Current Progress of CAR-T Cell Therapy in Lung Cancer

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Abstract. Lung cancer is one of the most common malignancies in the world. For lung cancer treatment, traditional surgery, radiotherapy, chemotherapy, monoclonal antibody therapy and other therapeutic methods can have a certain therapeutic effect, but the prognosis of these treatment methods is generally poor. The recurrence rate of patients treated with surgery is high, and chemoradiotherapy may cause damage to normal cells and tissues with serious side effects. Drug resistance is also a very serious side effect when using monoclonal antibody drugs. So, a better treatment is needed. CAR-T has been investigated in the past as a potential treatment for lung cancer. CAR-T for lung cancer is now undergoing clinical testing. Finding viable therapeutic targets is essential for CAR-T therapy for lung cancer, and there are numerous antigenic targets for this type of treatment. The advancement of CAR-T therapy, its relevant targets for lung cancer treatment, and the shortcomings of traditional methods will be discussed in this review.

Keywords: lung cancer; CAR-T therapy; progress of treatment.

1. Introduction

Lung cancer is a very common malignant tumor, and its morbidity and mortality are constantly on the rise. Lung cancer patients make up 11.6% of all cancer cases, while 18.4% of cancer-related deaths globally are lung cancer deaths, causing huge economic losses and health burdens to global society, about 80 percent of lung cancers are caused by smoking [1]. Lung cancer patients are unable to receive prompt and efficient therapy because of the absence of an early diagnostic platform and the delayed onset of symptoms. Because of this, lung cancer has a poor prognosis, which reduces available treatments and decreases survival rates. Effective methods of managing or treating lung cancer are therefore desperately needed.

Advanced lung cancer surgery, chemotherapy and radiotherapy are the three traditional anti-cancer strategies for lung cancer [1]. Currently, surgical excision plus adjuvant therapy is the primary treatment for lung cancer. Only a small percentage of patients, though, are candidates for surgical intervention, and the majority of them relapse and pass away following surgery. Radiotherapy can reduce the risk of surgery. However, due to the radiotoxic, it also damages healthy cells, tissues and organs during treatment. Chemotherapy is convenient and non-radiotoxic, but chemotherapy can also cause damage to healthy cells and may lead to drug resistance.

The prognosis of these traditional therapies is generally poor, which forces the emergence of new technologies to change this situation. There is already a lot of evidence that the treatment of lung cancer favors monoclonal antibody therapy. But monoclonal antibodies tend to lose epitopes. The efficacy of monoclonal antibody therapy depends on the expression of antigens that the tumor cells can bind to. Resistance to monoclonal antibody drugs is also a major concern, and previous monoclonal antibody treatments have demonstrated many resistance mechanisms. So, there is also a more top-of-the-line immunotherapy, chimeric antigen receptor T-cell immunotherapy (CAR-T). CAR-T is a new adoptive immunotherapy strategy, which not only provides a great help for the treatment of blood tumors, but also a more effective immunotherapy for lung cancer.

The immune mechanism of T cells is to recognize the surface by targeting cell surface antigens to play a killing role. But eventually, cancer-associated fibroblasts eventually interact with tumor-infiltrating immune cells and other immunological components through the release of various cytokines and other effector chemicals, resulting in the creation of an immunosuppressive tumor microenvironment and allowing tumor cells to elude the immune system's surveillance. The advent of CAR-T therapy overcomes immune escape. The brief process is to extract T cells from the first

patient, then modify them *in vitro*, then grow them, multiply them, and finally import them back into the patient. CAR-T has now reached its fifth generation, which is expected to overcome some of the limitations of previous generations.

CAR-T treatment has been the subject of numerous research for solid malignancies, particularly lung cancer. According to Jaspers, it is highly advantageous to increase patient survival using CAR-T that specifically target DLL3, a tumor-specific cell surface marker for small cell lung cancer [2]. Furthermore, carcinoembryonic antigens are highly expressed in lung cancer patients, according to Xiao's findings. Furthermore, advanced lung cancer has been shown to be eradicated by CAR-T that target carcinoembryonic antigens [3].

Despite several challenges, CAR T cell therapy can effectively cure lung cancer. It still has a lot of untapped potential. Several identified solid tumor CAR-T target antigens are being utilized in preliminary clinical trials. Numerous tumor-associated antigens have reportedly been employed to treat lung cancer; the majority of these antigens have shown potent antitumor cell properties.

This review will systematically discuss the limitations of traditional therapies, the development, challenges, and advantages of CAR-T, and the future outlook for lung treatment.

