

Engineering CAR T Cells for their Anti-tumor activity



Pratiksha Shanbhag¹, Rakhi Khabia², Renuka Maru¹,
Saurabh Maru^{1*}

¹ School of Pharmacy and Technology Management, SVKM's NMIMS, Babulde, Banks of Tapi River, Mumbai-Agra Road, Shirpur, Maharashtra 425405, India

² Acropolis Institute of Pharmaceutical Education and Research, Indore Square, Indore Bypass Rd, Manglaya Sadak, Indore M.P. 453771, India

skmaru@gmail.com

Introduction

Cell-based therapeutics involve replacing or repairing damaged cells by administering new, viable and healthy cells. It began in the nineteenth century and since then it has drawn significant attention from researchers as a promising treatment strategy for treating a wide range of diseases. With newer technologies and a vast imagination, its application has been wide and varied. It is generally used in the prevention of autoimmune and immunological diseases, along with regenerative medicine.(1)

CAR-T cell therapy

T cell is a part of immune system which recognizes foreign cells and kills it, thus protecting the body. This understanding of immune system can be used to fight against cancerous cells. The antigen on tumor cells gets recognized by T-cell receptors, which causes the cells to die. Certain developments have been made to increase the progression of immunotherapy one of them being CAR-T cell therapy which is also known as “a living drug”. It is used for the treatment of B-cell lymphoma, follicular lymphoma, multiple myeloma, blood cancer, HIV, acute lymphoblastic leukemia, hematological malignancies etc. (2)

Structure of CAR-T cell

There are two regions in this receptor located outside the cell firstly the antigen-recognizing region, derived from monoclonal antibodies containing a variable heavy as well as light chain which interact with antigen present on targeted cells. Secondly, the hinge region present above the outer membrane of the T-cell provides flexibility to the receptor. Thirdly a transmembrane region has an α -helix structure that is hydrophobic in nature functioning to stabilize the CAR. Fourthly an intracellular T-cell messenger region which sends signals to the cell when a foreign antigen is recognized. (Fig.2)(3)

