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Using Chimeric Antigen Receptor Modified T-Cell to Treat B-Cell Leukemias

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Abstract: Leukemias are cancers that happened in the blood of organisms, which affect the immune system's T cells and B cells. Therefore, investigating studies to cure this disease are crucial. Several researchers demonstrated the use of modified receptors of T cells to fight leukemia. CTL019 (tisagenlecleucel) is a type of therapy that transplants the CD19 receptors into T cells to help them recognize the CD19+ B cells. They are used to detect and kill the cancerous B cell leukemia cells. The chimeric antigen receptor-modified T-cell (CAR-T) is a method that extracts the blood cells from the patients to cultivate the CD19 receptors. Finally, the blood would be injected back into the patients. Even though this method effectively kills CD19+ B-cells, it may have some side effects, such as the quick release of cytokines that can cause high systemic effects. With these side effects, scientists should investigate a better way to reduce the side effects in the future.

Keywords: Lymphocytic Leukemias; Cytotoxic T-Cells; CAR-T; CTL019

1. Background

Leukemias are cancers that happen in a person's bone marrow. "According to estimation, 61090 people of all ages in the United States will be diagnosed with leukemia in 2021"(Cancer.net, 2021). Leukemia can be separated into two main types: lymphocytic leukemia and myeloid leukemia. Lymphocytic leukemias are caused by cancerous lymphocytes; myeloid leukemias are caused by cancerous myeloid cells. (Mayoclinic, Jan 13, 2021). There are two types of lymphocytic leukemia: acute lymphocytic leukemia and chronic lymphocytic leukemia. Acute lymphocytic leukemia is when leukemia cells in the bone marrow abnormally replicate without being controlled. They produce immature cells that block other healthy cells. Patients who have this disease may have symptoms such as gum bleeding, bone pain, fever, weakness, and frequent infections. (Mayoclinic, Feb 10, 2021) Chronic lymphocytic leukemia (CLL) also occurs in the bone marrow. CLL leukemias happen slower than other leukemias. They mostly happen in older adults, who cannot use bone marrow transplantation. The symptoms contain enlarged lymph nodes, fever, night sweat, fatigue, weight loss, and frequent infections. (Mayoclinic, 2019) The research question I have is how immunotherapy, especially CAR-T, works. How does CTL019 affect lymphocytic leukemia patients?

2. Cytotoxic T-cells

Cytotoxic T cells belong to the third defense line of the human body. They use cell-mediated responses to protect our bodies. They would target infected cells and kill them. (Humphrey, 2020)

2.1 Receptor Structure of T-cells

T cell receptors (TCR) are composed of 2 polypeptide chains. They are named as α & β, and sometimes γ & β. Both chains have sections called constant domains and variable domains. Their functions are similar to enzymes. When they bind to molecules, the variable sections would change the shape to bind to the antigen. T cells only have 1 binding site. (Humphrey, 2020) The T cell hinge regions that are composed of flexible amino acids link the peptide chain and the cell. They have a disulfide bond between them. (Adlersberg JB., 1976). There are also CD3 molecules in the cell. CD3s are used

for intracellular signaling. They can be phosphorylated within the cell and send the signal. (Wah, M., 2014) CD8 molecules are used as coreceptors of the cytotoxic cells. They are able to recognize MHC I molecules and bind to them. (Sharpe, 2015)

With these cytotoxic T cells, scientists are able to create modified cell-mediated immunity by using chimeric antigen receptor T-cells. They are generally called immunotherapy.

3. Chimeric Antigen Receptor T-cells

Immunotherapy for cancer was to use our immune system to fight against malignancies, which was different from chemotherapy. Scientists first used lymphocytes to treat leukemia. In the 1990s, Zelig Eshhar created the first chimeric antigen receptor for the treatment. Later on, many institutions - "Memorial Sloan Kettering Cancer Center , University of Pennsylvania and the Children's Hospital of Philadelphia , Fred Hutchinson Cancer Research Center , and the National Cancer Institute (NCI)" (Vairy, 2018)- started to investigate anti-CD19 therapies.

