A Review on Chimeric Antigen Receptor Therapy

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Abstract

Chimeric antigen receptor (CAR) has emerged as a very promising technology for the treatment of leukemia, lymphoma, and myeloma. It utilizes the functionality of T cells and the individuality of the affected cells to target the attack. Cancer cells have developed various adaptations to deceive the immune system of the body. Some of them include release of immunosuppressant by tumor microenvironment, expression of weakly immunogenic antigens, and down regulation of the manifestation of antigens. For this reason, the immune cells fail to recognize the cancer cells and perform the cytotoxic action. CAR can play an important role in the treatment of cancer. It is a genetically engineered molecule. Moreover, when infused with T cells, it directs them to recognize specific antigens that are presented on cancer cells. This therapy has shown encouraging results in its clinical trials for chronic lymphocytic leukemia (CLL), acute lymphocytic leukemia (ALL), and non-Hodgkin’s lymphoma (NHL). In this review, we focus on the structure of CAR and its functioning. In addition, we focus on its after and side effects. Along with this, we briefly about the application of CAR for multiple myeloma and acute myelogenous leukemia. This review briefs about the challenges faced by this incipient technology as well. We conclude with its future aspects.

Keywords: Acute lymphocytic leukemia; Chimeric antigen receptors; Chronic lymphocytic leukemia; Genetic engineering; Myeloma; Non-Hodgkin’s lymphoma

Introduction

In human body, all the mature blood cells (red blood cells, white blood cells, macrophages, granulocytes, and dendritic cells) arise from a single cell type, the hematopoietic stem cell (HSC). The HSCs are self-renewable cells that differentiate into different lineages through the process of hematopoiesis [1]. The two lineages in which HSCs divide are provided in the following:

1. Common myeloid progenitor (CMP), which includes red blood cells, macrophages, granulocytes, and macrophages.
2. Common lymphoid progenitor (CLP), which produces B lymphocytes, T lymphocytes, and natural killer (NK) cells.

After differentiation, HSCs loses its ability to renew itself. Myeloid cells and NK cells are a part of innate immunity; whereas, lymphocytes are a part of adaptive immunity. The response imparted by B cells is known as humoral immunity, and the response by T cells is known as cell-mediated immunity. During immune surveillance of the body, all those molecules that are identified as nonself (antigens) are destroyed. These antigens are presented by foreign as well as host cells. The tumor specific antigens (TSA) are recognized as nonself and are eliminated subsequently. However, the escaping tactics of tumor cells helps them to grow in the body. Few of them include weakly immunogenic antigens or down regulation of the expression of antigen so as to escape the immune system. Along with this, tumor cells synthesize various immune suppressants that inhibit the functioning of immune cells [2].

Leukemia, lymphomas, and myelomas are a class of tumors that are associated with hematopoietic cells. Leukemia is the abnormal proliferation of white blood cells and is broadly classified as acute and chronic leukemia. The leukemia of less mature cells is termed as acute, while the chronic leukemia is of mature cells. The acute leukemia include acute lymphocytic leukemia (ALL) and acute myelogenous leukemia (AML). The chronic leukemia includes chronic lymphocytic leukemia (CLL) and chronic myelogenous leukemia (CML). With recent developments in the field of immunology, novel technologies that utilize the cells of immune system and calibrate them to the level that is optimum for tumor cell eradication have been developed. The CAR technology has been developed by fusing the antibody binding domain with T cell activation domains. These molecules work independently of major histocompatibility complex (MHC) restriction and have also proved successful in its clinical trials. The encouraging results thus obtained from CAR are a proof that this technology has the potential to replace all the conventional methods of cancer treatment. However, this technology emerges with various side effects. Some of them include cytokine release syndrome (CRS) and tumor lysis syndrome (TLS) along with fever, nausea, constipation, and diarrhea. Treating cancer without any side effects is the major challenge faced by these therapies. In addition, the autologous nature of the therapy possesses a limiting factor to it. The patient’s own T cells are to be utilized for the purpose of this therapy, which is very costly and time taking. Universal chimeric antigen receptor (UCAR) is a solution for the problem where T cell receptor and CD52 are removed with transcription activator-like efficient nuclease (TALEN) technology. UCARs thus developed show the same antitumor activity as shown by the normal CAR cells. This incipient technology needs to be developed further to suppress its side effects.

