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Review Article

A Review on CAR-T Cell Therapy

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Abstract

A promising new cancer treatment called Chimeric Antigen Receptor (CAR)-T cell therapy entails genetically altering a patient's T cells so they can identify and combat cancer cells. An outline of the most recent findings and clinical studies pertaining to CAR-T cell therapy, along with the idea and uses of the treatment, are given in this article. The review also covers the drawbacks and possible adverse consequences of CAR-T cell therapy, such as its expensive nature and the possibility of neurotoxicity and cytokine release syndrome. Even though CAR-T cell therapy has demonstrated encouraging outcomes in the treatment of hematologic malignancies, more research is required to increase the therapy's safety and efficacy and extend its application to solid tumours. As long as research and development on CAR-T cell therapy continue, it could revolutionize cancer treatment and improve outcomes for cancer patients. [2025, 6(1): 1-8]

Keywords: Chimeric Antigen Receptor T (CAR-T) cell, Cancer immunotherapy, Cytokine release syndrome.

Introduction

Cancer is one of the leading causes of death worldwide (1). The process of cancer development is called as carcinogenesis. Carcinogenesis mainly occurs due to extrinsic factors and genetic susceptibility. A long standing chronic inflammation may release different mediators which cause formation of TME (tumour micro-environment). The inflammatory mediators also inhibit apoptosis. There are cytotoxic T cells which release perforin and granzyme and help in apoptosis. Chimeric Antigen Receptors T (CAR-T) cells are specialised cancer killing cells, which bind to their surface proteins. CAR-T is also called as first living drugs, as once they infused and activated, they present in the body and show prolonged results. In this therapy autogenous blood is drawn, autologous T cells are genetically modified and inject into same patient (2).

Mechanism

Autologous T cells are collected and then artificial genomic alteration done of those cells by introducing DNA of CARs with help of retrovirus (help to recognise tumour specific antigen) to form CAR T cells (3).

At first leukapheresis should be done. It is a mechanical process by which WBCs and platelets are separated. Second step is enrichment and washing

which help to separate T cells from leukocytes by magnetic separation with the help of monoclonal antibody coupled metallic beads. After that, CARs are coupled with lentivirus vectors which help in reverse transcription into DNA and insertion into genome of T cells. Then during culture viral vectors washed out with dilution and exchange of medium should insertion of RNA by mRNA transfection. After this, CD4, CD8 were separated, activated and cultured them with growth factors. Re-enginered CAR-T cells are cultured by bio-active culture system. Cells are then amplified to achieve number of effective clinical dose and it stored in frozen form. Before infusion patient undergo chemotherapy "lymphodepletion" which increase expansion of CAR-T cell by 2-3 times proper thawing performed. Then it perfuse to patient, cell amplify and it attack cancer cells (Fig 1) (4).

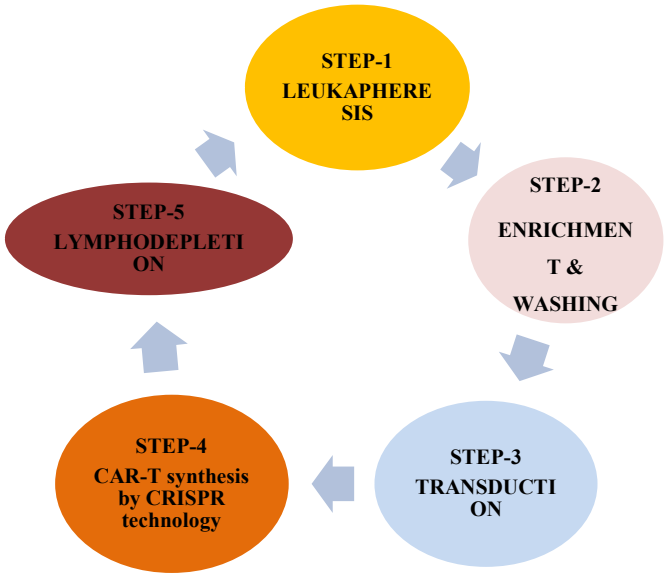

Fig1: Mechanism of CAR-T Cell 1

FDA Approved CAR-T Cells

Previously FDA approved mainly 2 trademarks of CAR-T therapies

1. Kymirah T™ (Tisagenlecleucel) 2.Yescarta™(axicabtageneciloleucel)

Since 2017, six CAR T-cell therapies have been approved by the Food and Drug Administration (FDA) (Table 1). All are approved for the treatment of blood cancers, including lymphomas, some forms of leukemia and most recently for multiple myeloma (5).

