

The Living Panacea: CAR T Cell Therapy



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Introduction

The conventional strategies for treating neoplastic diseases have shown limited efficacy with various side effects deteriorating patients' quality of life. Poor recovery rates, varying therapeutic outcomes and significant relapse rates are some of the major challenges while curing dreadful cancer. As conventional medicines are falling short of expectations, a new paradigm – 'Immunotherapy' has emerged in recent years, offering the possibility of curing cancer fully. The immune system involves potent T-lymphocytes, which can eradicate the neoplastic cells, but often several hurdles mitigate the T cell response:

1) Lacking Co-stimulation and Tumour antigen

Cancer cells frequently suppress the expression of Major Histocompatibility Complex (MHC) class I and Costimulatory molecules, which are critical for cancer cell recognition and antitumor response by T cells (1).

2) Anergy

In the malignant environment where costimulatory molecules are present in reduced numbers, T cells often lose the ability to generate cytokines, thus threatening their proliferation and cancer cell-killing activity (2).

3) Tolerance of T cells

Most cancer cells have self-antigens, whereas the T cells possess low-affinity T Cell Receptors (TCRs) designed to identify nonself antigens. This permits malignancies to avoid detection by immune systems (3).

Chimeric Antigen Receptor (CAR) T cell therapy helps to overcome these barriers by genetically altering T cell receptors with an antigen-binding domain of an immunoglobulin using modern gene cloning techniques. T cell receptors that have been genetically changed are known as chimeric antigen receptors (CARs), and T cells that have their receptors modified are known as CAR T cells. This revolutionary therapy was declared as 'advance of the year' by the American Society of Clinical Oncology in 2018 after the US FDA approval in 2017 for B cell malignancies by anti-CD19 CAR T cell therapy.

Structure

CAR TCells are synthetically engineered cells that combine the capabilities of B-cell antibodies and T cells, just like the chimera, a fire-breathing creature from Greek mythology. The structure of CAR as receptors shown in fig. 1 can be divided into 4 parts:

·Antigen Binding Domain (ABD):

This domain provides antigen specificity to the CARs by targeting the extracellular tumor antigens present on the surface resulting in MHC-independent T-cell activation. They are derived from variable light (VL) and heavy (VH) chains of antibodies that are joined by the single chain variable fragment (scFv) as a flexible linker. The characteristics of scFv decide the specificity and affinity of the CARs (4).

·Hinge:

This extracellular region, majorly derived from amino acid sequences of CD28, IgG4, CD8 or IgG1 connects the antigen binding domain to the transmembrane domain. They help the ABDs to access the target epitope while providing sufficient length and flexibility. Changes in them can also impact the recognition of epitopes, CAR expression and signalling (5).

·Transmembrane Domain:

Derived from natural proteins like CD4, CD28, CD3 ζ or CD8 α , the transmembrane domain anchors the CARs to the T cell surface. The characteristics of this domain are yet to be thoroughly studied, but some reports suggests that they might influence the stability, activity and expression levels of CAR (6).

·Intracellular Signalling Domain:

When CAR T cells recognize the antigen, the intracellular signalling domain also known as the signalling endodomain triggers an intracellular cascading signal to impart its activity (7).

