

CHIMERIC ANTIGEN RECEPTOR FOR CHRONIC LYMPHOCYTIC LEUKEMIA - A REVIEW

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ABSTRACT

Chronic lymphocytic leukemia cancer is a deadly one which affects the bone marrow from making it to produce more amounts of white blood cells in the humans. This disease can be treated either by radiation therapy, bone marrow transplantation, chemotherapy, or immunotherapy. In radiation therapy, the ionizing radiation is used toward the tumor cells, but the main drawback is the radiation may affect the normal cells as well. To overcome this drawback, immunotherapy chimeric antigen receptor (CAR) is used. These CAR cells will target only the antigen of the tumor cells and not damage the normal cells in the body. In this therapy, the T-cells are taken either from the patients or a healthy donor and are engineered to express the CARs which are called as CAR-T-cells. When these CAR-T-cells come in contact with the antigen present on the surface of the tumor cells, they will get activated and become toxic to the tumor cells. This new class of therapy is having a great prospect in cancer immunotherapy.

Keywords: Chimeric antigen receptor, Chronic lymphocytic leukemia, Immunotherapy.

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INTRODUCTION

Chronic lymphocytic leukemia (CLL) cancer is a deadly one which affects the bone marrow from making it to produce more amounts of white blood cells in the humans [66,67,73]. The lymphocytes consist of natural killer cells, B-cells, and T-cells. The bone marrow produces both T-cells and B-cells, but the maturation of T-cells takes place in the thymus and maturation of B-cells takes place in the bone marrow. When these lymphocytes are produced in large amount, they get accumulated in spleen, bone marrow, blood, and lymph nodes which results in enlargement of these organs and the hemoglobin content will get decreased [1,2]. This can be treated either by radiation therapy, chemotherapy, immunotherapy, or stem cell transplantation. In radiation therapy, the ionizing radiation is used toward the tumor cells, but the main drawback is the radiation may affect the normal cells as well. Initially, in bone marrow transplantation, the patient should be treated with either chemotherapy or radiation therapy and allogeneic bone marrow transplantation, but the infections are major complication [47]. Here, in this review, we have focused the structure, production, evolution of chimeric antigen receptor-T CAR-T-cells, and their role in treating CLL.

CAR-T-CELLS

The CAR-T-cells are also known as "living drug" [48] as they are engineered with receptor immune cell whose activity is specific toward the cancer cells. The targeting efficiency of these cells is very high as they target only cancer cells and harmless to the normal cells.

Of these therapies, the latest method is CAR-T-cells. In this method, the T-cells are taken either from the patient's blood or from a healthy donor, and then, these cells are genetically engineered to express an artificial T-cell receptor which will target and destroy the cancer cells [14,72].

CAUSES OF CLL

The CLL is caused by specific herbicide 2,3,7,8-tetrachlorodibenzodioxin. The tetrachlorodibenzo-p-dioxin (TCDD) is a fat-soluble compound in contact with the skin, the compound has ability to enter into the gene of the organisms [10,76]. The TCDD binds with the aryl hydrocarbon

receptor which is a type of protein present in the gene resulting in gene transcription [11,12]. The primary cause of ionizing radiation will break the double-stranded deoxyribonucleic acid (DNA) [13]. Furthermore, the risk factor of this cancer depends on age, family history, gender, and ethnicity. About 90% of people with CLL are above the age group of 50. First-degree relatives (parents, siblings, or offspring) of people with CLL have more than twice the risk for this cancer. CLL in male is more common when compared to a female. According to ethnicity and recent statistical report, the people in North America and European countries are more likely to be affected by this cancer [77].

AVAILABLE TREATMENT FOR CLL

Chemotherapy

To treat CLL, the drug rituximab, fludarabine, and cyclophosphamide against CD20 is used as it inhibits the synthesis of DNA resulting in the suppression of tumor cells. The drug cyclophosphamide is used along with fludarabine and has a side effect of reducing the white blood cell count when taken individually. Hence, the combination of this drug used to treat CLL [74,75]. Furthermore, the CLL can be diagnosed only when it is treated at initial stages [3-5].

