The Research Progress and Clinical Application of Chimeric Antigen Receptor T-Cell Therapy

Yunshi Wu

College of Clinical Medical, Jiamusi University, Jiamusi, Heilongjiang, 154007, China

Abstract. Immunotherapy, as an emerging method for tumor therapy, which offers numerous hopes for patients with tumor. Among these, Chimeric antigen receptor (CAR) T-cell therapy is one of the most remarkable ones, which have achieved huge progress in cancer therapy and with a compelling prospect. CAR T is a kind of T cells that mediated by antigen receptor, transmembrane domain, activation domain; rebuilt by viral vector like lentivirus, retrovirus, non-viral vector such as mRNA, DNA etcetera and modified by immunosuppressive microenvironment. In this article, I at first reviewed classical CAR T development, second, I analyzed non-viral CAR T, third I discussed in vivo editing system of CAR T, at last I look forward to the future prospects of CAR T therapy. This review wishes to offer some ideas and appreciates for CAR T therapy by analyzing classic CAR T, discussing in vitro CAR T cells, absorbing in vivo CAR T therapy.

Keywords: Immunotherapy; Chimeric Antigen Receptor; Target; Viral; in Vivo.

1. Introduction

With the developing of scientific acknowledge and patients' necessary to drugs of tumor therapy, researchers continuously finding ways for treatment, tumor immunotherapy is proposed as an emerging therapy modality. Chimeric antigen receptor (CAR) T cell, which is firstly successful used by Carl June as a useful cell for cancer immunotherapy. As the name suggests, CAR T cells are ex vivo recombination of single chain fragment variable (scFv) of identified tumor-associated antigen and immunoreceptor tyrosine-based activation motifs (ITMA) into recombinant plasmid, then transfect it into patients' T cells ex vivo. The primordial study of cancer immunotherapy started in 1890s when William Coley, a chaplain alleviates the pain of cancer patient by streptococcus pyogenes and Filtrate of Serratia marcescens. In 1957, Burnet first raised the definition of *doctrine of tumor immunosurveillance*, which leap the research of cancer immunotherapy. Furthermore, people started to study how to utilize T-cell treatment cancer since 1992 based on technology above and Hybridoma technology for monoclonal antibodies founded in 1975. After delving, the ancestry of CAR T cells, PD-1/PD-L1 inhibitor, the inhibitor which can recover T cells activate, was constructed by James. P. Allison and Tasuku Honjo who achieved 2016 Nobel prize for physiology and medicine by this foundation.

The succussed of CAR T is also a long journey. In 1989, Israeli scientist Zelig Eshar firstly raised CAR, in 1993 the earliest CAR T built with scFv and signal domain, it is made for solid tumor, such as ovarian tumor and melanoma, but activity duration of T cells isn't long, it has limitation in therapy. In the 2000s, American scientists Michel Sadelain and Carl June improved the technology of CAR T, they bring in costimulatory molecule to reinforcing T cells' ability of proliferation and survival. They are used for acute lymphoblastic leukemia (ALL) and diffuse large B-cell lymphoma (DLBCL) therapy, the complete response of ALL reached 70% to 90%, the remission rate of DLBCL is about 40% to 50%, which shows its remarkable efficiency to CAR T therapy. All these are centralized in *Nature Biotechnology* 2003 about CD28 stimulation area and Science Translation Medicine 2011 about 4-1BB stimulation area. In 2014, drugs make by CAR T cells are officially approved for marketing in worldwide, which gave people more wish for cure cancer. The mostly internationally ratified CAR T-cell medicines are Tisagenlecleucel and Axicabtagen ciloleucel. Tisagenlecleucel was published by Lentivirus, its Gene Editing Vector, with the promotor EF1a. This kind of drug always use to treat ALL and DLBCL. Besides, Axicabtagen ciloleucel works by its Gene Editing Vector Retrovirus and its promotor MSCV. It can use for treating Diffuse large B-cell lymphoma (DLBCL) and Non-Hodgkins lymphoma. Regrettably, both drugs are for hematological tumor, and both in high price, they are also the beginning of CAR T drugs. Even now, there are 8 CAR T drugs approved for marketing (Table 1), among these six are marketed by FDA in America, two are marketed by NMPA in China and five marketed by EMA of European Union. CAR T therapy that targeting CD19 is the most common ones, besides Tisagenlecleucel and Axicabtagen ciloleucel, Tecartus for treating mantle cell lymphoma (MCL) and lisocabtagene maraleucel for large B-cell lymphoma therapy have approved by FDA and EMA, two kinds of drugs local researched by China have approval by NMPA, they are Yescarta for DLBCL and Relmacabtagene autoleucel for r/r LBCL. Furthermore, BCMA as the key target of multiple myeloma also be focused research, Idecabtagene vicleucel and Ciltacabtagene autoleucel are for multiple myeloma therapy. The CAR T scale of market is huge, in 2022, it's market size is about 3 billion dollars, 5 billion dollars in 2023 and estimated to break 10 billion dollars in 2025. Table 1 summarized major drugs for CAR T therapy.

