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Clinical Implications of Anti-CD19 Chimeric Antigen Receptors (CAR) T Cell Therapy in Acute Lymphoblastic Leukemia (ALL)

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Abstract

Acute Lymphoblastic Leukemia (ALL) is a cancer of white blood cells that is most prevalent among young children. Several environmental and genetic factors contribute to the occurrence of ALL, including radiation exposure, ethnicity, gender, and other genetic traits. Traditional treatment options include chemotherapy, radiation, and bone marrow transplant. More recently, a form of antibody-mediated immunotherapy using genetically engineered chimeric antigen receptor (CAR) T-cells has been used in several clinical trials with encouraging results. CAR T-cells allow for highly targeted treatment by inducing activation of the modified T-cells and stimulating an apoptotic response in target B-cells upon ligation with a given antigen target. Typically, modified CAR T-cells are capable of specific recognition of the B-cell surface marker CD19, a universally expressed oncogenic antigen found in many forms of B-cell acute lymphoblastic lymphoma. Because CD19 is not expressed in hematopoietic cells, successful CD19-targeted treatment would eradicate cancerous cells without damaging the hematopoietic system. Hematopoietic cells can therefore regenerate normal CD19+ B-cells following treatment, as these are also depleted by anti-CD19 CAR T-cells. With limited toxicities and significant efficacy, CD19-targeted CAR T-cell immunotherapy is a promising treatment approach for ALL.

Keywords: Receptors; Chemotherapy; Leukemia; Immunotherapy; Signaling pathways

Introduction

Acute Lymphoblastic Leukemia (ALL) is a hematological malignancy that originates in the immature lymphoid cells of the bone marrow called lymphoblasts. The disease is associated with arrest in lymphocyte maturation of the clonal cells, and the induction of proliferation and accumulation of these cells in the blood and bone marrow, thus producing hematopoietic failure. ALL has a sudden onset and can progress quickly, and a number of environmental and genetic risk factors are associated with its development. The malignancy can be categorized into several subtypes, distinguished by the type of lymphocytes affected by the disease (B-cells, T-cells, or rarely, NK cells) as well as the stage of maturation of affected cells. Several treatment modalities exist, and the optimal approach is determined by taking into consideration the subtype as well as certain prognostic factors including age, initial white blood cell count, cytogenetics, molecular features, and response to initial treatment [1].

Radiation as a Risk Factor

Pre-B cell Acute Lymphoblastic Leukemia is principally found in young children, with the highest prevalence occurring in children between the ages of two and five, although half of all new cases are identified in adults [2]. Multiple factors may be responsible for the predominantly young demographic affected by this disease, including a higher sensitivity to radiation exposure, one of the risk factors of ALL. While no mechanistic relationship between radiation and ALL has been found, an increased risk of developing leukemia has been shown to occur in individuals who have been exposed to radiation. Factors that can affect severity include amount of exposure to radiation, age at which exposure occurred, and other environmental factors such as carcinogens [3]. Preconception irradiation and in utero irradiation may also be risk factors [4].

Genetics and Inheritance

In addition to radiation, certain genetic characteristics may cause an increased risk of developing ALL. Children with Down Syndrome, a chromosome 21 trisomy, are 10 to 20 times more likely to develop ALL when compared to children without Down Syndrome. Although several ALL-associated oncogenes are located on chromosome 21, a direct genetic link relating these genes with Down Syndrome has not yet been found [5]. Additionally, siblings of individuals with leukemia are more likely to acquire the disease, which further supports the notion that ALL is linked to genetic factors [6]. The risk of childhood ALL has also been shown to increase in children born to older parents, particularly in fathers over the age of 45 [7]. The genetic basis of childhood ALL may originate in utero, as the disease is frequently initiated by a prenatal chromosome translocation event. Based on studies of identical twins, this event may require a postnatal promotional event to cause clinical ALL [8].