Radiation therapy

Radiation therapy uses ionizing radiation, which controls the cell growth by damaging the DNA. Since CLL is a category of blood cancer, the success rate of radiation therapy is low because radiation therapy is done by introducing a specific area (where tumor cells are located) to the ionizing radiation as blood flows all over the body, it is not possible to treat with radiation. Most importantly, it has a probability of killing the normal cells as well [6].

Immunotherapy

This is the most promising treatment for CLL where genetically engineered lymphocytes (T-cells) are used to kill the tumor cells. This therapy is very specific as it targets only the tumor cells and it is less harm to the normal cells [9]. By seeing the drawbacks of other therapies, CAR-T-cells are the source of immunotherapy for treating CLL [7,8] effectively.

Structure of CAR-T-cells

CAR-T-cells are composed of three portions they are ectodomain, endodomain, and transmembrane domain [15]. The overall CAR-T-cells are composed of a light chain (V_L), a heavy chain (V_H), hinge region, and transmembrane domain which are a lipid bilayer. The structure of CAR-T-cells is given in Fig. 1.

Ectodomain

The ectodomain is a membrane protein present on the outer region of cytoplasm. It is composed of three parts; antigen recognition region, spacer, and signal peptide [16,79]. The signal peptide is a single-chain variable fragment (scFv) in which a portion of heavy and light chain of immunoglobulin (Ig) is fused by a linker [17]. The linker is hydrophilic, and for flexibility, the amino acids glycine and serine are present [18]. The antigen recognition domain is scFv with a basic ectodomain and more exotic recognition components. The tumor antigen will bind in the ectodomain region. The spacer is an intermediate which connects the transmembrane domain and ectodomain. The basic form of spacer is the IgG1's hinge region [20].

Transmembrane domain

The transmembrane domain plays an important role in the stability of the receptor molecule. The transmembrane is hydrophobic α -helix which is the secondary structure of protein where H bond from the N-H group is donated as a C=O group of the amino acid which is present around the membrane [19,22]. Initially, CD3- ζ was used as the domain as they resemble the normal T-cell receptor, but comparatively CD28 is the most effective one. These CDs are the proteins that are expressed in T-cells and they stimulate the signal which results in activation of T-cells [21].

Endodomain

The endodomain is present in the cytoplasm of the cell. It is the functional terminal of the receptor and is activated by CD3- ζ which presents in the ectodomain. The CD3- ζ contains immunoreceptor tyrosine-based activation motif and the efficiency is quite less, and hence, a costimulating signal domain is needed [68]. To satisfy these conditions, the molecules such as CD28, CD134, CD137, and CD27 are included in the CAR-T-cells to enhance the direct costimulation after the binding of tumor antigen to the CAR-T-cells [23].

Production of CAR-T-cells

The production of CAR-T-cells involves various steps. The most important step is quality control testing throughout the process. The process is briefly explained and the flow is depicted in Fig. 2.

Initially, the leukocytes are taken either from the patient or from the donor's blood from the process called leukapheresis [24]. In this

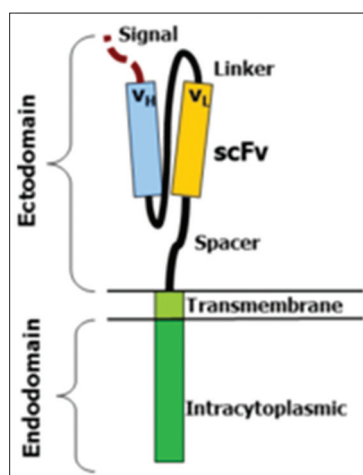


Fig. 1: Structure of chimeric antigen receptor-T-cells

process, the blood is drawn to the apheresis where the components of the blood are separated by centrifugation. Anticoagulants are added during this process [25] and the leukocytes are retrieved. Second, the T-cells are separated from other components of leukocytes by enriching and washing. This process helps in removing the anticoagulants that were added in the previous step. Now, the CD4 and CD8 subsets in the T-cells are separated by specific antibody beads conjugates which are also called as marker [26]. The CD4 is a T helper cell which has CD4 glycoprotein at their surface and CD8 is a cytotoxic cell which contains a glycoprotein. The T-cells will be activated for proliferation and growth. The activation of T-cells can be done by three methods; they are as follows [37].