2. Traditional treatment:

2.1. Surgical treatment:

Worldwide, lung cancer is a prevalent cancer. Because lung cancer is usually found at an advanced stage. it has a high death rate. Currently, surgery remains the primary treatment for lung cancer in its early stages. However, surgery is very limited, and it only has a practical effect on early and non-metastatic tumors. Dafeng Hospital in Shantou, Guangdong Province, China, has recorded 31 cases of lung cancer patients treated with surgery, according to reports. Among them, 28 cases survived 6 months after surgery and 21 cases survived 1 year. Complications occurred in 6 cases after operation [4]. These results demonstrate that surgical treatment of lung cancer is still imperfect.

2.2. Radiation Therapy

Radiation therapy is also a commonly used treatment for lung cancer. Moreover, it can be used in combination with surgical treatment and preoperative neoadjuvant chemotherapy to significantly improve the anti-tumor effect of radiotherapy. It can also improve the survival rate of patients and reduce the risk of death, and it can also be combined with immunotherapy. Radiotherapy can change the TME and increase the sensitivity of tumor cells to immune cells. The use of radiotherapy in conjunction with other treatments can greatly increase patient survival rates. In addition, in the course of radiotherapy for lung cancer, the position of radiotherapy generally includes the lung and mediastinal lymph nodes, which are very close to the heart, so the heart may be exposed to radiation, so some patients who use radiotherapy for lung cancer may suffer from radioactive heart injury (RIHD), which makes the prognosis of patients poor. Radiation lung cancer is a hazardous side effect of lung cancer treatment with radiation. Radiation pneumonia (RP) was reported in 44 out of the 106 lung cancer patients who underwent radiation therapy at the Affiliated Hospital of Xiangnan University in China. [5]. According to this research, radiation therapy is not the best treatment for lung cancer at the moment.

2.3. Chemotherapy

Surgery and radiation are commonly used to treat local and non-metastatic cancer, but when the cancer has spread throughout the body, these methods are significantly less effective. In comparison, chemotherapy is a better method. Platinum-containing chemotherapy drugs are important chemotherapy drugs, among which cisplatin has a favorable impact on lung cancer treatment. Cisplatin is selected because of its strong anti-tumor effect, but many patients will relapse due to drug resistance, so it can be combined with some other drugs for treatment. This has greatly improved the survival rate of patients. But chemotherapy also has serious side effects such as neutropenia stomatitis

mucositis diarrhea and vomiting [6], Central granulocytosis was particularly severe. FN (Febrile neutropenia) often forces patients to stop or delay continuing chemotherapy. Nausea and vomiting are the most worrying side effect of patients [6]. Drug resistance may also occur in the course of chemotherapy, which will lead to the recurrence rate increase and the prognosis deterioration. Chemotherapy drugs can also cause damage to normal cells.

3. CAR-T Cell Therapy

3.1. Introduction of CAR-T therapy

T cell receptors (TCRs) on the cell surface present antigens provided by MHC molecules, allowing T cells to distinguish between different cell types. However, cancer cells reduce or eliminate MHC molecules on their cell surface to evade T lymphocytes. CAR-T is a potential treatment for this disease. T cells are designed to specifically detect tumor cells regardless of whether MHC molecules are expressed. The CAR is the cornerstone of CAR-T therapy, which enables T cells to detect malignancies without the use of human lymphocyte antigen (HLA).

CAR is a special kind of antigen receptor that mimics the structure of the native TCR and has the ability to reprogramme T lymphocytes. The three domains that make up CAR are the transmembrane domain, the inner domain, and the outer domain. Spacer, antigen recognition site, and signal peptide are examples of the outer domain. The variable portion of immunoglobulin (IG) in the outer domain, namely scFv, is the antigen recognition site as the signal peptide. The transmembrane domain is employed to maintain the complex and can also be used as the co-stimulatory domain of T cells such as CD-28.

As shown in **Fig 1**, CAR-T therapy consists of five steps. Firstly, T cells are extracted from the patient, modified and expressed, then multiplied outside the body, and finally reinjected into the patient [7].