Table 1. Summary of approved CAR T drugs in worldwide

Drug name	Company	Obtain approval	Indication
Tisagenlecleucel	Novartis	FDA;EMA;NMPA	r/r ALL; r/r DLBCL
Axicabtagen ciloleucel	Kite	FDA;EMA;NMPA	r/r DLBCL
Tecartus	Kite	FDA;EMA	r/r DLBCL
lisocabtagene maraleucel	BMS	FDA;EMA	r/r MCL
Yescarta	FOSUNKAIROS	NMPA	large B-cell lymphoma
Relmacabtagene autoleucel	JW Therapeutics	NMPA	r/r LBCL
Idecabtagene vicleucel	BMS	FDA;EMA	r/r MM
Ciltacabtagene autoleucel	J&J/Legend	FDA;EMA;PMDA	r/r MM

With the commencement and in-depth of CAR T therapy, their application no longer be limited to tumor (Table 2), CAR T cells can use for autoimmune disease and similar diseases in this category, such as lupus erythematosus, refractory anti-synthetase and myasthenia gravis, these can be treated by pathogenicity clearance B-cells by CAR T. Diseases like nervous system disease, heart disease and arthritis including Alzheimer disease, Parkinson disease, myocardial fibrosis, heart amyloidosis and myocarditis can also treated by CAR T. The therapy of these diseases is still in exploration phase. Table 2 shown the diseases exploring in CAR T therapy.

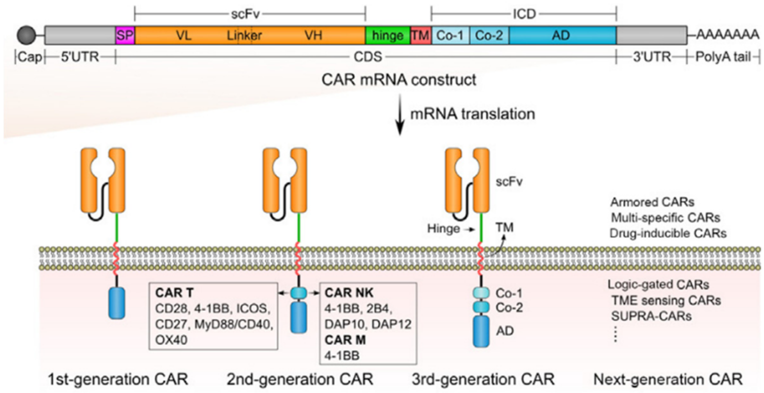
Table 2. A list of underway CAR T therapy diseases

Types	Disease
Autoimmune disease	lupus erythematosus
	refractory anti-synthetase
	myasthenia gravis
Nervous system disease	Alzheimer disease
	Parkinson disease
Heart disease	myocardial fibrosis
	heart amyloidosis
	myocarditis
Arthritis	

CAR T technology is not mature yet, there are still many hurdles to overcome and new technologies to discover. New research directions of CAR T are about general propose CAR T cells, which can both decrease the flexible and costs of CAR T treatment. Furthermore, there are also new usage of CAR technology by changing T cells into other immune cells such as NK-cell or macrophage. The article is for discussing the previous, currency and the aim of CAR T cells or CAR technic. We explored the development, opportunities and new thoughts of CAR T technic. We aim to combability the experience of CAR T cells, and researches more clinical possibilities for CAR technic.

2. Classic CAR T(viral)

Since CAR T have been found, there are lots of CAR T drugs hit the market, they can be separated into five generations by its difference in mode of action and types. These five generations of CAR T cells can be separated by their structure, they are initial attempt of CAR therapy and the basic of in vivo CAR T explorations.



