CAR T-Cell Therapy—A Review

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Introduction

According to experts, the mainstay of cancer treatment has been composed of four pillars: Surgery, radiotherapy, chemotherapy, and targeted drug therapies, such as tyrosine kinase inhibitors and HER2/neu receptor antibodies.[1] Recently, new research in immunotherapy has created what many are calling the "fifth pillar."[2] Immunotherapy is a treatment modality that uses the body's immune system to fight disease.[3] One of the building blocks of this pillar is adoptive T-cell therapy (ACT), which is a method of immunotherapy that isolates tumor-specific T-cells from a patient and expands them *in vitro*. These T-cells are then reinfused into the host with the intent of mediating tumor destruction.[4]

Chimeric Antigen Receptors

Although there are several forms of ACT, such as the expansion of tumor-infiltrating lymphocytes (TILs) or genetic engineering of peripheral blood lymphocytes (PBLs) to target tumors, it is the development of chimeric antigen receptors (CARs) that have generated great interest as potential game-changers for treating refractory or relapsed cancers.[5] Unlike TIL treatment, which relies on isolating T-cells from cancer biopsies and amplifying them *ex vivo*, a procedure both labor-intensive and time-consuming, CAR therapy is based on genetically engineering T-cells to target specific tumors.[6] A less rigorous approach, CAR therapy is also advantageous because it has the potential to target a greater variety of cancer cells than other therapies, which are bound by several restrictions.[7]

CAR T-Cells: MHC Independent Recognition, Antibody-Mediated Targeting

One such restriction is the MHC restrictions of normal T-lymphocytes, which requires foreign antigens, such as a bacteria or virus, to be presented on the T-cell receptor by proteins called the major histocompatibility complex, or MHC. Only if the peptide-MHC complex is presented will T-cells activate and carry out their functions.[8]

In terms of treating cancer, MHC restriction poses many complications for therapies which rely on natural T-cell receptors (TCR). Firstly, tumor cells must express adequate MHC.[7] According to researchers, cancer cells can downregulate or completely shut-off their expression of MHC class I. This is a subtype of MHC which displays peptide fragments to T-cells to induce an immune response, and helps cancer cells evade detection from the host immune system.[9] Also, so far, there are a limited number of peptide-MHC complexes identified for tumor cells, which narrows the therapeutic range for these types of treatments.[7]

On the other hand, CAR T-cell therapy bypasses the MHC class restriction altogether by modifying T-cells with an extracellular tumor-specific antibody fragment.[4] In this way, T-cells are directed by cell surface antigens rather than peptide-MHC complexes.[6] These antigens can

include peptides, carbohydrates, phospholipids, and inorganic compounds of different composition and structure.[7] This makes CARs more universally applicable to targeting cancer cells, as they have a much wider variety of potential targets.[7] They can potentially target every surface molecule on a tumor cell, even MHC presented antigens.[7]

Structural Advantages of CAR T-Cells

Aside from the benefits of MHC-independent signaling, CARs possess other unique advantages over natural T-cells. CAR T-cells are hybrid receptors in that they combine both antigen-binding and T-cell activating functions into one receptor.[8] The extracellular portion of a CAR is an antigen recognition domain and its intracellular portion is a T-cell signaling domain, which activates T-cells upon binding of an antigen.[6] Both the antibody domain and the T-cell signaling domain are synthesized to enhance CAR ability and function. For example, the antibody domain is modified to increase its binding affinity to surface antigens up to 20-fold greater than that of TCR affinities for the peptide-MHC complex.[7]

The unique aspect of the CAR T-cell signaling domain is the fact that it has a built-in costimulatory molecule. This is important because, without costimulatory signaling, T-cells cannot function properly. In fact, tumor cells can induce antigen-specific tolerance or anergy without certain costimulatory signaling domains.[10] Moreover, studies have shown that costimulatory signaling in CARs can enhance cytokine production and T-cell proliferation in response to tumor-associated antigen (TAA) compared to CARs without costimulatory signaling.[10] Researchers have even created CARs with two costimulatory molecules, which have shown greater activation and proliferation compared to those with one.[11]

Furthermore, because CARs are synthetic receptors, scientists can engineer them to target antigens in a very specific way.[8] This eliminates the problem of cross-reactivity of endogenous antigens that normal TCR can sometimes exhibit, and which some researchers believe is a causative factor in autoimmune disease.[12]

Synthesis and Implementation of CAR T-Cells

T-cells used in the production of CARs are harvested from the blood of a donor or a patient. After designing the CAR, it is introduced permanently into T-cells using a viral vector.[13] Usually, this includes γ-retrovirus or lentivirus, both of which are replication-defective retroviruses. After T-cell transduction, the RNA genome of both viruses is reverse-transcribed into DNA, which integrates into the host genome. Additionally, since viral vectors can potentially be oncogenic and require careful testing and extensive monitoring, non-viral gene transfer systems are under investigation as an alternative CAR delivery system.[13] Moreover, these systems are easier to manufacture, potentially safer, and have lower costs.[13]

After the CAR gene insertion, T-cells are expanded in culture for several days to weeks. The goal is to inject enough cells back into the patient to produce a sufficient antitumor response. Usually, this is around 10^{11} cells and it takes approximately 7 to 14 days to reach.[13]

To date, CAR T-cells consist of the more differentiated, heterogenous PBC population from patients or donors. Therefore, the final product usually consists of cells that have characteristics similar to the intrinsic attributes of the individual donors circulating PBCs. However, researchers have found that the less differentiated, or naïve, cell populations may have increased persistence and proliferative capacity *in vivo*, and better survival after transfer back into a patient, compared to the more differentiated ones. Yet, the question about which cell line is best for adoptive T-cell transfer is still an ongoing topic of research.[13]

