generation of CAR T-cells have additional genetic alterations to improve their potency and security. To increase their anti-tumor effectiveness, some fourth-generation CAR T-cells may express cytokines or other immune-modulating substances. Others have suicide genes that can be turned on to destroy the CAR T-cells if they start to cause problems. Although fourth-generation CAR T-cells are still in the preclinical stages of development, preliminary research has yielded encouraging results [10].

3.3. CAR-T cell in clinical application

With apparent benefits, universal CAR-T has established itself as an unstoppable trend in the field of cell therapy immunity. Numerous domestic and international businesses have created the groundwork for it, and an increasing number of general-purpose CAR-T products with both safety and efficacy have appeared, giving more tumor patients hope. After a large number of clinical trials and large-scale real-world studies after marketing, CAR-T cell therapy is becoming more and more mature.

BCMA (B-cell maturation antigen), is another interesting target for CAR-T cell treatment. ALLO-715, a novel therapeutic for multiple myeloma (MM), is a universal CAR-T therapy that targets BCMA. The results of the universal investigation, which involved 35 patients with R/R MM, revealed a positive correlation between the dose of CAR-T cells and the efficacy of ALLO-715 and its expansion in patients. Patients receiving BCMA-targeted CAR-T cell therapy had an ORR of 81% and a complete response rate (CRR) of 39%, according to research by Berdeja et al in 2021.

Clinical trials are looking into a number of different targets, such as CD22, CD30, and EGFRvIII, in addition to CD19 and BCMA. B-cells express the protein CD22, and CAR-T cell therapy that specifically targets CD22 has showed promise in treating B-cell cancers. Five of the six R/R B-ALL patients recruited in the study with CTA101, a universal CAR-T cell therapy product that targets CD19 and CD22 dual targets, were CR/CRi, and all of them tested negative for MRD. Not all patients experienced GvHD and ICAN episodes, and one patient experienced grade 3 CRS. Hodgkin's lymphoma and some T-cell lymphomas display the protein CD30 on their surfaces. The epidermal growth factor receptor, known as EGFRvIII, is expressed on the surface of glioblastoma cells. There is tentative evidence that EGFRvIII-targeted CAR-T cell therapy is effective in treating glioblastoma. CAR-T cell therapy has been associated with several challenges, including the risk of CRS and neurotoxicity, as well as the high cost and complexity of manufacturing the CAR-T cells. However, ongoing research is exploring ways to optimize and refine the therapy to make it safer and more accessible. These efforts include developing new targets for CAR-T cell therapy, improving the manufacturing process, and identifying strategies to mitigate the side effects of the therapy.

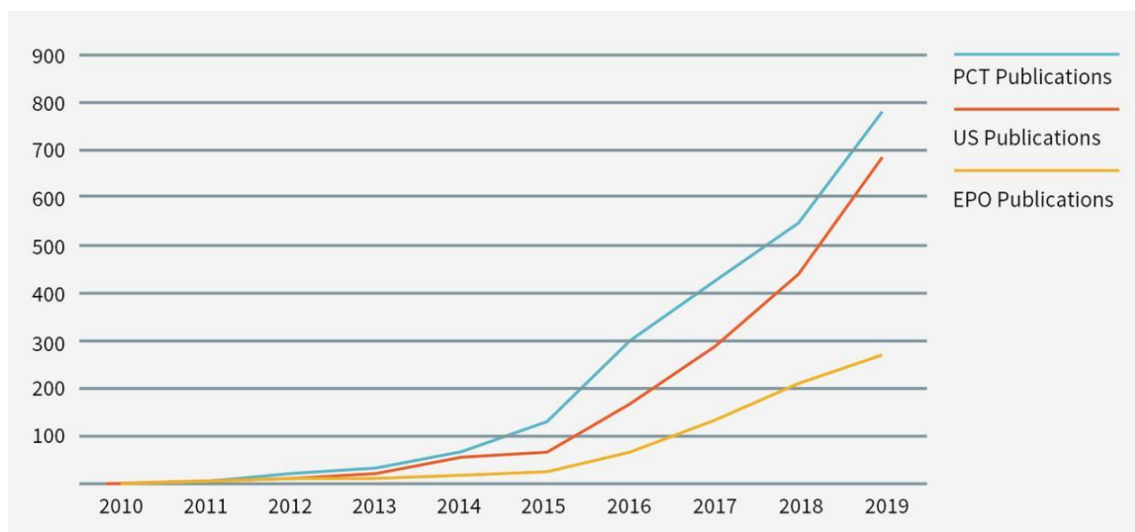


Figure 2. Patients reports on using the CAR-T

Solid tumors pose a number of challenges for CAR-T cell therapy, including the absence of a particular tumor antigen that the CAR-T cells can specifically target, the CAR-T cells' restricted ability to penetrate the tumor mass, and the presence of an immunosuppressive tumor. Figure 2 shows that the usage of CAR-T cell treatment has increased significantly. As more and more patients are eager to employ this type of medical treatment, this further demonstrates the great triumphs in this type of technology [11]. CAR-T cell therapy is currently being intensively investigated for the treatment of solid cancers. Targeting antigens that are expressed on both healthy and cancerous cells, but at higher levels on the latter, is one strategy that may lower the risk of toxicity to healthy tissue. Another strategy includes simultaneously attacking numerous antigens, which may increase the therapy's specificity and lower the danger of tumor escape.

