

Current Targets For CAR-T Therapy Used in Experiments for The Treatment of Thoracic Cancer

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Abstract. Cancer can progress rapidly and cause death within months. Methods of treatment have merited attention. Cart-t cell treatment has been proven to be an emerging approach towards malignant tumours. Until now, successful results have been showing in treating blood tumours while targets or drugs for solid tumours are still under experiments. In this article, some common and potential targets for better treatment effects against thoracic cancer have been introduced. Thoracic cancer consists of those malignant tumours that originate in the chest cavity, including lung cancer and breast cancer.

Keywords: Chimeric antigen receptor therapy; Lung cancer; Breast cancer.

1. Introduction

Cancer generally refers to malignant tumours, with abnormal cell differentiation and multiplication, loss of growth control, infiltration and metastasis and other biological characteristics. It is a major reason of death worldwide, representing about 10,000,000 death cases in 2020 [1]. Due to various reasons, such as poor health conditions of patients, or the diseases being discovered in their advanced stages, treating cancers can be hard and exert pressure on patients themselves and their families. The cancer burden grows continuously worldwide, exerting excessive emotional, physical and financial strain on patients, families, and the country's public healthcare. It is usually ill-prepared for the public health care system in low-income and middle-income countries to manage this problem. There is a shortage of timely access to efficient diagnosis, treatment and prognosis for large numbers of individuals diagnosed with cancer worldwide. In high-income countries, survival rates for various cancer types are modifying due to access to early detection, treatment and aftercare.

The occurrence of cancer is a result of multi-factor and multi-step. It is divided into three phases: carcinogenic, cancer-promoting and evolutionary, which it's occurring is closely related to living habits, occupation, environmental pollution, biological and natural factors, chronic stimulation and trauma, iatrogenicity, genetics, immunity and endocrinology.

Of all cancers, lung cancer has a high incidence and mortality rate. In females, breast cancer has a higher mortality rate. These cancers that occur in the chest cavity involve vital organs of the body and exert huge pressure on the physical, emotional and financial resources of patients and their families. Some cancers do not have obvious or specific features in their early stages, so it is difficult for patients to get treatment when it is easiest to treat. Some cancers come back and metastasize easily, making it difficult to settle once and for all. Humans have been exploring ways to treat cancer and have achieved some results, but it is not enough to cure cancer. Among the various cancer treatments, immunotherapy is an emerging and effective method, including immune-checkpoint inhibitor (ICI), monoclonal antibodies (MABs), cancer vaccines, adoptive cell transfer (ACT) and cytokines. Tumour immunotherapy is a treatment method that actively or passively causes the body to produce a tumour-specific immune response and exerts its function of inhibiting and killing cancer cells, which has the advantages of specific and efficient treatment that protects the body from harm. Different from traditional treatment methods such as surgery, targeting, radiotherapy and chemotherapy, immunotherapy does not directly kill cancer cells but mobilizes immune cells in the body that can recognize tumours, improves the fighting ability of the immune system in the human body, and relies on them to indirectly kill and control cancer, with few side effects, safe and effective. With the in-depth research of tumour immunology theory and the continuous advancement of technology, it is

expected to achieve a breakthrough in radical tumour treatment and become the mainstream method of tumour treatment. Currently, CART therapy is an emerging approach to treating different types of cancers. CART therapy equips T cells with molecules that can quickly recognise cancer cells by binding to their surface antigen in cell surveillance. These modified T cells are called CAR-T cells. Like normal T cells, they move around the human body, and once the TCR on their surface binds complementarily with cancer-associated antigens, CAR-T cells are activated. They launch, attack, and destroy the signalled cancer cells. It has shown successful outcomes in treating some blood tumours. CAR products for solid tumours are still in progress. Some problems are difficulties in finding appropriate targets and unavoidable side effects.

In this review, the focus is CART therapy attempting to treat thoracic cancers. Thoracic cancer includes cancers occurring in the chest cavity, some common ones include lung, oesophageal, mediastinum and breast. Breast cancer is the cancer that is the most diagnosed in 2020 and is the fifth reason for deaths caused by cancers. In American females, it ranked first in risk of developing and occupied the second position in terms of risk of death, it is relatively rare in American males. Lung cancer is cancer is the cancer with the second largest number of people diagnosed in 2020 and is the leading one among all the death cases caused by cancers [2].

2. Introducing CAR-T therapy

2.1. Development

The CAR-T cell treatment was first proposed by Zelig Eshhar and his colleagues in the late 1980s. The concept of genetically altering T cells to express synthetic receptors that could recognize and target particular cancer cells was first presented in the publication that covered the topic in 1989. Dario Campana and Michael Jensen made substantial advancements in preclinical tests in the late 1990s, proving the capability of CAR-T cells to target and kill cancer cells.