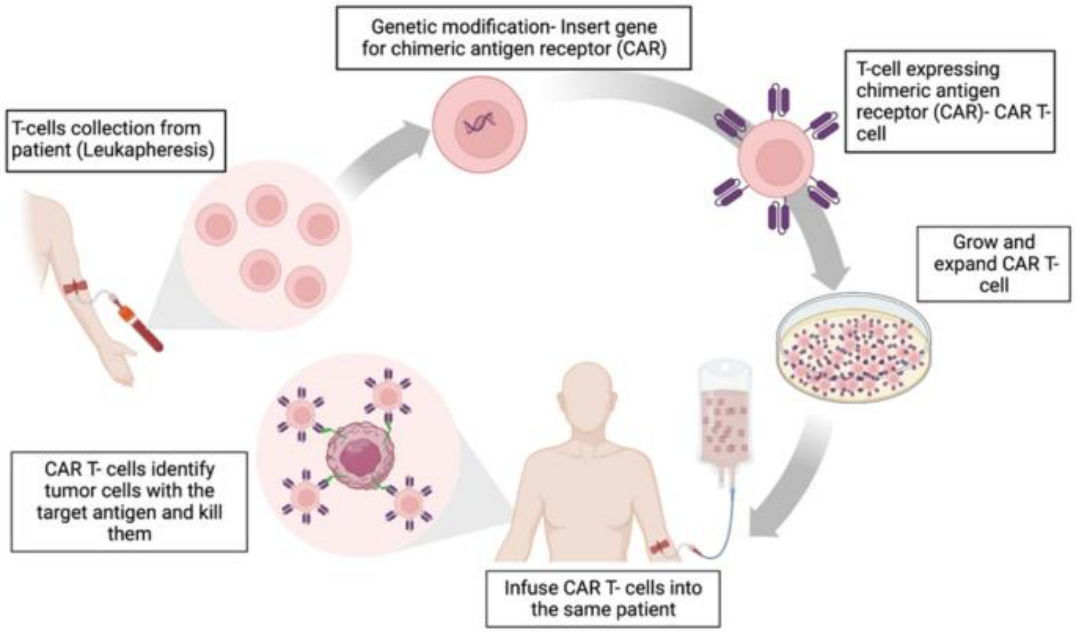

Fig. 1. Diagram of CAR-T treatment process

3.2. The development of CAR-T cell Therapy

CAR-T therapy has been in development for many years and is progressing rapidly, with many drugs being developed to treat a wide variety of tumors, and it is currently in its fifth generation. The development process of CAR is shown in Fig 2. The first generation of CAR refers to the fusion of scFv with transmembrane structure and intracellular structural unit: CD3zeta chain. However, the first generation lacks the expression of co-stimulatory molecules, so there is no significant clonal

expansion and anti-tumor performance. Adding a co-stimulatory molecule (CD28) to the first generation increases the anti-tumor efficaciousness and duration of its action [8]. The third generation CAR combines two co-stimulatory chemicals at the same time to enhance long-term proliferation and anti-tumor characteristics, maximizing its efficacy. Because antigen-negative cancer cells degrade tissues, fourth-Generation CARs express transgenic immunological alterations, like interleukin (IL)-12, to counteract antigen-negative cancer cells' effect. The fifth-Generation CAR works more efficiently by adding a few structures to recognize multiple antigens.

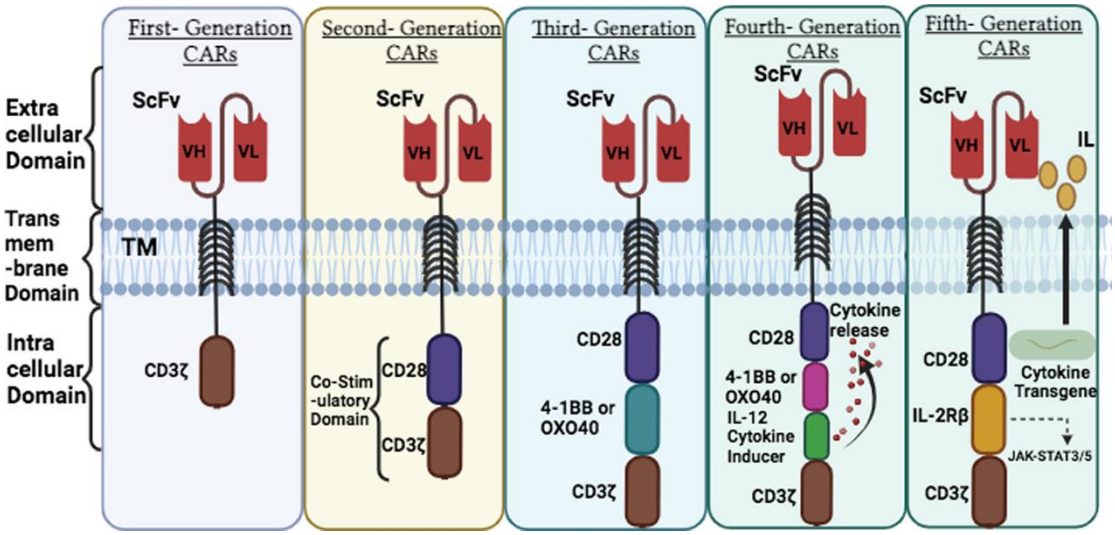

Fig 2. Components of different generations of CAR [8]