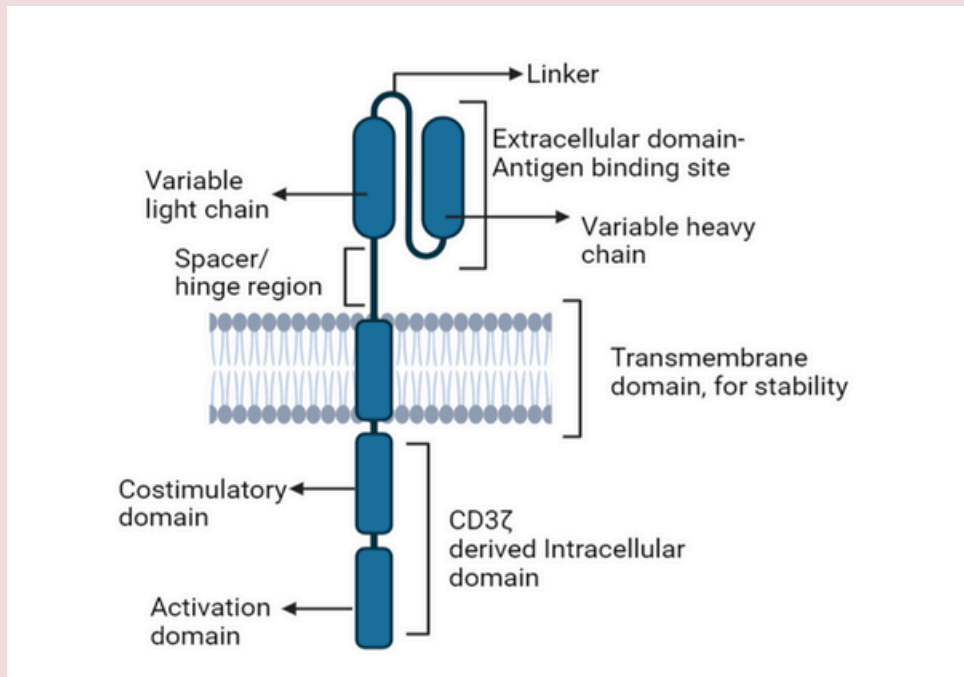


Figure 1: Structure of CAR

Mechanism

This treatment involves the modification of the T-cell, blood is removed from the patient to isolate the T-cells this procedure is known as pheresis. Then these T-cells are genetically altered with the help of a virus known as lentivirus containing the genetic information that encodes for the chimeric antigen receptor further these t cells are infected with the viral vectors which inserts the CAR gene causing the formation of chimeric antigen receptors (CARS) on the surface of T-cells. These CAR-T cells are further multiplied ex vivo and administered back to the patient intravenously. In the body these cells multiply, the receptors get attached to the target antigen and accumulation of CAR-T cells begins which leads to drying out of the tumor cell. (Fig.1) This therapy is customized for every patient according to the type of antigen present in the cancer cells. For instance, the cancer cells in the case of acute lymphoblastic leukemia have an antigen known as CD10. These CAR T-cell therapies are designed in a manner so that they attach the specific antigen only, in this case, the therapy won't work for cancer cells that don't have CD10 antigens.

The CAR T-cell therapy, not just only arms T-cells to inhibit the growth of tumor cells but also triggers T-cells to proliferate and multiply. For months, these cells stay inside the body of the patient and keep the tumor cells from growing. For improved results, it is applied in conjunction with radiotherapy and chemotherapy. It has shown promising results in patients with the reoccurrence of cancer.(4)

Side effect and treatment of CAR T-cell therapy

·*Cytokine Release Syndrome (CRS)*

As soon as the CAR T-cells start multiplying in the body, as a part of the immune system it releases cytokines into the bloodstream causing serious side effects including difficulty in breathing, high fever, nausea, diarrhea, vomiting, low blood pressure, etc. The emergence of CRS ensures that the therapy is 'on point' and it gets severe in case of patients with higher stages of cancer.

·Immune Effector Cell Associated Neurotoxicity Syndrome (ICANS)

The actual cause of these neurological side effects is yet not clear, the symptoms include twitching, impaired hearing, loss of balance, confusion etc. Other side effects include anaphylaxis, increase risk of getting serious infections due to a weak immune system, low sodium and potassium levels, and a decrease in RBC count which may elevate bleeding, infections and the patient is frequently exhausted.

·Tumor Lysis syndrome (TLS)

The lysis of tumor cells leads to the release of cell contents in the surroundings which cause damage to other organs like kidney failure, metabolic abnormalities, arrhythmias, etc.

·Tumor toxicity

The attack of CAR T-cells on the healthy cells having the same antigen as that of tumor cells causes tumor toxicity for e.g.- HER2(Human epidermal growth factor receptor), MSLN(Mesothelin), PSCA (Prostate stem cell antigen) are the antigens which are present in both healthy as well as tumor cells. The target specific approach is needed for the success of CAR T-cell therapy

The treatment strategies are still under extensive research, currently, tocilizumab (used to in the case of arthritis to suppress the activity of cytokines and IL-6) and steroids like dexamethasone are the treatment of choice. Methylprednisolone, siltuximab, and anakinra are used for severe cases of neurotoxicity associated with CAR T-cell therapy. Monitoring patients in ICU is advised for patients undergoing therapy. (5)

In cases where CAR T-cells toxicity (CRS, ICANS, TLS) is at a peak level immediate reversal of the therapy is required which is not fulfilled by the drugs used generally like rituximab. In order to address these challenges and increase the efficacy and application of CAR-T cell treatments, several new possibilities are being developed one of which being an ‘OFF SWITCH’. These off switches will immediately inhibit the activity of CAR T-cells, eg- Protein derived SMASH (small molecule assisted shutoff CARs, introducing a tyrosine kinase inhibitor (Dasatinib) which prevented the activation of CAR-T cells in mice models.(6)