1. Monoclonal antibody and interleukins (ILs)
2. Magnetic beads coated with antibody
3. Artificial antigen presenting cells.

Monoclonal antibody and IL

In this method, a monoclonal antibody anti-CD3 and IL-2 which is a type of cytokine is added to the T-cells to develop proliferation [27,84].

Magnetic beads coated with antibody

Anti-CD3 or CD28 is coated on magnetic beads resulting in the artificial antigen presenting particles [80]. The superparamagnetic beads are of diameter 4.5 μ m and are removed efficiently with a strong electromagnet, leaving <100 residual beads per 3×10^6 cells at the end of production. The beads are used continuously to proliferate the T-cells during the expansion [39]. This method results in a very strong activation of T-cells when compared to the use of monoclonal antibody and IL. The usage of magnetic beads is more convenient as they are removed easily after the proliferation of T-cells [38,40].

Artificial antigen presenting cells

The other ways to activate the T-cells are using non-viable antigen presenting cells. These cells present the antigen on its surface and stimulate the T-cells [28,100].

GENE DELIVERY

Gene delivery will be done either by viral method of gene transfer or through the plasmid mode of transfer. In CAR-T-cell therapy, viral vectors are used in gene delivery, where either lentiviruses or retroviruses are used for gene delivery [99]. The vector expresses the gene of CAR which is formed by combining the head of the antibody (V_H and V_L) and the T-cell signaling motif [37].

Viral transduction

High efficiency is obtained from viral transduction [41,78]. Since the retrovirus transduces the divided and proliferated cells, where activated T-cells are used. The CD28 signaling domain and CD3- ζ are combined together with the scFv to form the CAR gene and then transduced to the lentiviral vector. This is specific to the CD19 cells that are mainly present in B-lymphocytes, which are the chronic lymphocyte leukemia tumor cells. The viral vector encoding CAR gene after the entry of the target region, it results in CAR-T-cells [42].

Plasmid-based gene delivery

In plasmid-based gene delivery, the gene transposon system is used. A transposon is the sequence of DNA, which can change its position either by insertion or excision within the genome by transposase enzyme. The CAR transgene is inserted into the plasmid, and then, the plasmid is inserted into the T-cell genome. After the transformation studies, CAR-T-cells are produced and the cells are injected into the patient's body [43-46]. The CAR-T-cells lyse the CD19-positive targets, and the release of cytokines will activate the cellular components of the adaptive and innate immune system for enhancing the tumor rejection [83].