2.2. Structure

The CAR consists of 3 domains: the extracellular one, the transmembrane one and the intracellular one (or endotoxin). The transmembrane domain is responsible for signalling in the body. A signal peptide, which consists of a variable light chain and a heavy chain is parted by a flexible junction and is tethered to the transmembrane region through a spacer that can be cut from the mature chimeric antigen receptors expressed at the surface of the cell.

The alpha helix of the transmembrane domain is important for the surface expression and durability of the CAR. The third domain is the intracellular one. The signalling proteins are recruited and phosphorylated following the binding of the antigen. Several functional units can be found in the endodomain.

2.3. Mechanism

The patient's T cells are adjusted to present a synthetic receptor. This is the chimeric antigen receptor or CAR. CARs show fragments of a specific antibody fused to a T-cell signalling domain. The modified T cells are injected back into the patient. There is a smaller range of potential targets for CARs when compared with TCRs. The general working mechanism of CARs mainly consists of seven procedures: collection of T cells, genetic modification, expansion of modified CAR-Ts, pre-conditioning chemotherapy, CAR-Ts infusion, targeting cancer cells and persistence and memory. Leukapheresis is used to extract the T cells of the patient, which are then subjected to an ex vivo procedure that involves activating the T cells, transducing them with a viral vector encoding a CAR, and expanding the CAR-expressing T cells before patient infusion. It is then followed by apheresis without the addition of granulocyte colony-stimulating factor (G-CSF). The reason why G-CSF is excluded is that it can disturb T-cell proliferation and responsiveness [3]. A tumour antigen-recognizing antibody-derived fragment and co-stimulatory molecules that encourage T cell proliferation and persistence make up CARs.

The components of CAR consist of spacer, ITAMs, CD3z domain, ScFv, transmembrane domain and genome-edited CAR T cells. Immunoreceptor tyrosine-based motifs (ITAMs) are signalling endo-domains including CD28, 4-1BB, and mimic co-stimulation (ICOS) that is provided during TCR recognition by APCs. The major transmitter of signals from endogenous TCR is the CD3z domain. ScFv has high-affinity CARs that can result in an on-target off-tumour effect. Accurate CAR design should be improved to minimise this while maintaining efficacy. The transmembrane domain currently is the most stable and frequently used, which is derived from the CD28 receptor. Genome-edited CAR T cells offer novel solutions to old problems. Editing out FAS was shown to be effective in decreasing levels of the automatic implantable cardioverter defibrillator (AICD) [4].

2.4. Applications

A typical example is acute lymphoblastic leukaemia. CAR-T cells can detect and bind to a highly expressed antigen in leukaemia cells. CD19 is frequently used main antigen for B-cell ALL. As a result of the CAR-Ts attaching to the CD19 antigen, several intracellular signalling pathways take place inside the CAR-T cell. These signalling domains, which are frequently produced from T-cell receptor signalling molecules, activate the modified cell. The activated CAR-Ts release cytotoxic substances to kill leukaemia cells, which express CD19 on their surfaces. One of the primary cytotoxic substances released by CAR-T cells is perforin, which rips holes in the cell membrane of the target cell. Granzymes, which are proteases, enter the target cell through these holes once they have developed. Once inside, granzymes set off a series of biological processes that eventually result in cell death. The leukaemia cell is destroyed as a result of caspases, enzymes involved in apoptosis (programmed cell death), being activated by granzymes. Additionally, the activation of CAR-Ts has the possibility of causing an inflammatory reaction in the surroundings. As a result of the inflammatory response, other immune cells, such as macrophages, are drawn to the area of CD19-expressing cells. These immune cells also help to eliminate leukaemia cells.

Until now, there are 5 FDA-approved CART drugs for treating different types of cancers.

In August 2017, Kymriah/Tisagenlecleucel/tisa-cel was approved to treat ALL (acute lymphocytic/lymphoblastic leukaemia). ALL is a type of blood cancer that progresses rapidly and produces immature white blood cells. This type of cancer is commonly found in children and is mostly curable while that found in adults shows less effect of treatment. Tisa-cel is the first CART product that passed all safety tests and has a satisfying effect on treating ALL. In 2018, tisa-cel was approved to treat refractory or relapsed LBCL (large B cell lymphoma). This type of cancer is formed when mutated B cells proliferate uncontrollably and affect normal cells. The lymphatic system is a network of lymph vessels and tissues that carries fluids through the human body. Lymph contains WBCs called lymphocytes. B lymphocytes produce antibodies to fight against infections. In normal tissues, damaged or old B cells are programmed to death to make room for new and healthy B cells. In advanced stages of LBCL, cancer can metastasise to other regions of the human body such as the spleen and bone marrow.