Company name Brand name Generic name	Date of approval	Target antigen/ Antibody	Targeted cancers	Outcomes
Novartis Kymriah Tisagenlecleucel	Aug 30, 2017	CD19 Mouse FMC63	R/R CAYA BALL	81% overall remission rate (6)
Kite Yescarta Axicabtageneciloleucel	Oct 18, 2017	CD19 Mouse FMC63	R/R LBCL	58% complete response (7)
Kite Tecartus Brexucabtageneautoleucel	Jul 24, 2020	CD19 Mouse FMC63	R/R MCL	67% complete response (8)
Juno Breyanzi Lisocabtagenemaraleucel	Feb 5, 2021	CD19 Mouse FMC63	R/R LBCL	53% complete response (9)
Bluebird Abecma Idecabtagenevicleucel	Mar 26, 2021	BCMA Mouse BB2121	R/R MM	33% complete response (10)
J&J and Legend CarvyktiCiltacabtageneautoleucel	Feb 28, 2022	BCMA dual camel singledomain antibodies	R/R MM	82.5% complete response (11)

Table 1: List of CAR-T cell therapies 1

[R/R: Relapsed or Refractory, CAYA: Children And Young Adults, LBCL: Large B-Cell Lymphoma, MCL: Mantle Cell Lymphoma, MM: Multiple Myeloma]

Generations wise structure of Chimeric Antigen Receptors

CAR consists of four components- 1. An antigen binding domain (only present in modified CARs use in immunotherapy), 2. A hinge domain, an extra cellular structure allow extensions of antigen binding domain and hinge domain, 3. A transmembrane domain (combines intracellular and extracellular domain), 4. Intracellular signalling domain (12,13).

The difference in each generation CAR mainly depends on the structure and function of the Intracellular Domain (Table 2).

CARs with the model scFv (single chain fragment variable)-spacer-CD3z(CD3-zeta chain, a key transmembrane signalling component of T-cell receptor) layout are known as "first-generation CARs." It can only activate the receptors. In order to improve in vivo persistence, proliferation, their ability to secrete cytokines and resistance to apoptosis, second and third generation CARs are synthesized. Higher generations bear co-stimulatory domain like CD28 and/or 4-1BB (14).

The third generation of CARs has improved tumorlysis and in vivo survival compared to previous generations. TRUCKs (T cells redirected for antigen unrestricted cytokine initialled killing), also known as armored CARs, are the most advanced fourth-generation vehicles. TRUCKs integrate domains within the endodomain, enhancing anti-tumor activity. These include costimulatory ligands and cytokines. Enzymes can dissolve the extracellular matrix of tumors (15).

Features		Generation				
		1ST	2ND	3RD	4TH	5TH
Extracellular Domain	scFV	Present	Present	Present	Present	Present
Transmembrane Domain	hinge	Present	Present	Present	Present	Present
Intracellular Domain	CD 3 signaling domain	Present	Present	Present	Present	Present
	Additional Constimulatory Domain (CM)	Absent	4-IBB or CD or OX40	CD28 &41BB both	4-1BB Or CD28 Or OX40	4-1BB Or CD28 Or OX40
	Interleukin Inducer	Absent	Absent	Absent	IL-12 inducer	Absent
	Interleukin 2 receptors	Absent	Absent	Absent	Absent	IL-2receptor beta chain domain
	Binding site for Transcription factor STAT3	Absent	Absent	Absent	Absent	Present

Table 2: List of different generation CA 1

Potential therapeutic application

i. Acute lymphoblastic leukaemia

B-cell acute lymphoblastic leukemia (ALL) responds best to CAR-T cell therapy. Anti-CD-19, a biomarkeriv. of B-cell lineage, is the most effective CAR in treating ALL. B-cell ALL is characterized by higher expression of this marker. Other potential targets include anti-CD20 and IG light chains (16).