Another strategy being researched by researchers is the use of nanoparticles or other delivery systems to help CAR-T cells penetrate solid tumors more effectively. To improve the effectiveness of CAR-T cell treatment, they are also looking for strategies to alter the immunosuppressive tumor microenvironment. There have been some encouraging findings in clinical studies, despite the fact that CAR-T cell treatment for solid tumors is still in its early phases of research.

Myeloid malignancies include a variety of antigen targets that could be treated with CAR-T cells, including CD33, CD123, LeY, CD44v6, CLL1, and others. For instance, the shared target CD123 is overexpressed in myeloid blasts while being under expressed in hematopoietic stem/group cells and normal myeloid cells. ADC medications that target CD123 have demonstrated some benefit in human clinical studies and have a low level of toxicity to hematopoietic stem cells. However, there is considerable bone marrow/hematopoietic damage in CD123 CAR-T cell treatment studies. Meanwhile, Wang's group reported a preclinical study on CD123-CD33 complex CAR-T cells with novel single-stranded antibody variable region fragment (scFvs) antigen-binding domain in acute myeloid leukemia, which has effectively maintained hematopoietic function and is anticipated to start the following clinical study.

4. CRISPR/Cas9 potential to overcome potent challenges of CAR-T cell-based therpaies

Scientists have posed high hopes in this new technology, believing its liability and also commercializing this therapeutic product, so that it is more affordable for groups of people.

The first-time usage of CRISPR came when it treated lung cancer in humans. The scientists first extracted T cells from the patient and brought it to the lab for engineering (gene deletion using CRISPR cas-9). After the successful deletion of the specified sequences, it was then injected back into the patient. These T cells would be able to detect antigens and kill off the cancer cells. Finally,

after the treatment, there are no side effects that are shown, and also the modified T cells are still abundant after a 9 month stretch. Not only this but microorganism infections are also able to be treated using CRISPR tech. This is when it comes to treating the HIV and AIDS. In May 2017 a group of researchers have identified that the HIV-1 is able to stop its replication if the genome is extracted from the target cell. Not only that, CRISPR cas-9 are also capable of blocking the HIV virus out completely preventing one from further infection. This process is made by CRISPR cas-9 editing CCR5 genes in the specified cells. In addition, later on research from China had proved that the therapy of CRISPR cas-9 had zero toxicity. This also then indicates that this tech has successfully blocked out the HIV virus. It is being researched in medicine for malignancies, HIV, and gene therapy for conditions including sickle cell disease, among others.

5. Conclusion

In conclusion, both the CRISPR and CAR-T are current methods when it comes to the therapy of gene engineering. From the upmost procedures that were posed above, it has been made clear that us humans have already been making large steps into the area of genetic engineering. From all the technology that exists in the present, one is able to see the progressing nature of the development. Indeed, many challenges were faced through the process, many challenges are faced with the usage of CRISPR and CAR-T. Especially CAR-T technology. Due to the fact that this tech just recently emerged in a short period of time, the technology is still not as developed and CRISPR. On the other hand, one is still clearly able to see that the advantages brought forth by genetic therapy had shaken the world and altered many previous medical perspectives on infectious diseases. In addition, it is unquestionable that genetic engineering has served crucial roles in medical therapy [12]. General-purpose CAR-T therapy, when compared to autologous CAR-T therapy, simplifies the complexity of cell therapy in cell preparation, quality control, and clinical administration, lowers product costs, but also introduces new challenges. Additionally, the immune transplant rejection brought on by allogeneic T cells has always hampered the clinical application and development of general-purpose CAR-T. Over 30 years have been spent developing CAR-T cell therapy, and positive clinical outcomes have also been attained. Risks and opportunities do, however, coexist, and CAR-T cell treatment also exhibits potential toxicity and drug resistance, which hastens the process of identifying new tumor targets, researching signaling processes, and creating new technologies. Future improvements in CAR molecular design, transduction techniques, and choice of the best cell type may transform the way cancers are treated, and cell therapy will likely become more common.

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