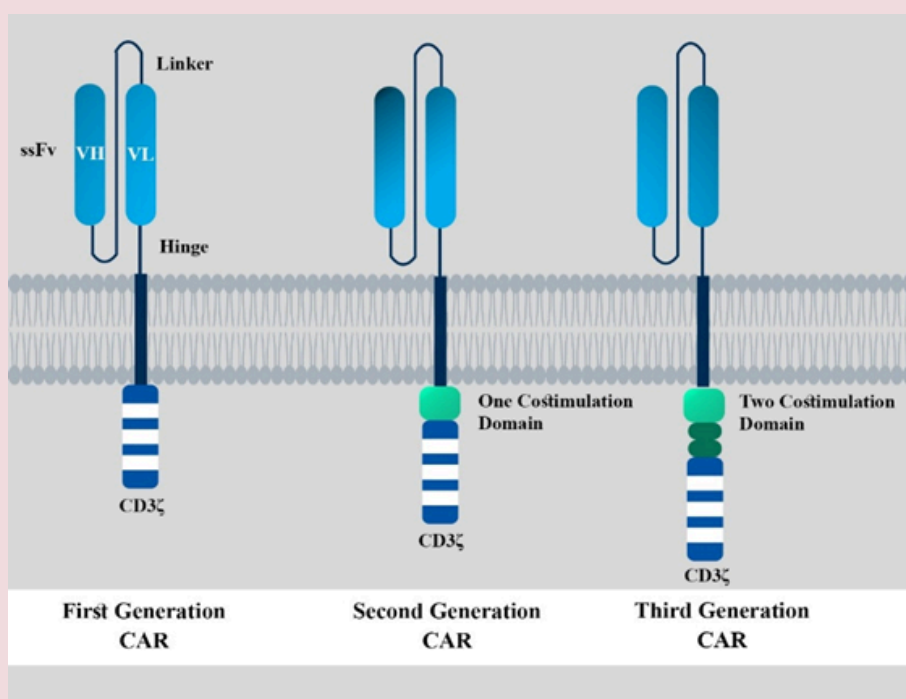


Figure 1: Structure and Generations of CARs

Generations of CARs

In the late 1990s, first generation CARs were produced with a CD3 ζ chain transmitting the signals from endogenous TCR, but they did not carry costimulatory molecules. In the clinical trials, it was seen that their persistent exposure resulted in therapeutic effects, but due to lack of activation by signalling had resulted in inadequate anticancer activity (8). To enhance the safety and efficacy, second generation CARs are designed with dual signalling – activation via CD3 ζ and costimulation by integrating costimulatory molecules like inducible T cell costimulator (ICOS), CD28, CD137, OX40 or 4-1BB to the tail of the CAR. Adding such costimulatory domains not only helps to produce a significant complete response for B-cell malignancies but also enhances the T cell accumulation and effector cell functions (9). In order to increase efficacy, two signalling domains are introduced in the third generation CARs with a CD3 ζ chain. Infiltrating and lysing the tissues in chronic lymphocyte leukemia, CARs with CD-28 and 4-1BB molecules showed complete remission rates. They aid in prolonged proliferation, enhanced signalling and increased production of cytokines (10).

Productions of CAR T Cells

At the clinical level, the production starts with the recruitment and screening of patients with stage IV cancer as shown in fig. 2 and then they undergo apheresis so that Peripheral Blood Mononuclear Cells (PBMCs) can be isolated. Ex vivo cultures activate and increase the PBMCs until the required T cell number is attained. Further, the T cells are modified by infecting them with CAR-retroviral particles and these CAR-modified T cells are then expanded in an ex vivo culture while monitoring their changes. Finally, the prepared CAR T cells are reinfused into the patients and their antitumor response is monitored (11).