Chimeric Antigen Receptors

Approximately 25 years ago, the concept of CAR developed so as to add additional benefit of target specificity to adaptive T cell therapy. This chimeric antigen receptor (CAR) is a molecule that when bound to T cell increases its ability to recognize the antigens present on the surface of other cells. Along with antigen recognition domains, CARs even have T cell activation domains [2]. The antibody domain is designed such that it binds to the CD-19 protein sequence of antigens.
These CD-19 sequences are specific to B cells malignant or normal, and are not present on bone marrow stem cells. Thus, CD-19 is made target for binding and is used for the treatment of diseases such as ALL, CLL, and non-Hodgkin's lymphoma (NHL), where the immune B cells themselves become cancerous [3].

The structure of CAR has undergone several changes since its genesis. The first generation CARs were the chimera of single chain variable fragments (scFv), derived from monoclonal antibodies and CD3-ζ chain. This N terminal scFv directs the T cells to the cancer antigen in an MHC independent manner, that is, the antigen does not need to be processed for the recognition to happen [2,4]. The CD3-ζ chain, which is the C-terminal endodomain of the CAR, transmits the activation signals [4]. However, owing to poor in vivo persistence ability of the first generation CAR, the addition of co-stimulatory molecule of CD137 (4-1BB) was performed. The new molecule has been shown to increase the persistence time and proliferation capacity of T cell and rapid antitumor activity. This new CAR with CD137 co-stimulatory domain is termed as second generation CAR. Further development in this technology leads to the formation of two co-stimulatory domains, a combination of CD27, CD28, 4-1BB, ICOS, or OX40, which are termed as third generation CARs [3]. CAR therapy resembles with autologous bone marrow transplantation. The patient's blood sample is taken, and the T cells are separated from rest of the blood by leukapheresis. After this, a gene which encodes the protein for the recognition of the CD19 antigen of B cell is transferred to the cell with help of a lentivirus vector [5]. Retrovirus was not selected for the purpose of gene transfer because of the risk of insertional mutagenesis. Lentivirus proves to be a more promising tool for genetic engineering of T cells [6]. Moreover, various stimulating molecules are added for the proliferation of modified cells. When the cells are grown in sufficient amount, they are transferred back to the patient. Hence, these cells came to be known as CAR T-CD19 cells [5].

The presence of CD19 on normal cells is associated with depletion in the number of normal B cells, and it has been observed in many cases. This is called as B cell aplasia, and the activity is known as on-target off-tumor toxicity [3]. To provide a solution for this purpose, an additional feature that has been added to CAR is the “suicide switches,” which eliminates the T cells that have been overly expressed. This suicide gene is iC9. This gene encodes for an altered caspase 9, having a trimmed caspase recruitment domain (CARD). FK506, which is the drug binding domain, is also present in this gene in a mutated form. The injection of AP1903 molecule dimerizes caspase9, which activates the apoptotic mechanism of the cell. This iCasp9 proved more successful than herpes simplex virus-thymidine kinase (HSV-TK) suicide gene system, because it is less immunogenic than the latter. In addition, its functioning is much more rapid than other suicide inducing system, as it induces the apoptotic system of the cell and does not alter the DNA replication mechanism [7,8].

Figure 1: Structure of CAR [3]

Figure 2: Procedure for CAR therapy [5]
This CAR technology has been even applied for the treatment of multiple myeloma. However, different from CD19, the targets here are CD138, CD38, and CD56. Precisely similar to CD19 CAR T cells, the on-tumor off-target toxicity was shown by these myeloma targeted CAR cells as well. In fact, the toxicity level of myeloma CAR T cells was even worse, as these antigen targets were present on the other important cell lineages as well. Similar to CD138, which is present on bronchial epithelia, CD38 is present on HSCs, NK cells, dendritic cells, prostate, and pancreas islet cells, and CD56 is present on neurons and NK cells. Research is being going on to determine the potential antigen targets to increase the specificity and reduce the toxicity. The application of CAR technology for AML is also under study, where the targets are CD33 and CD123. Among these two, CD123 is found to be more efficient than CD33 because of its limited expression on hematopoietic progenitors, good proliferation rate, and apoptosis resistance quality. Moreover, its interaction with normal monocytes and endothelial cells expressing low CD123 is less. The inclusion of suicide gene (iCasp9) in CARs for myeloma and AML is under testing as well [9].