3.1 CAR-T Cell Therapy

Chimeric antigen receptor T cells (CAR-T therapy) is a therapy that modifies the patients' cytotoxic T-cell and uses it to kill cancer cells in patients' bodies. People genetically modify the lymphocytes to target certain diseases and inject them back to the patients. The cytotoxic T cells injected back to the body would target the infected cell to defend the body. One of the benefits of this treatment is that there wouldn't be much graft-versus-host disease (GVHD) because it uses the patient's own immune cells. (NCI Dictionary of Cancer TERMS)(Sharpe, 2015)

3.2 Structure of CARs

Chimeric antigen receptors are composed of three regions: antigen binding domain, a transmembrane domain, and an intracellular signaling domain. The antigen-binding domain would bind to the antigen, and they usually originate from antibodies. The intracellular domain would signal to the cell to perform the anti-tumor effect. The first-generation CARs only have CD3 as a signaling domain. However, in the second generation, coreceptors, such as CD28, were added to the cell too. The coreceptors would aid the cell to perform a stronger response. (Sharpe, 2015)

3.3 The Procedure of CAR-T Therapy

The patient's blood is extracted out to pass the apheresis machine that removes the white blood cells, including the cytotoxic cells. The rest of the blood would be sent back to patients. The cells that are extracted are modified by adding a certain virus inside. The modified cytotoxic T cells would express the receptors on their surface. Scientists would grow the cells in vitro. After the modification and cultivation, the T-cells are injected back to the body of the patients by infusion to enhance their immune system. (NCI Dictionary of Cancer TERMS)

4. CTL019

CTL019 uses CAR-T technology to apply the receptors on the T cells. They would target CD19 cells to treat leukemia. (Vairy S., 2018)

CD19 are proteins on the surface of normal B cells and leukemia B cells, but they are not on other normal cells' surfaces, which reduce the destruction of the cytotoxic T cells. It is acceptable to fight the tumor by killing the normal B cells. (Vairy S., 2018)

4.1 Structure

The basic CAR-T structure has "an intracellular T-cell activation domain, an extracellular hinge region, a transmembrane domain, and an extracellular antigen-recognition moiety" (Vairy S., 2018) The second generation of CAR-T has a costimulatory domain. CD 28 and CD137 (4-IBB) raise the production of cytokine, proliferation of cytotoxic cells, and persistence of the CAR-T cells. CTL019 has CD8-α as the hinge part of the cell, sc-Fv (anti-CD19) as the recognition section, CD137 as the costimulatory domain, and CD3ζ as the signaling domain. (Vairy S., 2018)

4.2 Mechanism

The 4-IBB (CD137) binds to TNF(tumor necrosis factor)-receptor-associated factor 2 (TRAF2). It sends signals to NF-kB via proteins p38 mitogen-activated protein kinase (MAPK) and c-Jun N-terminal kinase (JNK). 4-IBB also raises the anti-apoptotic factors, stimulates the proliferation of T cells and cytokine secretions, provides more cytolytic potential (power to destroy cells), and elongates the cell's lifetime. Intracellular signals phosphorylate CD3ζ, interact with the TCR, and activate the MAPK molecule. With more cytotoxic T cells, the T cells can easily detect the CD19 cells. Then, they will bind to it and release the cytotoxin to kill the cell. (Vairy S., 2018)

4.3 Side Effects

CAR-T cells cause toxicity which damages normal cells that have that antigen. Cytokine-release syndrome and neurotoxicity can occur. Patients may also have other symptoms like fever, hypogammaglobulinemia, viral infection, loss of weight, diarrhea, and vomiting. Scientists are trying to investigate the best way that reduces the disadvantages of CTL019 and improve the antitumor effect. Doctors should track the adverse reactions, determine what grade the patient was at, and use a reliable treatment to reduce the adverse reaction. (Vairy S., 2018)