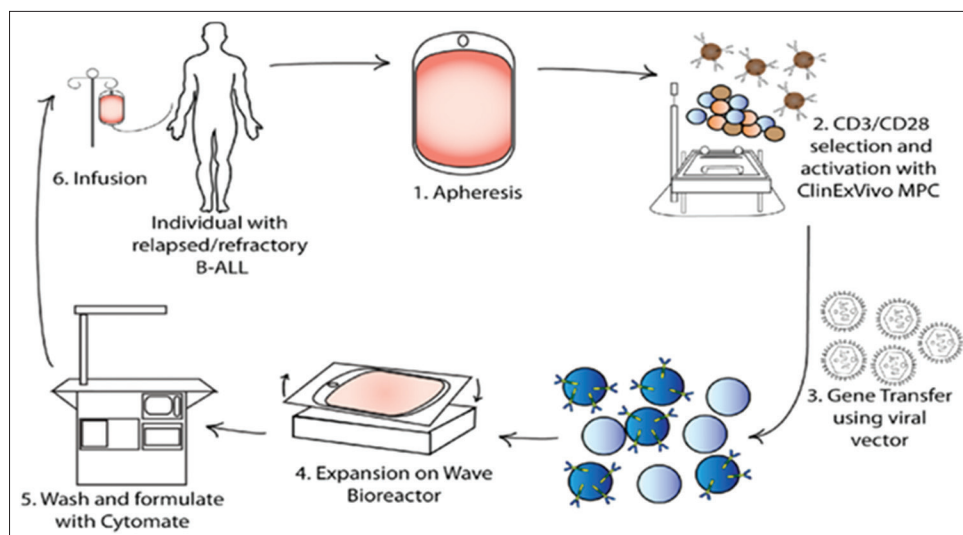


Fig. 2: The production process of chimeric antigen receptor-T-cells

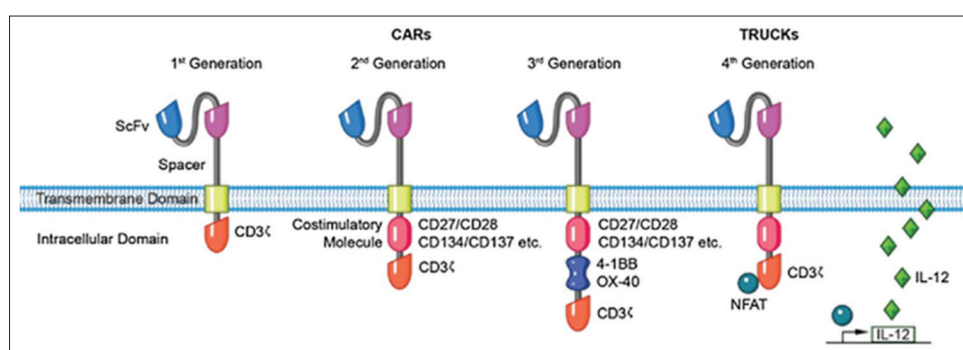


Fig. 3: Evolution of CAR-T-cells

CAR-T-cell evolution

The CAR-T-cells have been evolving since the initial development of immunotherapy. There are four generations of CAR-T-cells, which is depicted in Fig. 3. The structure differs from each other by the changes in the position of endodomain.

First generation

The first generation consists of simple structure, CD3- ζ which is present in the endodomain, a primary signal transmitter [29,30,69]. This type of CAR-T-cells is less efficient as they cannot produce enough amounts of cytokines. Hence, IL-2 should be added to it. Thus, the first-generation CAR-T-cell which is transfected with single chain receptors is benefitted only by the accompany of cytokines [31]. The transmembrane domain of CAR-T-cell consists of either homologous or heterologous dimer of CD3, CD8, and CD28 [32]. However, the first-generation CAR-T-cells did not give satisfied outcomes due to insufficient production of cytokines.

Second generation

In the second generation, the CAR-T-cell dual signal has been used for T-cell activation. Three receptors are included in this generation; they are antigen receptor, cytokine receptor, and costimulatory receptor. The T-cell antigen receptor is present on the ectodomain where the first signal is received after the antigen presenting cells bonded with it. The costimulatory receptor is present in the endodomain which contains CD28/CD137/CD27/CD134 [33,34,85,101]. The CD137 can maintain and strengthen the production of the IL-2 cytokine to destroy the tumor cells [82].

Third Generation

In this generation, an extra signaling domain has been added to already existing second-generation CAR-T-cells. This signal domain is of OX40 or 41BB to increase the potential of the production of the cytokines [35]. This CAR-T-cell is predominantly used in the treatment of lymphoma and colon cancer [36,81].