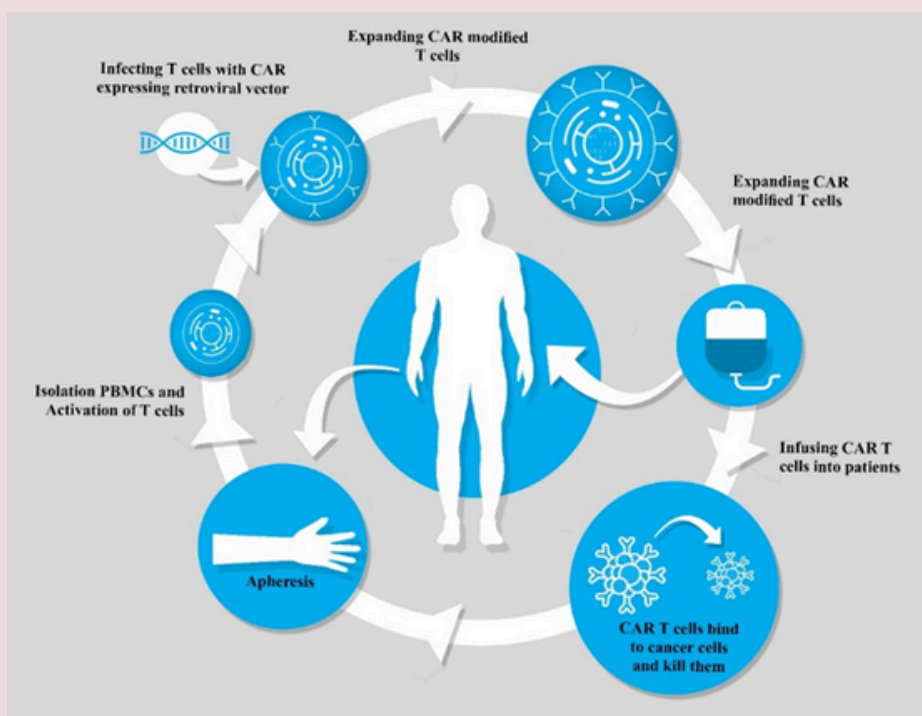


Figure2: CAR T cells production at clinical level

CAR T cell Therapy Advantages

The biggest advantage of this treatment is the single infusion of CAR T cells which impart their action over decades after 2-3 weeks of sufficient care and observation and can destroy the tumour even during relapse. It is also seen that refractory blood cancer comes back despite various transplants, the CAR T cell therapy successfully eliminated the disease. Moreover, maintaining the patients' quality of life, they provide a living without any significant risk of relapse (12–14).

Limitations of CAR-T Cells

On-target off-tumour effects –

It is seen that antigens expressed on solid tumors are also present in normal tissues at different levels. CAR T cells targeting such antigens can cause an on-target off-tumor effect by attacking healthy cells. Thus, to avoid such toxicity, tumor-restricted post-translational modifications like overexpressed truncated O-glycans for solid tumors can be targeted (15).

Antigen escape –

In this phenomenon, CAR T cells showing high response rates for a target antigen can lose their activity by tumor resistance development. In such cases, malignant cells can show partial or complete antigen expression loss which were targeted by CAR T cells. Recent studies demonstrate the development of resistance in patients with recurrent disease after the treatment by downregulation of CD19 antigen in 30%-70% of patients. Furthermore, downregulation or deletion of BCMA (B-Cell Maturation Antigen) expression has been reported in multiple myeloma patients receiving BCM targeted CAR T cells. Thus it is essential to select a target antigen which can reduce the antigen escape (16,17).

Immunosuppressive Microenvironment -

Many cell types that cause immunosuppression can infiltrate solid tumors, including Tumour-Associated Macrophages (TAMs), Myeloid-Derived Suppressor Cells (MDSCs) and Regulatory T Cells (Tregs). Such tumour cells and infiltrates stimulate tumor generation, facilitating chemokines, growth factors and cytokines. Also, the immune checkpoint routes act to decrease anticancer immunity. It's been proposed that co-inhibitory pathways cause the development of T cell exhaustion. As a result, combined immunotherapy with CAR T cells and checkpoint blockade is anticipated to be the next immunotherapy frontier since it delivers both the infiltration and the PD-1/PD-L1 blockage required for significant immune responses (18). Other than this, CAR T cell toxicities and trafficking are some of the other problems that had to be resolved for safe and effective tumor treatment (19,20).

Future Prospects

FDA approved CD19-specific therapies in the form of Yescarta (axicabtagene ciloleucel) by Kite Pharma/Gilead Sciences and Kymriah (tisagenlecleucel-T) by Novartis. More than 220 trials have been conducted to identify CAR T cell therapy targets. It is marking a paradigm shift for curing cancer using a self-replicating and living drug, but the overall cost for the treatment and hospitalization is around \$1,500,000 per cancer patient which is significantly high. Thus, research must be conducted to optimize the treatment and find solutions to reduce the costs. Other than that, problems associated with the treatment, transportation, manufacturing and storage of these living drugs possess a big challenge which have to be overcome in the near

future. Effective preclinical models are also needed to evaluate the efficacy and safety of medications before human studies. CAR T cell therapy has shown effective treatment in hematological cancers, but this is just the beginning. Several features of this therapy are yet to be uncovered and its potential to cure recurrent non - hematological, metastatic and resistant cancers is yet to be explored.

Abbreviations:

CAR - Chimeric Antigen Receptor

MHC - Major Histocompatibility Complex

TCR - T Cell Receptors

scFv - Single Chain Variable Fragment

ABD - Antigen Binding Domain

PBMC - Peripheral Blood Mononuclear Cell

BCMA - B-Cell Maturation Antigen

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