Blood cancer treatment has advanced significantly with CAR-T therapy. According to reports, William Paul Ludwig of the University of Pennsylvania, who had chronic lymphocytic leukemia, was the first person to get CAR-T treatment. She went into remission for over ten years following treatment in 2010, and COVID-19 ultimately claimed her life in 2021 [9]. The first drug for CAR-T to receive FDA approval is Tisagenleucel, It is CAR-T targeted for the treatment of B-cell acute lymphoblastic leukemia (ALL). Its emergence marks a major advance in CAR-T therapy, which is used to treat ALL in adults and minors.

3.3. CAR-T Therapy for Lung Cancer

Many researchers have employed CAR-T to treat solid tumor lung cancer because of its positive effects. For CAR-T to be effective in treating lung cancer, the right tumor-specific associated antigen (TAA) must be selected. However, in solid tumors like lung cancer, it is more difficult to build CAR-T, so an ideal therapeutic target is very difficult. Currently, a few promising targets for the therapy of lung cancer have been identified. This section will explain several CAR-T therapies that have been developed for particular lung cancer targets.

3.1.1. PD-L1

The immunoassay molecule programmed death-1(PD-L1) is significant because it controls how T cells behave. When it is overexpressed, T cell activity is suppressed, allowing tumor cells to evade the immune system's observation. By focusing on PD-L1 signaling, lung cancer treatment has advanced significantly. PD-L1 enhances anti-tumor immunity and inhibits T cell proliferation. In addition, PD-L1 targeted CAR-T cells showed strong anti-tumor efficacy both in *vivo and in vitro* And CAR-T drugs targeting PD-L1 have already entered clinical trials, with some success. Sun Yat-sen University Cancer Center showed a clinical trial (NCT03330834) on the treatment of lung cancer with CAR-T drug targeting PD-L1. The patient had side effects with different symptoms during hospitalization, but was discharged in a stable state after 65 days with symptomatic administration [10]. Lung cancer treatment has been shown to benefit greatly from CAR-T medications that target PD-L1. I

3.1.2. EGFR

Extracellular growth signals are transferred into cells by epidermal growth factor (EGFR), sometimes referred to as human epidermal receptor (HER-1). Over 60% of lung cancer cells that are not small cells overexpress EGFR. It can therefore be used as a prerequisite for CAR-T targeted lung cancer therapy. Furthermore, EGFR-targeting CAR-T has been the focus of a great deal of clinical research. Based on viral vector technology, EGFR-targeting CAR-T is a feasible and safe treatment for lung cancer. On the other hand, the dangers associated with viral vectors are higher than those of non-viral vectors such as the piggyBac transposon carrier system. In vitro and in mice xenotransplantation, CAR-T treatment targeting EGFR based on the piggyBac transposon carrier system has had some promise in earlier trials for lung cancer [11]. A Phase I clinical trial (NCT03182816) was conducted in 9 EGFR-positive patients with lung cancer at the Fifth Hospital in Ningbo, China. None of the 9 patients had serious adverse reactions. The overall survival rate was 15.6 months, indicating that CAR-T therapy based on piggyBac transposon carrier system targeting EGFR is feasible for the treatment of lung cancer. Another clinical trial (NCT01869166) also demonstrated its feasibility. Two patients had partial remission and five had stable illness during the 2-to 8-month trial, which involved 11 patients with positive EGFR expression [12]. This suggests that EGFR-targeting CAR-T is a promising treatment option for lung cancer.

3.1.3. MSLN

A tumor-associated antigen, mesothelin (MSLN) is only weakly expressed in healthy tissues and on the mesothelial surfaces of the peritoneum, thoracic cavity, and heart. MLSN does not seem to be significant in normal tissues, as evidenced by the lack of effect that MLSN knockout in mice has been shown to have. On the other hand, it is highly expressed in ovarian, pancreatic, lung, and other malignancies [13], so it is possible to treat lung cancer and other diseases by targeting MLSN. CAR-T targeting MLSN is a very superior method for treating lung cancer. Injecting MSLN-targeting CAR- T into the tail vein of mice with tumors, the researchers found that they significantly reduced tumor development and showed anti-tumor effects [14]. A number of clinical trials (NCT02414269, NCT02580747) are exploring CAR-T targeting MSLN in treating lung cancer cases. All of these suggest that therapies targeting MLSN are feasible.