Fourth generation

This CAR-T-cell has scFv in ectodomain, CD3- ζ in transmembrane domain, and in endodomain CD3- ζ , CD28, as a costimulatory and additionally has a modified inducible expression cassette for a transgenic protein-like cytokine is present. These are called T-cell redirected for universal cytokine (TRUCKS)-mediated killing. It activates T-cells and also activates and engages the innate immune cells to terminate the antigen-negative tumor cells. To engineer these TRUCKs, two transgenes require one for the CAR and the other for inducible cytokines. Therefore, the CAR-T-cells were additionally engineered with a nuclear factor of the activated T-cell-responsive expression cassette for the inducible expression of a transgenic cytokine, for example, IL-2. These TRUCK T-cells can also be used for treating viral infection, autoimmune diseases, and metabolic disorders [70,71].

Various antigens being targeted by CAR-T-cells

The CAR-T-cells are not only for treating CLL but also used for the treatment of various cancers.

Table 1 gives the information about the various cancers that can be treated by CARs [48-65,86-98].

Table 1: Cancer and their corresponding CAR-T-cell type

S. No.	Cancer type	Antigen to be targeted	Receptor type
1	Ovarian cancer	α -Folate receptor	scFv-Fc ϵ R1 γ
2	Acute lymphocytic leukemia	CD19	scFv-41BB-CD3- ζ
3	Renal cell carcinoma	CAIX	scFv-CD4-Fc ϵ R1 γ
4	Pancreatic adenocarcinoma	CD24	scFv-CD28-Fc ϵ R1 γ
5	Breast cancer	Erb-B2	scFv-CD28-CD3- ζ
6	Cervical cancer	CD44v7/8	scFv-CD28-CD3- ζ
7	Ovarian cancer	FBP	scFv-Fc ϵ R1 γ (alloantigen)
8	Hodgkin lymphoma	CD30	scFv-CD3- ζ
9	Neuroblastoma	G _{D2}	scFv-CD28-OX40-CD3- ζ
10	Prostate cancer, colon cancer	Erb-B2	scFv-Fc ϵ R1 γ
11	Lymphomas	CD20	scFv-CD28-CD3- ζ
12	B-cell malignancies	CD20	scFv-CD4-CD3- ζ
13	B-cell lymphomas	CD20	scFv-CD3- ζ
14	CLL	CD23	scFv-CD28-CD3- ζ
15	Epithelial cancer	α -Folate receptor	scFv-41BB-CD3- ζ
16	Non-Hodgkin lymphoma	CD38	scFv-41BB-CD3- ζ
17	Colorectal cancer	CEA	scFv-Fc ϵ R1 γ
18	Osteosarcoma	IL-13R α	scFv-CD28-CD3- ζ
19	Melanoma	HMW-MAA	scFv-CD3- ζ
20	Glioblastoma	IL-13R α 2	IL-13-CD3- ζ
21	AML	CD33	scFv-CD28-CD3- ζ

CLL: Chronic lymphocytic leukemia, scFv: Single-chain variable fragment, AML: Acute myeloid leukemia, HMW-MAA: High-molecular-weight melanoma-associated antigen, CAR-T: Chimeric antigen receptor T

CONCLUSION

The treatment for cancer using CAR-T-cells has given many promising outcomes. Not only CLL but also various cancers, viral disease and genetic disorders can also be cured without any side effects. The CAR-T-cells have been successfully used for treating all kinds of hematological cancers. The functionality of the CAR-T-cells can be effectively increased by modifying the domain of the CAR.

FUTURE PERSPECTIVES

The CAR-T-cells are "living drug," by manipulating their domain structures the applications can be broadened. These can be used for treating many genetic disorders and immunodeficiency disorders. The CAR-T-cells are more specific in nature when compared to chemotherapy and radiation therapy, and the side effects are also less when compared to others. Hence, the application of these cells can be used in various clinical trials for further development.

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AUTHORS' CONTRIBUTION

Both the authors have contributed equally for bringing this review article effectively.

CONFLICTS OF INTEREST

The authors do not have any conflicts of interest.

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