From: Chimeric antigen receptor therapy meets mRNA technology.

Figure 1. Structure evolvement of different generation classic CAR T

First generation The exploration of first generation of CAR T cell start with the thought of the mixture of the advantages between immune cells (T cell) and tumor cells, in order to create a kind of cell have both abilities to eliminate cancer cells and keep alive outside. After delving, people found the earliest CAR T. The first generation is the simplest ones, it merely has 6 peptide chains and only separate into three parts, extracellular area, transmembrane area and intracellular area. People recombine the gene of single chain fragment variable by tumor-associated antigen and immunoreceptor tyrosine-based activation motifs (mostly CD3ζ). Extracellular compose by single chain fragment variable or variable domain of heavy chain of HCAb and tumor associated antigen synchondrosis; transmembrane is for transmembrane parts, including CD4, CD8, CD28, CD3ζ, which are for connecting antigen binding domain and intracellular signal domain; intracellular area aims to transduce like TCR signal to intracellular. Come as no surprise, the first generation of CAR T did not meet expectation. The first generation of CAR T mostly happen in 2006 to 2008, all the CAR T therapy methods have the same problem that CAR T cells can't continuously active in vivo, this generation of CAR T cells can only alive for about one week to six weeks. What is worse that CAR T cells possibly doesn't effective, some patients haven't any enhancement after therapy. The turn happened in 2008, though CAR T cells can still live only six weeks in vivo, tumors turn smaller after six weeks treatment. This succeed can prove the efficiency of CAR T and shows more possibility of CAR T. After the survey of patients, the problem has been found that this generation of CAR T is lack of strong stimulation and activation so it's' easy to die because its energy deficit. At the same time the problems such as tumor antigen selection, gene transfer methods and T cells amplification technic in vitro still need improve.

Second generation Draw on the experience of the first generation, people realized that CAR T cells with only three parts can't display its intended standard. After exploring, they found that there need two extra signals to help T cells active. The first signal is the stimulus of TCR or CD3 complex; the second ones is costimulatory signal made by CD28 and its ligand B7. The two signals are in the same importance, T cells can clone, active, proliferation then start to immune response only by two signals exist together. Among these, the first signal is use for accept antigen stimulate and the second one can help immune response for active T cells. After exploring, CAR T of second generation need a costimulatory numerator added urgently. We can found numerus of costimulatory numerators to choose. By comparison, 4-1BB is defined as the most suitable ones as it has more persist antitumor activity. In clinically speaking, it succeeds. With the help of costimulatory numerator, CAR T cells have longer lasting ability to antitumor activity, and live longer. In 2017, two kinds of drugs against tumor by CAR T officially launched, Kymriah by Novartis, using 4-1BB as its costimulatory numerator and Yescarta by Kite using CD28 as its T cells ability. Until now, these two drugs are still in widely used. Grudgingly, these two drugs have the same side effect, Cytokine release syndrome Neurotoxicity, the inevitable complication in lymphoma. This generation of CAR T therapy is the most successful ones in clinical application.

Third generation Based on the succeed of the second generation, some may think that more costimulatory numerators in CAR T cells may shows better expression efficient and longer survival time, thus, an extra or more costimulatory numerator attached on the third generation. Choosing from exiting costimulatory numerators, there are two kinds of matchings rise above others, CD28 OX40 combination and CD28 4-1BB combination. OX40 can largely change the property of CD28. When CD28 will stimulate L10 secreting and inhibit CAR T activity, the composition of CD28 and OX40 can inhibit L10 secreting, which can improve the therapy of CAR T cells. T cells with two costimulatory numerators, CD28 and 4-1BB added is confirmed with more proliferation, behave better than only one costimulatory within. These experiments corroborate that this generation of CAR T technic is better in therapy, duration time for longer and this envision is feasible theoretically.

Unfortunately, when it turns to clinic application, the design of two costimulatory numerator in a CAR T cell failed. The patient with advanced cancer treated by the third generation of CAR T cells died in five days after therapy. The problem is that single chain fragment variable was overstimulated. The target used in CAR T cells also presence in vivo patient, as the patient is too week that most of its organizations are in low expression. When CAR T cells are in overexpression, they will recognize and attack these low expression organizations together with tumor cells, which will cause cytokine release syndrome. All the evidence shows that these overexpression CAR T cells lead to serious sequelae to patients, which will result in their death soon after surgeries.