In October 2017, Yescarta/Axicabtagene Ciloleucel/Axi-Cel was approved to treat refractory or relapsed LBCL. (large B cell lymphoma)

In July 2020, Tecartus/brexucabtagene autoleucel was approved to treat refractory or relapsed MCL. It is another subtype of LBCL. Also starts in the B cells in the lymph nodes but originates from the mantle zones. This type of cancer grows slowly with hardly any symptoms before it starts to be aggressive. In advanced stages, the cancer can metastasise to other regions of the human body such as the digestive system and bone marrow.

In February 2021, Liso-cel was approved to treat r/r DLBCL (diffuse large B cell lymphoma). It's the subtype of LBCL that is the most diagnosed which is often found in lymph nodes and progresses rapidly. There are more than 70 subtypes of LBCL found in human bodies.

In March 2021, Abecma/idecabtagene vicleucel/ide-cel is approved. This drug is used in treating refractory or relapsed multiple myeloma (MM) after 3 or over 3 times of therapy including a proteasome inhibitor (PI), immunomodulatory drug (IMiD) and an anti-CD38 antibody and

developing resistance to these methods of treatment. MM is a type of blood cancer characterised by unusual proliferation of bone marrow plasma cells with monoclonal immunoglobulin overproduction. Abnormal plasma cells accumulate in the bone marrow and can form tumours in multiple places in the body. Not only are these cells unable to function properly, but the antibodies they produce can also prevent the bone marrow from producing healthy blood cells. In addition, patients will suffer multiple osteolytic damage, anaemia, renal impairment, and hypercalcaemia. Although there are currently multiple myeloma therapies, there are still many patients who are resistant to all approved therapies, and it is important to develop innovative therapies for multiple myeloma [5].

3. CAR-T therapy in thoracic cancer

So far, amazing results have been witnessed in treating blood cancer with CART therapy with a few drugs approved and used by the market. However, when it comes to solid tumours, investigations need to be thoroughly done to ensure the efficiency of CART therapy.

3.1. Lung cancer

This type of cancer often starts in the epidermal cells of the bronchi and the lung such as the alveoli. There are two main types of lung cancer. Over 80% of lung cancers are non-small cell lung cancer. Small cell carcinoma, large cell carcinoma, and adenocarcinoma are some of the main subtypes of the disease. These subtypes of cancer can originate from different types of lung cells, but they are grouped due to their similar treatment. Small cell lung cancer (SCLC) accounts for the rest 20% of cancer. It can be called oat cell cancer due to its shape. It is more likely to grow and spread than NSCLC. Chemotherapy and radiation therapy work well for this cancer since it grows quickly. Most people will get cancer at some point.

Currently, around 50 interventional clinical trials using CARs to treat lung cancers are being conducted around the world.

3.1.1 MUC1(mucin1)

Abnormal overexpression of mucins can be a sign of cancerous cells growing and metastasising. MUC1 is a cell membrane glycoprotein and is a commonly targeted antigen overexpressed on the surface of lung cancer cells, especially on NSCLC. The research found that MUC1 contributes to angiogenesis to ensure the nutrients supply to cancer cells and the invasion of cancer to another part of the body. [6] The anti-MUC1 CAR gene is found in a lentiviral vector that is constructed by transfection into T cells. Modified CAR-Ts have been constructed and activated by IL-12, which improves the proliferation of CAR-Ts. In this way, they are capable of killing cancer cells [6].

3.1.2 EGFR (epidermal growth factor receptor)

EGFR has proved to be found on the surface of metastasised lung cancer and brain invasion and thus has been used as a novel marker in some clinical trials for NSCLC and presented excellent results in terms of cytotoxicity. Research proved that EGFR variant III CAR-T can efficiently recognise and eradicate EGFRIII+ cancer cells by releasing cytokines [7].

3.1.3 FR (folate receptor)

Normal cells have low expression levels of FR. Another approach that can be used to control the CAR-Ts function in tumours is the use of a bispecific small molecule switch. Recognising FITC molecules, anti-FITC-CAR-Ts bind to them and kill cancer cells. This process is assisted by bispecific T cell engager (BiTE). They help to direct CAR-Ts and target cancer. The anti-FITC-CAR-Ts can be conjugated to different anti-tumour antibodies. [8].

3.2. Oesophageal cancer

The oesophagus is a long, hollow tube that connects the human's throat to the stomach and is responsible for propelling the chyme towards the stomach to be digested. Mutations that enable cells

to proliferate uncontrollably can happen in any part of the oesophagus and most of them start from the lining of the tube.