ii. Chronic lymphocytic leukemia

Leukaemia has a diverse clinical course and chemotherapeutic prognosis (17). CD19 CAR-T therapy has recently been used to treat relapsed chronic lymphocytic leukemia (CLL). Besides CD19, other treatment targets being explored include tyrosine-protein kinase transmembrane receptors and CAR-T cells (18).

iii. Multiple myeloma

Evidence suggests that CAR-T cell therapy may be beneficial in myeloma, as it has been shown to reduce myeloma through grafting. It has an effect on patients undergoing allogenic stem cell transplantation (19).

CAR-T cell therapy in head & neck cancer

CARs must enter the tumour microenvironment (TME) to be effective. HNSCCs must be treated similarly to other solid tumours. Vascular remodelling reduces immune cells' ability to penetrate blood vessels and reach tumours (20). The stromal barrier in solid tumours can have abnormal extracellular matrix (ECM) or mismatched chemokines. Immune cell receptors and tumour-derived chemokines can inhibit T cell migration. Cancer-associated fibroblasts (CAF) in the TME contribute to the accumulation of abnormal ECM around the tumor, resulting in a compact fibrotic environment.

The ErbB(erythroblastic oncogene B) (EGFR-Epithelium Growth Factor Receptor) family is a promising target for CAR-T treatment in HNSCCs.

The family of EGFR refers to Human Epidermal Growth Factor Receptor 2 (HER2), ErbB3 (HER3), and ErbB4 (HER4) (20).

Specific ligands can activate each of these receptors. The receptors form homo- or hetero-dimers, activating their intrinsic tyrosine kinase activity and triggering downstream signalling pathways that regulate cell growth, differentiation, and survival. CAR-T cells targeting EGFR regulate cell growth, differentiation, survival, and migration. A preclinical study conducted by a team that had previously built and verified EGFR-CAR-T cells confirmed their cytotoxic function against hypopharyngeal squamous cell carcinoma.In study it has been seen that EGFR-CAR-T cells had a target cell lysis rate of 52.66% (21).

Many healthy tissues express members of the ErbB family, on-target off-tumor toxicity is one of the most difficult obstacles to T4 immunotherapy application. prior to clinical evidence suggested that the intra-tumoral route may be used to reduce this toxicity, and a phase-I clinical study involving 13 patients with advanced HNSCCs has been developed and shown to be safe for administering intra-tumoral T4 (22).

Although CD70 expression was high in 19% of HNSCC tumor biopsies, Park et al. used a retroviral human CD70 CAR construct to generate CD70 CAR-T and discovered that it has the ability to efficiently eliminate CD70-positive HNSCC cells (23).

Radioresistant HNSCC cells exhibit high expression of CD98hc, a T cell activation marker. The CD98hc-targeted UniCAR-T cell .The developed method effectively eliminated tumour cells in a 3D setting, specifically in HNSCC tumorspheroid (24).

Limitations

1. **Antigen escape**

One major drawback of CAR-T cell treatment is tumour resistance to single-antigen targeting CAR designs. Although initially, a single antigen targeting CAR-T cells can provide significant response. Many patients treated with CAR-T cells saw partial or complete elimination of target antigen expression in their malignant cells. This phenomenon is called antigen escape (25).

2. **On target off tumour**

Targeting solid tumour antigens might be challenging due to their varied expression levels in normal tissues. So, antigen selection is important for effective CAR design requires minimizing "on-target off-tumour" effects while maintaining therapeutic efficacy. Tumor-restricted post-translational modifications are required.

3. **CAR-T cell trafficking and tumour infiltration**

The capacity of CAR-T cells to enter solid tumours is limited due to immunosuppressive tumour microenvironments and physical barriers like tumorstroma. To overcome these limitations, local administration of CAR-T cells can be used instead of systemic delivery. This eliminates the need for CAR-T cells to travel to disease sites and limits on-target off-tumour toxicities by focusing on tumour cells and minimizing interactions with normal tissues (26).