Fourth generation Facing the failure of the third generation, people give up inserting more costimulatory numerator in CAR T cells. They start to turn attention to the other route to enhance CAR T technic. The complications after therapy are always inevitable, both CAR T cells in the second and the third generation bring about annoying complications.

The solution provided in the fourth generation was connect cytokine expressed sequence and CAR gene into autocrine cytokine, TRUCKs.

In several choices of autocrine cytokines, including IL-7, IL-12, IL-15 and IL-18 autocrine IL-7 CAR T cell and autocrine IL-12 CAR - cell are more effective choices. IL-7 is used for cell signaling by activate signal pathway. In mouse experiment, the conclusion shows the effectiveness of IL-7 CAR-T cell. The cooperation of IL-7 and CCL19 in CAR T cells shows a new way improve the survival of CAR T cells in tumor. IL-12 provenance from B-cells and macrophage, action to T cells and NK-cells. It can stimulate activated T cells proliferate and make T cells perform well in therapy. Same with IL-7, autocrine IL-12 CAR - cell also shows well in mouse experiment.

Nevertheless, after experiments, a new problem appeared, people found that though CAR T cells stay longer in vivo, it hardly vanishes in vivo, which made these CAR T cells out of control, it can't be eliminate if it variates into harmful cells. In response of this issue, an innovative experiment shown a new scheme, they designed a new kind of fragment variable called SUPRA CAR, which look like a pair of zip. The SUPRA CAR have two parts, one is scFv with Azip named zipFv, the other include Bzip and signaling domains named zip CAR. In this CAR, T cells can activate only when two parts connects together, moreover the zip CAR will metabolism naturally, CAR T cells can reactivate only by inject zip CAR again. All these steps can design a zip between single chain fragment variable and immunoreceptor tyrosine-based activation motifs for the purpose of control the response and apoptosis of CAR T cells. It's not a mature idea but a new direction for CAR T exploration.

After that, a new discovery called Split CAR appeared, it is also controlling CAR T cells by separating it into two parts. The experiment uses a protein called TIM3 to control it, when there is no TIM3, CAR T cells are in low activation, when TIM3 connected with other parts of T cells, they will in full activation. All these are for the regulation of CAR T cells.

Presently, there are many hospitals and academies are preparing for clinical experiment of the fourth generation CAR T technic, the widely used of TRUCKs may fulfil.

Fifth generation In widely believed, the development of CAR T technic only go through four generations, the CAR T cells studying now can be define as the fifth generation though it is still an aim. With the maturating of CAR T technic, the thought of solve the problem of the cost of CAR T therapy appeared. As we can see, listed CAR T medicines all in high price, it mostly because of the personal relevance of CAR T therapy. According to this drawback, the development of universal in vivo CAR T cells begin. Until now, the in vivo CAR T delivery by biomaterials is maturating day and day, though their defectives can't be ignored, it is a promising method.

With the in-depth study of classic CAR T cells, the failure since the third generation of CAR T mostly based on their security problems, which pushed researchers finding later CAR T therapy methods.

3. Non-viral CAR T

Accommodate with security problem, so begin to use non-viral methods such as mRNA and DNA to build CAR T as a safter therapy way.

In vitro mRNA CAR T Based on the basic maturity of the CAR technology treating lymphoid lineage tumor, overcome solid tumor become the next target. When one sense the efficiencies of mRNA therapeutics in coronavirus disease 2019 (COVID-19) and shows its capacity in CAR T-cell therapy, the mixture of CAR and RNA became a new way of thinking. Cause mRNA offers a unique approach to generate CAR cells in vivo without gene integration, which can be used for translate into any target protein and lower the risk of mutation.

In the process of exploration, an in vitro CAR mRNA method first sign of scale. It can be divided into several steps. Firstly, disjunctor T cells in vivo by leukapheresis. Secondly, active T cells by beads. Thirdly, CAR mRNA should be metastasized into T cells. There are two universal manners to solve it, electroporation and with the help of biomaterials, what is worth noting that electroporation may cause massive cell death. Eventually, these T cells will send back to patients after amplification and detection.

mRNA CAR T cells has its dominance that it allows multiple drug delivery, which can reduce potential risk of long-term expression, it is the other way to degrade the likelihood of complication.