CAR T-Cell Therapy Clinical Trials

As of 2016, there were 220 CAR T-cell therapy trials completed, 188 ongoing, and nine requiring long-term follow-up.[14] Mostly trials are evaluating safety profiles and dosage, however, there are several phase 2 trials assessing the efficacy of treatment, as well. However, the breakthrough CAR trial, which many believe will launch CAR T-cell therapy into the mainstream of cancer treatment, used CD19-specific CAR T-cells against B-cell malignancies.[13,14] It showed partial or complete response for single individuals, as well as, the majority of patients in several trials.[14]

Subsequently, the most frequently targeted tumor type for CAR T-cell trials today are hematological and lymphoid malignancies, such as acute lymphoblastic leukemia (ALL) and non-Hodgkin's lymphoma (NHL). The most targeted antigen under investigation is CD19, followed by CD22, CD30, Ig k, and Lewis-Y. Solid tumors have displayed fewer encouraging results in terms of treatment efficacy, however, several antigens are under trial for these types of cancer, as well. For instance, CEA is being investigated as an antigen for breast cancer, colorectal cancer, and gastric cancer. Other trials are targeting ErbB2/Her2, GD2, and GPC3, for breast cancer, neuroblastoma or sarcoma, and hepatocellular carcinoma, respectively.[14]

In terms of therapeutic success against B-cell malignancies, it seems that tumors in this cell line selectively and homogenously express CD19 and CD20, and CAR T-cells have easier access to these antigens. In five trials evaluating CAR T-cell therapy for ALL and NHL, 85% of patients reached complete response of tumor burden. These included patients with varying grades of detectable disease inside the bone marrow, in extramedullary sites, or cerebrospinal fluid.[14] Moreover, those patients with low disease burden after treatment, especially in ALL, lived significantly longer.[14]

Overall, CAR T-cell therapy is extremely variable and complex. Many factors such as disease burden, CAR construct and design, production and amplification of CARs, and administration doses can vary between trials and severely affect results. In this way, the focus of many future studies is to identify the most relevant parameters for positive clinical outcomes.[14]

Toxicities of CAR T-Cell Therapy

CAR T-cell therapy is not without drawbacks. Given the extreme potency of this treatment, it is no surprise that it has significant toxicity potential.[5] These toxicities can include life-threatening

conditions such as cytokine release syndrome (CRS) or macrophage activation syndromes (MAS) to “on-target, off-tumor” toxicity, neurotoxicity, tumor lysis syndrome (TLS), and anaphylaxis/allergy.[5,15]

CRS is the most prevalent adverse effect of CAR T-cell therapy. The symptoms of CRS include fever, fatigue, myalgia, hypotension, tachycardia, nausea, capillary leak, and cardiac, renal, or hepatic dysfunction.[15] Moreover, increase cytokines, such as interferon-gamma, granulocyte-macrophage colony-stimulating factor, IL-10, and IL-6, are the hallmark of the syndrome. Research has shown that, in regards to hematological malignancies, those patients who respond to treatment with CAR T-cells show at least mild CRS. Furthermore, patients with higher tumor burden at the time of T-cell infusion exhibit a more severe form of CRS. Treatment of CRS aims to control symptoms without affecting antitumor efficacy of the infused T-cells.[15]

Other toxicities, such as neurologic toxicities occur in patients receiving CD19-specific CAR T-cells, as well. However, it is unclear as to whether it may occur with the targeting of other tumor-associated antigens. The symptoms include confusion, delirium, expressive aphasia, and even seizure. The cause is unknown, and to date, cases have been reversible. There is also "on-target/off-tumor" toxicity, which occurs when CAR T-cells target normal tissue that shares the same antigen as the target antigen. Usually, the cause is reversible, but in some cases, this toxicity has led to death.[15]

Conclusion

The first CAR T-cell products have entered commercial markets, however, treatment outcomes for all patients cannot be assumed.[16] Several changes to CAR T-cell design and implementation have been proposed to combat adverse effects and increase specificity and control of CAR T-cells. One study is investigating the drug rimiducid as a costimulatory molecule. Instead of an antigen alone acting as an activation switch, the separate drug does as well, leading to better control of CAR T-cells.[17] Additionally, a safety switch, or suicide gene, has been proposed that would selectively deplete genetically modified cell populations in the case of an emergency.[15]

CAR T-cell therapy has implications even beyond cancer treatment. Due to their broad targeting specificity, the range of their therapeutic potential is extremely wide. One recent study has proposed CAR T-cell therapy in treating multiple sclerosis (MS). In this study, scientists used CARs to target myelin oligodendrocyte glycoprotein (MOG) in mice. MOG is involved in axon myelination in the central nervous system. Results showed encouraging signs of disease suppression and a decrease in symptoms.[17] CARs have also been considered in other diseases such as inflammatory bowel disease, HIV, and pemphigus Vulgaris.[17]

In terms of commercial use for cancer therapy, CAR T-cell success will depend on sustained treatment responses and manageable toxicity profile.[14,16] As an experimental treatment, there are several unknown variables, such as appropriate dosage to elicit a complete response, defining the optimal target antigens, or efficacy beyond hematological malignancies. These and

other questions add to the risk profile of CAR T-cell therapy.[14] However, the advantage of engineered cells is that their programming can be debugged and improved as often as necessary, to create a more safe, stable, and precise treatment for patients.[18]

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