4.4 Cytokine Release Syndrome (CRS)

CRS is an inflammatory reaction that was caused by the raised level of cytokines. It was caused when T-cells proliferate massively in the body. It causes patients to experience symptoms like high fever, fatigue, malaise, and capillary leak. It mostly happens in patients who received infusions within 14 weeks. Interleukin-6 (IL6) was used to produce the C-reactive proteins, which mediates the CRS effect, but it causes other cytokines to increase, such as IL-10, IL-5, and interferon-gamma. (Vairy, 2018)

4.5 Treatments

Tocilizumab (Atlizumab [Chugai Pharmaceutical Co., Tokyo, Japan], Actemra [Hoffmann-La Roche, Basel, Switzerland]) is a humanized monoclonal antibody against the IL-6 receptor (IL-6R). It binds to IL-6 to prevent it from signaling. However, it may increase the level of IL-6 as it binds to it, causing neurotoxicity in the central nervous system. (Vairy S., 2018) "Siltuximab (CNTO 328, Sylvan; Janssen Pharmaceutica, Beerse, Belgium) is a human murine chimeric monoclonal antibody against IL-6." (Vairy S., 2018) It causes many patients to have rapid reversal symptoms. Corticosteroids are useful for CRS, CRES, HLH/MAS, but it inhibits the function of T-cells and causes T-cell apoptosis. (Vairy S., 2018)

5. Clinical Examples

5.1 Acute Lymphocytic Leukemia

According to the journal from Grupp, S. and his team, two children with relapsed and refractory pre-B-cell ALL had experienced CAR-T therapy. Both of them had experienced two relapses. Patient 1, a 7-year-old girl, used chemotherapy, but her cancer recurred, and further chemotherapy didn't work. Patient 2, a 10-year-old girl, received cord blood transplantation from a stranger, but she GVHD after transplantation. She didn't receive any immunosuppressive therapy, and the target antibody treatment didn't work for her. Finally, they used CTL019. The CTL019 quantity expanded 1000 times in their bodies. The cells were found in the bone marrow and had lasted in the cerebrospinal fluid for more than 6 months. However, both of them experienced fever after the infusion, which was probably caused by the cytokine-release syndrome. Patient 1 experienced a high fever on day 4, and she was transferred to the pediatric intensive care unit. 2 weeks later, both patients' lymphocytes had increased. Most of their T cells had expressed the CARs. About 1 month later, morphologic remission of ALL in both children was achieved. The remission in patient 1 was associated with molecular remission that lasted 9 months. Unfortunately, patient 2 had a relapse about 2 months later. (Grupp, S., 2013)

5.3 Chronic Lymphocytic Leukemia

According to the journal that was published by Kalos, M in 20,16, Science Translational Medicine, Porter, et al. presented the data of 14 CLL patients treated at the University of Pennsylvania on September 2, 2016, some patients' clinical activity experienced logarithmic expansion, contraction, and long-term persistence in T cells, with side effects like cytokine-release syndrome. The final result was that the immunotherapy was viable for treating CLL. 3 CLL patients were in late-stage, and they had a strong anti-tumor response after the CTL019 treatment. Their long-lasting T cells expanded a lot in vivo. The engineered T cells mediate the profound activity in CLL patients. However, ⅓ of the patients didn't respond to the therapy. CTL019 is able to mediate complete molecular remission of CLL patients. CLL response to CTL019 is lower than ALL's response, but the expansion of cells is still effective in treating CLL. (Kalos, M., 2016)

6. Conclusion

CAR-T, as a new technology, opens the door to using immunological cell-mediated therapies to treat diseases. It allows scientists to modify the cell's genes, proliferate the cells, and target special infections with great efficacy. It is safer since the patients wouldn't receive the GVHD. A special type of therapy that uses CAR-T, CTL019, has been created to treat lymphocytic leukemias. It functioned well in some patients to proliferate their cytotoxic T cells, but some patients, especially those who had CLL, didn't respond to it. This therapy still needs to be optimized in later research.

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