On an average, it was found that each infused CAR T cell and their progeny eliminated approximately 1000 leukemia cells in vivo in patients with advanced chemotherapy resistant CLL. CAR T19 cells showed encouraging growth and were reported to be present in blood and bone marrow serum for at least 6 months; apart from this, they also maintained their anti-CD19 effector function along with a memory phenotype. Persistence of CAR T cells is proof of absent humoral and cellular immune response against them [6]. The rapidity provided by CAR therapy is one of the main advantages [10]. Moreover, CAR recognizes the tumor antigens without any human leukocyte antigen (HLA) restriction. It shows that processing and presentation of antigen on MHC is not required. Therefore, CAR can be constructed for tumors expressing many histological characteristics [2,11].
After Effects

Through quantitative polymerase chain reaction (qPCR) assay, it was observed that CAR T19 cells expanded and persisted in the blood of patients for at least 6 months. Precisely, it increased to approximately 1000-10,000 folds during the first month after the inclusion [6]. It is to be noted that the level of proliferation of CAR T19 cells depends greatly from patient to patient. Blood and bone marrow serums were collected and were batch analyzed to quantitatively determine the cytokine levels. It was found that a collection of cytokines, chemokine, and other soluble factors had increased the level in serum from baseline. This increase in level was essential to substantiate the functioning of CAR T19 cell. Few members of that collection include four cytokines (IL-6, IFN-γ, IL-8, and IL-10), five chemokines [macrophage inflammatory protein-1α (MIP-1α), MIP-1β, monocyte chemotactic peptide-1 (MCP-1), CXC chemokine ligand 9 (CXCL9), and CXCL10], and soluble receptors IL-1Rα and IL-2Rα. IFN-γ had the largest deviation from the baseline [6,11]. Owing to the presence of CD19 on normal cells, depletion in the levels of B cells was observed. Moreover, except for B cells, the levels of neutrophils, erythrocytes, T cells, and NK cells recovered to the normal levels in few weeks after the infusion. When measured by flow cytometry and immunohistochemistry, the absence of CD19+ cells was observed. After 14 weeks of treatment, the CD79a+ cells were undetectable in bone marrow. However, their count was soon detected after 36 weeks of inclusion. CD79 was expressed in B cells even before CD19 suggesting that B cell lineages can be recovered soon after the inclusion. Talking precisely about immunoglobulins, IgG and IgA decreased in serum. The level of IgA went down to a significant level. Apart from this, the level of IgM was below the level of detection from 9 to 36 weeks after the inclusion [12].

Side Effects on Patients

Identifying for an antigen that is present only on malignant cells and not on normal cells is a major challenge. As CD19 is present on normal cells as well, this treatment shows on-target off-tumor activity. During clinical trials, genetically modified T cells have shown reactivity toward those cells that expressed antigens even at low levels. Owing to this, there was continuous depletion in the normal B cells. A severe toxicity associated with this therapy is CRS, which is an on-target, on-tumor toxicity and happens because of sudden increase in T cell proliferation. Increase in T cell proliferation increases the cytokines released. Cytokines such as IFN-γ and IL-6 are released in high amount. IFN-γ is a cytokine that is produced by cytotoxic T cells, NK cells, and T helper cells. Functions such as macrophage activation, major histocompatibility complex induction, and T[α] differentiation is performed by IFN-γ. Any deviation in the amount of its release directly affects the efficacy of CAR T cells. Another cytokine, IL-6, was found at elevated levels along with IFN-γ, as high as 160 times the baseline levels [11]. IL-6 is produced by macrophages, dendritic cells, T cells, fibroblasts, endothelial cells, keratinocytes, adipocytes, mesangial cells, and osteoblasts. To control the level of IL-6, tocilizumab, which is an IL-6 receptor inhibitor, was used. This drug proved successful in clinical trials, as it did not affect the efficacy of the therapy [3].

TLS is another on-target, on tumor toxicity, which is a result of CAR T cell therapy. It is defined as the lysis of cancer cells [11]. It can occur naturally or in response to any therapy. The lysis of cancer cells results in release of potassium, phosphorus, nucleic acids, and cytokines more than the handling capacity of body. The severity of this toxicity depends on cancer mass, overall health of patient, and the medication received. Renal inefficiency is the direct effect of TLS. Owing to the increased potassium levels, patients are at risk of cardiac dysrhythmia and are advised to maintain their phosphorus and potassium intake low. Moreover, the condition of hypocalcemia, which can lead to dysrhythmias and neuromuscular irritability, is also observed [13].

Apart from above, the short-term effects such as fever, chills, diaphoresis, myalgia, headache, fatigue, nausea, thrombocytopenia, neutropenia, and lymphopenia were observed [11].