3.1.4. ROR1

Tyrosine kinase-like orphan receptor 1 (ROR1), a tumor fetal glycoprotein that inhibits apoptosis, is overexpressed in ovarian, lung, and breast malignancies. Therefore, ROR1 can be used as a targeted therapy for lung cancer, and it is negatively associated with prognosis. Furthermore, CAR-T targeting ror1 was safe. Berger confirmed the viability and safety of ROR1 CAR-T cells in macaques [15]. Wakkstabe et al. found that CAR-T targeting ROR1 had significant anti-tumor effects [16]. According to the aforementioned research, lung cancer can be effectively treated using CAR-T which specifically target ROR1.

4. Limitations and Challenges

There's a lot of interest in using CAR-T to treat lung cancer, and there are multiple clinical trials (NCT01583686, NCT01935843, NCT02713984) are ongoing. This proves that CAR-T for lung cancer is positive. However, there are also some difficulties in using CAR-T to treat lung cancer. At present, the main problems of CAR-T for lung cancer are the immunosuppression of tumor microenvironment(TME), the difficulty for T cell to infiltrate tumors, and the side effects of CAR-T, especially cytokine release Syndrome(CRS) [17].

4.1. Immunosuppressive Tumor Microenvironment

Due to abnormal factors secreted by solid tumor cells, it is difficult for T cells to infiltrate into tumor cells, which leads to low anti-tumor efficiency. In the TME, immune cells, endothelial cells, stromal cells, and tumor cells secrete many immunosuppressive substances, such as chemokines,

cytokines, and suppressor molecules. These cells support or inhibit each other, thus coordinating the highly immunosuppressive tumor environment. Armoured cars that contain immunosuppressive substances like IL-12 and IL-18 have been proposed as workable solutions to solve this problem and conquer TME. Super2 and IL33 CAR T have recently been shown by Brog to dramatically enhance anti-tumor effects by promoting anti-tumor immunity in a variety of solid tumor models [18].

4.2. The difficulty of T Cells Infiltrating into the Tumor

The difficulty of T cell tumor infiltration is similar to the immunosuppressive tumor microenvironment, which prevents T cells from moving to tumor cells and exerting anti-tumor effects. Therefore, to promote T cell infiltration into tumor cells, researchers have studied some cars that express IL-8 receptors, which contributes to tumor infiltration. Studies that used the interleukin-8 (IL-8) receptor CXCR1 or CXCR2 in αvβ6-targeted CAR-T cells, as created by Wilding, showed that these armed CAR-T cells had a notable antitumor activity against pre-existing ovarian or pancreatic tumor xenografts and enhanced T cell invasion into the tumor[19].

4.3. Cytokine Release Syndrome

The injection of T cells into the patient will result in a significant quantity of cytokine precipitation, which will cause CRS, even if CAR-T therapy has shown to have notable benefits for treating lung cancer. Fever is typically the initial symptom of CRS. After that, low blood pressure, respiratory failure, and potentially fatal capillary leakage can occur. However, there are currently treatments for CRS, and anti-cytokine therapy that targets IL-6 is one of the successful strategies [20]. Tocilizumab is a monoclonal drug that acts on the IL-6 receptor and is very effective in treating CRS.

5. Conclusion

Lung cancer has always been one of the most harmful malignant tumors in the world, which has caused great damage to the world's economic development and social stability. Some traditional therapies, such as surgery, radiotherapy and chemotherapy, are not the best options when treating lung cancer. CAR-T therapy for lung cancer has shown a good prospect. CAR-T is more targeted than traditional therapy, it is based on the patient's situation to create CAR-T cells, so its anti-tumor effect is stronger, and its anti-tumor aging is long and has stronger targeting. Several clinical trials including CAR-T therapy are now being conducted to treat lung cancer. There has been some success with these clinical trials. Notwithstanding its enormous promise, Lung cancer treatment with CAR-T is not without its challenges. Among them, the immunosuppressive TME and the CAR-T side effects—particularly cytokine release syndrome—have grown to be challenging issues during the course of treatment. To address these issues, researchers have looked into a number of intriguing approaches, including combining medication therapy and altering CAR-T. In the future, these issues will be resolved in a more straightforward and effective manner, which will encourage the use of CAR-T in treating lung cancer.

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