Lately, an experiment on llama shown some thinkings about the progress of lymphoid lineage tumor, and shown the convert from fundamental research to clinical application. In the experiment, people made an implantation of tumor cells into llamas, as they are exotic cells, llamas secret nanobodies in order to against it, after panning, we can find the purpose nanobody directly against cancer cells.

As a result, people extracted llama's nanobody contact with CAR T cells, which can generate the most fitness nanobody for CAR T cells. At last, corresponding tumor can be implant into mouse in order to view the effect of nanobody just found. It is a useful and importance technic for finding new target and new breakthrough for cancer therapy.

Though benefits of mRNA CAR T cells are conspicuous, there are also lots of hurdles to cross, including specificity, safety, flexibility, clinical experiment and what we may noticed in future.

DNA CAR In DNA CAR, DNA is used to insert CAR gene into T cells, to realize stable expression of CAR. DNA CAR has its risk that it may lead insertional mutation and result in security problem. The manufacture progress is complex, which come with high costs. DNA CAR used to applicate in blood system malignant tumor and lung cancer.

Compare with mRNA, it is more stable but has higher risk, the possibility of DNA CAR insertional mutation may be the most salient problem for DNA CAR, nowadays, with the continuously optimal for CAR T therapy, the efficiency and security will be improved.

Though mRNA CAR and DNA CAR have the advantage of security, they survive short in vivo, their therapy efficient is in challenge. There may be more study to solve these problems in future.

4. Primer CAR T

Since the harm of electroporation of its killing power to cells when electroporation was the mainstream methodology to metastasize CAR mRNA into T cells, a new way should be found to solve this problem. People started to put their eyes on biomaterials making efforts to find alternatives of electroporation. In the progress of finding the alternatives of electroporation, a better way to treat cancer by CAR was found, it was about in vivo CAR T by biomaterials. Simply speaking, in vivo CAR T cells generation is using biomaterials such as lipid nanoparticles and polymer nanoparticle, deliver CAR mRNA into in vivo immunocyte directly.

People start delving in vivo CAR T technology mostly because both the same of its advantage and the disadvantage of in vitro CAR T technology. In vivo CAR T technology can help reducing the unnecessary of lymphocyte clearance chemotherapy and its relevant sequelae, it can also effectively prevent the escape of CAR target antigen, the high cost of in vitro because of the difference between patients can also be solved by in vivo CAR T.

4.1 In vivo CAR T

With the high limitation and high costs of in vitro CAR T technology, more and more probing about in vivo CAR T technology have appeared currently. In vivo CAR T, as the name implies, aim to renounce all of the in vitro synthesis steps of CAR T cells. As an uncontrolled delivery method, it should be noticed to make sure that CAR gene should only expression in T cells and focus on the risk of insertional mutation and long-term expression. Researchers have found many different ways to solve these problems.

Viral vector Viral vector can be defined as a failure attempt and an important foundation. It can help CAR gene transcription and translate into any other host gene. It has its mere benefit as it can repair forever by only once drug. However, its defects are far more than its benefits. Coexisted with its advantage viral vectors extinguish the possibility of multiple drug offer, which may lead to the risk of malignant tumor, at the same time, viral vector will disrupt the expression of integration host cell. What make one surprise that viral vector also in high requirements to the style of cells. LNPs is also one of the options when exploring the alternative of viral vector in order to solve its problem of its insecurity and high costs.

Nowadays, when searching for general CAR T cells, the mixture of gene editing technology and viral vectors is also a worth trying route.

LNPs peridium Lipid nanoparticles (LNPs) were found in this context, they make up by three parts, cation lipid, helper lipid and polyethylene glycol.

How can LNPs rise above others in such number of biomaterials? Compare with other biomaterials, they have their unique benefits. First, they did well in biology safety, as the non-viral vector, they perfectly avoid the risk of gene mutation. They are easy to metabolize as their degradation products are non-toxic, when other some biomaterials may cause immunological reaction and limit the possibility of multiple drugging. Second, they are the most safety ones in those have high efficiency of gene delivery. Third, LNPs are easy to prepare and in a lower cost. Meantime, LNPs' targeting ability can help cargo spared degradation and clearance, escorting CAR coding nucleic acid to T cells and macrophages.

4.2 Other Biomaterials

Though LNPs are the best choice for metastasize, there are also many other biomaterials can finish this duty, they have their own usage on in vivo CAR delivery.