3.2.1 CD276

It greatly helps cancer to proliferate, invade and metastasise. CD276 is extremely overexpressed in both ESCC and EAC but shows a few expressions on normal tissues, which indicates that it can be a potential target for treating oesophageal cancer and is worth testing in trials [9].

3.3. Mediastinal cancer

The mediastinum is the space between the two lungs that protects the heart and other organs. Common type of mediastinal cancer includes thymoma and thymic carcinoma, thymic cysts, lymphoma, germ cell tumour and thyroid mass. Currently, the most common way of treating mediastinal cancer is by surgery removing.

3.4. Breast cancer

Breast cancer is a cancer that occurs in the breast and can affect both men and women, but the proportion of women with the disease is much higher than that of men. Although many risk factors associated with breast cancer have been identified, the cause of breast cancer remains unclear. Estrone and oestradiol are known to be hormones associated with breast cancer incidence. Many factors can increase or prolong oestrogen exposure. Genetics is a high-risk factor for breast cancer. The risk in the general population is 23 times higher than in relatives with a history of breast cancer. Some genes increase the risk of breast cancer. Radiation therapy to the chest in childhood is one of the factors that contribute to breast cancer. In addition, lifestyle factors can also increase the incidence of developing breast cancer.

3.4.1 Human epidermal growth factor receptor 2 (HER2)

It is a member of the EGF family and can be activated. In addition to being associated with 20%~30% of breast cancer, HER2 is also associated with endometrial cancer, ovarian cancer, and fallopian tube cancer. HER2 stops cells that should have died in nature from dying, allowing them to revitalize and proliferate. In tumour cells, HER2 causes them to grow, divide and repair themselves. Breast cancer and gastric cancer are two types of cancer that HER2 can promote. It can help with the rapid growth, proliferation, differentiation and metastasis of cancer. HER2 overexpression is a suitable target for CARs. Humanised HER2 cells reacted against HER2-positive breast cancer cells. The efficacy and feasibility of HER2 CAR-T were proved in a phase I clinical trial. [8]

3.4.2 Mucin1 (MUC1)

Overexpression of MUC1 can be found in over 90% of breast cancer and thus serve as a potential target for treatment. Preclinical experiments on mice have been done but careful investigations should be done to make sure the safety and efficiency before clinical trials are done [10].

3.4.3 Epidermal growth factor receptor (EGFR)

It is expressed on both normal cells and tumour cells but with a large quantity on the surface of cancer. It was shown that third-generation EGFR CART cells were effective in blocking triple-negative breast cancer with limited cytotoxicity to normal breast cells. Clinical trials suggest that anti-EGFR CAR T cells are safe and effective for the treatment of metastatic pancreatic cancer. [10].

4. Conclusion

Using MUC1, PD-L1, HER2, EGFR and other antigens as targets, CART therapy shows amazing effects in treating thoracic cancers in preclinical and clinical experiments

Despite being a potential treatment for cancers, some limitations have been shown in preclinical and clinical experiments. The first limitation is CAR failure: The CAR T cell product can't be manufactured, or the generated CAR T cells don't expand enough in vitro/vivo, which may contribute

to cancer relapse. The second limitation is cancer cells developing resistance against CART cells. Antigen loss or down-modulation contributes a lot to it. Some cancer cells may lose their tumour-associated antigens due to reasons like alternative splicing and cannot be recognised by CART cells thus escaping from them. In this case, even with a successful remission induction using CART cell therapy, there is a high risk of cancer relapse, since some of the cancer cells remain invisible to the immune system. The third limitation is the on-target off-tumour effects: Tumour-associated antigens (TAA) do not only exist on the surface of cancer cells but also in ordinary tissues, leading to CART cells binding to normal tissues and activating, leading to toxicity on normal cells.

In typical CART cell manufacture, autologous T cells are extracted and modified. Although there have been excellent results from clinical trials, the high costs, long duration and potential failure remain challenges for the public healthcare system. The current manufacturing process lasts for about 3 weeks, during this period, patients may lose eligibility due to reasons like highly proliferative disease progression. Also, since they are derived from the patients themselves, the T cells may be previously suppressed by the tumour microenvironment and unable to show sufficient activity. Allogeneic CAR T cell therapy can potentially overcome the drawbacks mentioned above. It derives T cells from healthy donors that allow for cryopreservation. Thus, allogeneic T cells can be offered immediately. Moreover, the T cells extracted from a single healthy donor can produce batches of modified T cells that can be a constant and steady supply in CAR T cell therapy for several patients and redosing for the same patient.

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