4. **Immunosuppressive microenvironment**

Immunosuppressive cells, such as myeloid-derived suppressor cells (MDSCs), TAMs, Tregs can infiltrate solid tumours. Immune checkpoint, including PD-1 and CTLA-4, can reduce anticancer immunity. Poor T cell multiplication and short-term T cell persistence are two of the most common causes for minimal response to CAR-T cell therapies (27).

5. **CAR-T cell-associated toxicities**

Although CAR-T cell therapy has revolutionized cancer treatment, its high toxicity rates and potential for mortality have kept it from being a first-line treatment.

6. **High cost of manufacturing autologous CAR T cells**

Current CAR T cell therapy is limited by the expensive expense of producing autologous cells, which can cost up to $500,000 for patients with severe CRS.
Autologous CAR T cell production typically takes

21–35 days. During the waiting time, patients may require bridging therapy or succumb to disease progression without receiving CAR T cell therapy.

Furthermore, T cells from sick patients may become exhausted and less active compared to healthy donors (28).

CAR-T cell therapy related toxicity

The cart therapy may cause cytokine-driven effects such as macrophage activation syndrome, cytokine release syndrome (CRS). Higher the dose of cart causes higher the incidence of CRS.

Neurological toxicity- It includes delirium, anxiety, sleep disorder, headache, dizziness tremors, seizures, mutism, aphasia, peripheral neuropathy and CRES (CAR-T cell related encephalopathy). CRES mostly occurs within 5 days of drug administration and the initial symptoms are diminished attention, impaired handwriting and language disturbances. For neurological assessment10 score provided by the (CARTOX-10) CAR-T cell therapy toxicity (29).

Infections (serious)- CAR-T therapy may cause impaired humoral immunity. Lead to Hepatitis B viruses activation cause hepatic failure & death. It was suggestive to do viral markers test.

Type I hypersensitivity- The Type I hypersensitivity may develop after infusion of Axicabtagene and Tisagenlecleucel. It can cause anaphylaxis reaction.

Prolonged cytopenias- Prolonged lymphodepleting chemotherapy and drug infusion may cause cytopenias in patients. Prolonged neutropenia increases the risk of infection. Myeloid growth factors, including Granulocyte-macrophage colony-stimulating factor, should not be administered for the first 3 weeks after the drug infusion or until the CRS is re-established.

Hypogammaglobulinemia- Tisagenlecleucel and Axicabtagene infusion can cause B-cell aplasia, after remission of treatment. Hypogammaglobulinemia is reported in pregnant women, so, it is recommended to monitor IG levels in newborns of mothers receiving CAR-T therapy.

Secondary malignancies- Long-term monitoring is recommended due to the high rate of secondary cancers and recurrence in many patients.

Black box warnings-The US FDA has approved these drugs with a black box warning for CRS and neurotoxicity (30).

Future perspective of car t therapy

The success of CAR T cell treatment has prompted researchers to investigate altering other immune cells, including natural killer (NK) cells, NKT cells, macrophages and neutrophils are used for medicinal purposes (31,32).

CAR-NK cell treatment has demonstrated promising results in clinical trials. Although these immune cells may be less susceptible to graft vs host illness, making them appropriate for off-the-shelf products, they have drawbacks such as short lifespan, restricted multiplication, and inability to develop memory cells. T cells can be tailored to target tumours using tumour-neoantigen specific TCRs. TCR-T cell treatment offers the benefit of targeting non-membrane antigens, but well-designed CAR can also be effective. T cells can be engineered to target tumours using tumor-neoantigen specific TCRs. TCR-T cell treatment offers the benefit of targeting intracellular neoantigens within MHC complexes, in addition to membrane antigens (33,34).

Conclusions

CARs are modular, synthetic receptors. CAR-T cells have revolutionized therapy for some haematological malignancies. However, hurdles persist in this review article structure and generations of CAR T cell receptors mechanisms ,synthesis of CAR-T cells, FDA approved agents, potential therapeutic application and limitations of CAR T cell therapy related toxicity were discussed.Despite this obstacles, new ideas and solutions are emerging, perhaps leading to safer and more effective therapies in the future.

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