Polymer nanoparticle Besides LNPs, polymer nanoparticle is also a good choice, which can realize targeting modification by conjugate antibody, such as CD3 or CD8 antibody and improve its targeting ability to object immunocytes. In terms of clinic experiment, polymer nanoparticle is a terrific choice, but there are also some disadvantages that some of polymer nanoparticles have high cytotoxicity, need to release its toxicity by grooming it. Compare with LNPs, its inadequate in expression, preparation, and penetration also should be notice.

4.3 Alternate Utilizes of CAR

After the success of CAR T researching, people begin to explore more possibility of CAR, many collocations of CAR immunocyte appeared. After the failures such as CAR NK-cells, a succeed attempt happened.

CAR macrophage CAR macrophage, as the name implies, using macrophage targeting phagocytosis tumor cells which CAR-Ms engineered and decompose by CAR-M. It can sometimes be defined as the mixture of CAR T and CAR macrophage, with the help of the characteristic of macrophage, they have ability of both active T cells for attacking tumor cells and decompose tumor cells their selves. According to the animal experiment, CAR macrophage is feasible, but compare with CAR T, it still just begins.

Solid tumor Though the technic of tumor therapy is continuously developing, but the current situation of solid tumor therapy is not optimist.

CAR T cells are also occupying a dominant position in solid tumor therapy, but solid tumor need longer treatment. Before the commencement of solid tumor therapy, there are three steps should be prepared, including the screening of mutation, Ommaya and apheresis, corresponding separate and drainage technic and mix with lactate dehydrogenase (LDH), which will lasts 11 days in total. After these preparations, directed CAR T have been manufactured, CAR T cells will be injected in day0, through the intensive toxicity monitoring for 28 days, then deciding whether it is feasible to infuse ICV CAR T. All these procedures are work for the safety and the efficiency of the therapy scheme. However, it is comforting that this method works well, tumors turn smaller with the speed visible to the eyes.

According to resent study, we can find that though there are useful targets for solid tumor for every part of body, most of the targets are repetitive. Which may result in the problem that it is hard to attack the tumor site exactly, these may bring about serious adverse reaction.

As we can know, there are about 10^9 tumor cells pre one centimeter in caliber solid tumor, it is hardly to complete resection, in the meantime, tumor cells have ability of metastasis, which made it is almost impossible to control by operations. CAR T cells are also useless in this situation, as the quantity of CAR T cells same with tumor cell will lead to excessive risk in complication.

Even if we have capacity to control such amount of CAR T cells, it will also fail. CAR T cells have their own limitation in solid tumor, physical barrier and immune micro-environmental suppression, both of them are the obstacles for CAR T invasion. Among these, physical barrier works with throttle the transportation of CAR T cells out of tumor.

CAR T-cell therapy complication Since ancient times, the born of many incurable diseases not only because itself is hard to treat, but also its large mortality rate in complication after therapy. Of cause, cancer counterparts the first one, but as a serious disease and its complex process of treating, CAR T cells therapy have serious complication inevitably. Common complication of CAR T-cell therapy including cytokine release syndrome (CRS), CAR-T cell related encephalopathy syndrome (CRES), anaphylactic response, hemophagocytic lymph histiocytosis (HLH) and gene integration mutation. These complications may cause fever, vomiting, headache, tachycardia, hypotension, anoxia, lymphocytosis and so on, severely may also cause nervous system symptom. In the other side, the happen of complications means CAR T cells works, as long as the complications can be cure, complications are good signals. Focusing on these complications, IL-1, IL-1inhibitor, atazanavir, and serum IL-6 are all terrific ways. Not like IL-1, IL-1inhibitor and atazanavir which can merely use for CRS and CRES, IL-6 is a unique reagent that have two sides. IL-6 in low level will progress cis-signal transduction and amply anti-inflammation action. In the other hand, Il-6 in high level progress trans-signal transduction, which can amply anti- proinflammatory action. As an inimitable drug, its two-sided usage based on its positive correlation with CRS severity index and the quantity of CAR T cells.

After the comparisons between the quantities of CAR T cells injected and the severity of complication, we can find that severe complications mostly cause by overwhelming CAR T cells, what we should notice by the result is to control the quantities of CAR T cells more carefully.

In relation to the above only, biomaterials or only LNPs are favorable choice for CAR therapy, but as we can see, there isn't any mature treating means work like this, from this, we can see, biomaterials still have many inadequacies we have to notice. As we know, biomaterials in vitro are in well deliver efficiency, but relatively, they don't do well in vivo, they cause the accumulation of mRNA. Besides, its safety and efficiency still need to observe and detect.