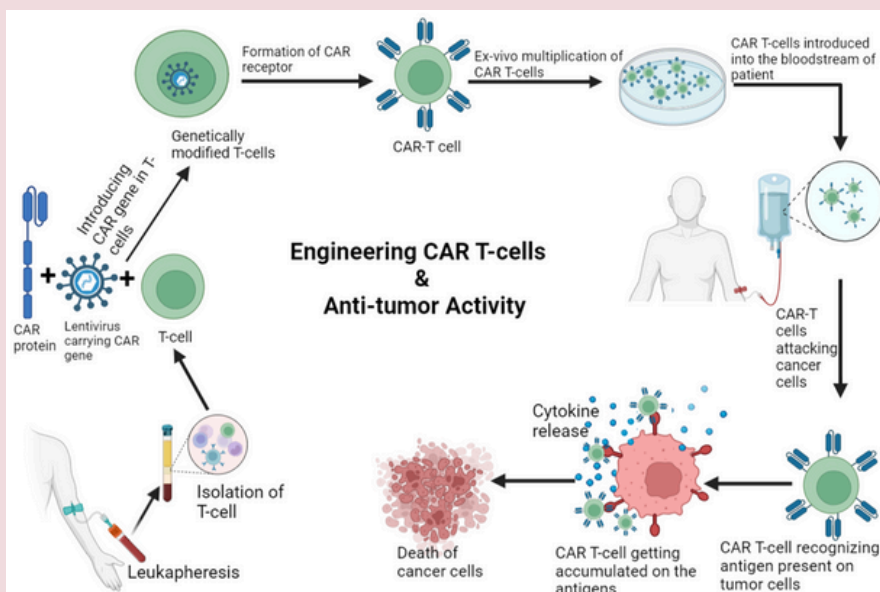


Figure 2: Engineering CAR T Cells and its Anti-tumor activity

Advancements in CAR T-cells

·Modulation in the structure of CAR T-cells

Improving the structure of CAR T-cells reduced the side effects according to a study done by National Cancer Institute, the substitution of the hinge and transmembrane regions with a protein fragment found in mice similar to that found in humans caused decreased release cytokines and was found to have the same efficiency to that of general CAR T- cells, remodeled CAR T-cells were able to attach to the antigens followed by lysis of tumor cells.

·Improving resistance to CAR T-cells

According to a study it was found that myeloma cells get rid of their surface antigens BCMA and protect themselves by getting attacked by CAR T-cells. To resolve this issue researchers introduced gamma-secretase inhibitors which increased the number of BCMA on tumor cells that were successfully killed by the CAR T- cells.

·Navigation of CART T-cells through solid tumors

The antigens present on solid tumor cells and those present in healthy cells are difficult to be distinguished. Other than this the tumor microenvironment (TME) is hostile towards CAR T- cells which makes their penetration into the cancer cells strenuous. A clinical study was conducted on the treatment of brain tumors with CAR T -cells by targeting the disialoganglioside (GD2) antigen present on tumor cells. With the subsequent treatment regimen small doses of modified CAR T-cells with GD2 were administered into the brain directly, which resulted in decreased tumor size and controlled other symptoms related to cancer. The ongoing duties of the T-cells are inhibited by the hostile tumor microenvironment due to hypoxic conditions, the release of toxic metabolites and reactive oxygen species (ROS), etc. To survive in these conditions “armored T-cells” are developed which release catalase into the TME to kill ROS.

·Controlling the metabolism of CAR T-cells

Several amino acids and their metabolites like L-arginine and ornithine respectively are required for the proper functioning of T-cells it prevents the metabolism and promotes their survival. There is an ongoing approach to developing ex vivo CAR T-cells having arginine residues, other attempts made in the same direction include genetic alteration of CAR T-cells with arginine synthesizing enzymes so that it can survive in the TME. Studies are still going based on the manipulation of glutamine metabolism in TME to promote the survival of CAR T- cells.

Vectors such as Lentivirus are used to deliver the genetic material to the T-cells but with development and research other vectors such as NK (natural killer) cells and CRISPR are under trials for their use

There are specific T-cell receptors that not only recognize antigens on the surface of tumor cells but also attack cancer cells by attaching to the antigens present inside the tumor cells. These receptors are under trials for use in conditions like sarcoma. (7)

Table 1. FDA-approved drugs under CAR T-cell-based Therapy(6)

Drug	Brand Name	Targeting antigen	Type of cancer
Lisocabtagene maraleucel	Breyanzi	CD19	High grade B cell lymphoma, Follicular lymphoma
Brexucabtagene autoleucel	Tecartus	CD19	Acute lymphoblastic lymphoma (ALL), Mantle cell lymphoma
Ciltacabtagene autoleucel	Carvykti	BCMA	Relapsed multiple myeloma
Idecabtagene vicleucel	Abecma	BCMA	Multiple myeloma
Axicabtagene ciloleucel	Yescarta	CD19	Follicular lymphoma
Tisagenlecleucel	Kymriah	CD19	Refractory B cell acute lymphoblastic lymphoma

Conclusion

Over the past few years, CAR T-cell therapy has been proven to be a highly effective treatment for patients with acute lymphocytic leukemia, hematological malignancies, HIV, etc. Several obstacles still hampering the treatment including off-target tumor toxicity, heterogeneity of tumor cells, and difficulty in penetration of CAR T-cells in solid tumor cells. Research is going on to use T-cells obtained from a healthy donor which will enhance the efficacy of CAR T-cell therapy. The newly designed CAR T-cells are taking the wheel for safer and more efficient use in treating lung and brain cancers.(8)

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