Challenges Faced

Cancer cells perform their best to survive. They develop various tricks to sustain themselves. One of them being secretion of Treg cells, which inhibit the functioning of immune cells. The current research focuses on increasing the effect of T cells, while depleting the level of regulatory T cells so as to enhance the anti-tumor activity. Modifying the T cells to secrete IL-12 can increase the resistance to Treg [2]. In addition, the expression of few antigens on both normal and malignant cells is a challenge as well. For example, HER2, a kind of biomarker, which is also present on heart cells and bronchial epithelial cells, was targeted with anti-HER2 CAR. Binding of CAR T cells to HER2 resulted in respiratory distress and multiorgan failure, ultimately leading to death because of “cytokine storm” [8,10].

During anti-CD19 CAR T cell therapy for ALL, it was observed that cancer cells down regulate the expression of targeted antigens. These antigens are tumor specific. Hence, these glycoproteins are becoming attractive targets for immunotherapies. However, in many cases, cancer cells keep on altering the glycoproteins they display by increasing their production or by changing the branching of glycans structure. Cancer cells displaying mutated epitopes are being targeted in clinical trials [2].

A major limitation of CAR T cell therapy is that it is autologous. This means, that the T cells that are to be used for the treatment should be of that specific patient only and not of anyone else, otherwise it will trigger graft-versus-host reaction. This raises a problem as there might be few patients, whose T cells have poor proliferation ability and functionality [10]. Hence, this procedure requires considerable time, trained clinicians as well as money, as the production of patient specific drug will be expensive [3].

Future Prospects

It is clear from above that CAR therapy, although very effective, has many side effects that should be corrected. The side effects are sometimes so severe that they prove to be lethal. Hence, the changes in therapy should be performed such that the efficacy is increased and toxicity is decreased. Reduction in inflammatory cytokine level is the major target, as the level of cytokines in blood serum is directly related to toxicity [6]. In addition, the optimum amount of signaling domain that is to be included in CAR is still unknown [14]. The calibration of the optimum amount of signaling domain is important so as to increase the proliferation of CAR T cells in vivo without any hindrance of regulatory T cells. Apart from this, the autologous nature of this therapy, as mentioned above, is problematic and is a major domain of concern. To eradicate this, the UCAR has been introduced. At Cellectis, which is a bioengineering company in Paris, scientists have restricted the expression of T cell receptors (TCR) and CD52. CD52, which is a glycosylphosphatidylinositol, is present on lymphocytes. With the use of TALEN system, which is a very precise genome editing method, TCR and CD52 have been eliminated from the T cell, hence removing the chances for graft-versus-host reaction. This UCAR method is under clinical trials and has provided successful results so far [15].
CAR for the treatment of solid tumors is also under investigation [10]. For this purpose, the detection of appropriate tumor markers should be extensively studied. Tumor markers such as carcinoembryonic antigen (CEA), C-reactive protein (CRP), and alpha-1 antitrypsin (A1AT) have gained attention because of their relation with esophagus cancer. CEA is a polysaccharide protein complex that functions as a cell adhesive. It facilitates metastasis of circulating tumor cells in liver and has some structural similarity with immunoglobulins and hence has some immunoregulatory functions such as suppression of T cell activity, inhibition of NK cell cytolyis, and inhibition of T-B cell cooperation. This CEA has been found at the elevated levels in heavy smokers. It was observed that the increase in the CEA levels resulted in increased tumor size. Owing to its metastasis activity in liver, it is used for predicting clinical response in gastric cancer patients. Along with CEA, another marker such as CA19-9 or CA50 can also be used for the detection of gastric cancer, as they improve the sensitivity of CEA [16,17]. Another work going on is for the prostate cancer in which the levels of prostate specific cancer (PSA) were analyzed and were found to have elevated levels and were directly related to serum glucose, total cholesterol, triglycerides (TGs), low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-C), and BMI. Marking PSA as tumor marker can be another potential method to target prostate cancer [18].

**Conclusion**

CAR therapy is a very effective therapy for the treatment of malignancies such as CLL, ALL, and NHL. It utilizes the presence of specific surface markers called cluster of differentiation to identify the target and function accordingly. This technology is in its budding stage and hence is faced by many challenges. Owing to the presence of CDs on other than target cell, the on-target off-tumor reactivity is observed. In addition, the activation of inflammatory cytokines results in side effects such as fever, nausea, and diarrhoea. The intensity of side effect is different for different patients, depending upon the initial health. The number of cancer cases reported is increasing per year, and there is a need for a reliable treatment that is cost effective and is also free of long-term side effects. CAR therapy has the potential to replace the present treatments to yield better results.

**References**