5. Concluding Remarks and Future Perspectives

Since the succeed of immunotherapy, CAR T therapy have experienced several generations, the adjustment of in vitro to in vivo and viral to non-viral. Drugs are continuously floated in the worldwide for treating tumor, autoimmune disease, heart disease and arthritis. Since always, cancer is the highest mountain modern medicine have met, in the process of therapy cancer, people meet many obstacles, facing with much failure, but at the same time, more and more methods and experiences have been discovered by people's efforts. Since CAR T technology was invented, cancer medical technology usher in great leap, the patients with malignant tumor start to have possibility of curable. In the progress of CAR technology, CAR T has been the mean way treating cancer and the basic of cancer drug development. The study of CAR gene never stopped and always with new thoughts.

The therapy of CAR T have experienced succeed and dilemma, its future development may be four points. Firstly, finding new targets, when some of the lymphoid lineage tumor diseases have mature therapy flow, we also need to focus on the problem that as the vast majority in cancer, solid tumor, they still lacks of its effective solution. Towards cancer, especially solid tumor, there are full of foresight in future development. People need to focus on the exploration of new target, refine CAR design and choosing antibodies, in order to minimize off-target effect. As we can know, all the targets and inhibitors including PD-1 and PD-L1 can only use for positive tumor cell immunotherapy, how to eliminate negative tumor cells may be the breakthrough for malignant tumor therapy in future.

Secondly, build a better CAR T structure, the failure of adding several stimulation molecules on CAR T means the importance of discovery new structure, TRUCKs are an emerging try, but still with defectives. CAR T cells can't live long inter solid tumor mostly direct to the problem of structure, CAR T cells should be rebuilt into ones' which can withstand tumor environments and affect the organizations, cells and blood supply around tumors. Finding a better structure is not an easy work, but as we have the ideas and way to effort, it will finally be solved.

Thirdly, using more effective transfection ways, in the progress of CART, many different ways of transfection have been tried. Including physical methods such as electroporation and gene gun; chemical methods like liposome transfection and polymer; biology method like viral vector transduction, they each has its own merits and demerits. The production of new type vector, explorations of gene editing technic, optimal of in vivo transfection technic may be the future aim in order to raise its precision, efficiency and security.

Besides these, we can actively enquire into diseases, though CAR T is a more mature technology, it has high expressions, in future development of CAR T, it needs to make sure its efficient expression in objective cells, and planning for clinical experiments. In order to focus on different types of tumors, CAR T cells which suitable for different diseases should be design. After that, it should have some ideas of using CAR T therapy to more diseases, which can be an efficient method for diseases not only kinds of tumors have been widely used, but also more kinds of autoimmune disease, infectious disease, fibrosis and senescence. Most of the un succeed CAR T envisions have the same problem that they have a absent of malignant tumor and enough clinical application.

The price of malignant tumor therapy is always what make people denounce and unaffordable, after exploring the efficient and more possibilities of CAR T, how to make the costs of CAR T lower should put on the agenda. The study of general-purpose CAR T cells hasn't landed yet; besides that, we may think of how batch production, it may be the most effective way of lower costs.

Cancer, now in public is still a disease that hardly to therapy, there still need enough clinical experiments and numerus of success stories to prove it's succeeded and change publics' mind. That's not only what cancer researchers should done, but also all the diseases whether in past or future have done and should done.

References

[1] Blache U, Tretbar S, Koehl U, et al. CAR T cells for treating autoimmune diseases[J]. RMD Open, 2023,9:e002907.

[2] Wu J, Jiacai, et al. Chimeric antigen receptor therapy meets mRNA technology[J]. Trends in Biotechnology, 2023, 42(2): 228-240.

[3] Short L, Lauralie, et al. Direct in vivo CAR T cell engineering[J]. Trends in Pharmacological Sciences, 2023, 45(5): 406-418.

[4] Xu C, Ju D, Zhang X. Chimeric antigen receptor T-cell therapy: challenges and opportunities in lung cancer[J]. Antibody Therapeutics, 2022, 5(1): 73-83.

[5] Singh AK, Anurag, et al. CAR T cells: continuation in a revolution of immunotherapy[J]. The Lancet Oncology, 2020, 21(3): 168-178.