Gender and Race

Gender and race can also influence the nature of ALL. Males are more likely to develop the disease, particularly during childhood, and generally have inferior prognoses compared to females [9]. In a study

comparing prognostic factors in 4,000 children in a series of trials spanning 18 years, the recovery rates of both genders increased over the course of the study, but females had a consistently higher 5-year survival rate than their male counterparts [10]. These results were later replicated in other studies, demonstrating that females are more responsive to ALL treatments and thus have slightly better treatment outcomes relative to those of males [11]. Survival rates also vary significantly by race. In the United States, 5-year survival rates are highest among Caucasian children, followed by Asian and African American children, while Native American and Hispanic children have the lowest survival rates. Disparities in survival rates are especially pronounced in children diagnosed between the ages of 1 and 9. [12]. Additionally, there is a greater incidence of ALL observed among African Americans after the age of three in comparison with Caucasian children of the same age. This may suggest that one or more race-associated environmental factors are responsible for the difference in incidence rates [9].

Treatment Options

There are several treatment options for ALL based on combination chemotherapy. Studies have shown chemotherapeutic agents in cases of relapsed ALL are more effective in adults than in children. In one such trial in adult patients, a high dose treatment of L-asparaginase was administered along with teniposide and cytosine arabinoside, resulting in 88% of the patients achieving complete remissions. A follow-up examination after a period of 77 months showed that 42% of the patients remained disease-free. Though L-asparaginase-associated toxicities are common in older adults, the side effects observed in the study were usually not life-threatening [13]. For children with relapsed ALL, however, chemotherapy treatment is not optimal. Studies comparing chemotherapy and bone marrow transplantation treatments found that children given a bone marrow transplant after a second remission had a relapse rate of 45%, while those given chemotherapy alone had a much higher relapse rate of 80% [14]. Cranial radiation therapy is also frequently utilized in prophylactic ALL treatment. This treatment may be insufficient to eliminate ALL and can cause significant adverse effects on the brain. In one clinical trial, a high dosage of radiation treatment, at 2400 centigray (cGy), caused neurological problems in patients. Additional trials sought to avoid complications by reducing the amount of radiation to 1800 cGy, however the lowered radiation exposure was still harmful, causing neurological impairments and decreased intelligence [15].

Antibody-mediated treatment, which targets a highly specific set of cells, may be a safer and often more effective alternative to high-dose chemotherapy or radiation, although its effects may not address the central nervous system. Antibodies that target the B-cell transmembrane signaling antigen CD19 can be used to target B-cell ALL [16]. CD19 is a key target molecule because it is expressed in all stages of B-cell development beginning with the late pro-B-cell stage and it is present in most cases of acute lymphoblastic leukemia [17]. Targeting CD19+ B-cells to treat ALL allows for effective eradication of diseased cells while avoiding damage to other types of cells and healthy tissues. Components of antibodies may also be combined with other mechanisms to target CD19, as with anti-CD19 chimeric antigen receptor (CAR) immunotherapy [18].

Chimeric Antigen Receptor T-cell Immunotherapy

Chimeric antigen receptor T-cell immunotherapy is an antibody-based therapy that can target ALL cells through the genetic modification of T-cells. CARs are expressed on the surface of T-cells, functioning to identify hematologic malignancies and direct T-cell activation. CARs bind to a particular antigen on tumor cells and trigger activation through subsequent signaling pathways inside the T-cell. This activation prompts the release of toxic enzymes such as granzyme and perforin that induce apoptosis in the cancerous cells, thus allowing for direct killing of the neoplastic target. The modified T-cells have a wider range of targets because they contain antigen-binding domains that are capable of substitution synthetically. Furthermore, infused CAR T-cells function independently of human leukocyte antigens (HLA). CARs can be made to target CD19, which is universally expressed on certain hematologic malignancies including most cases of B-cell acute lymphoblastic leukemia (B-ALL) [16]. Although normal CD19+ B-cells are also targeted, the treatment does not threaten hematopoietic stem cells, which do not express the antigen. Thus, depleted normal B-cells can be regenerated from hematopoietic cells, making CD19 an ideal target for immunotherapy [18].

Design and Manufacturing of CARs

The CAR construct is composed of intracellular signaling domains and co-stimulatory receptors in addition to an extracellular antigen-binding domain. The antigen-binding domain stems from a single-chain fragment of variable region antibody (scFv) and a co-stimulatory receptor that is derived from the T-cell receptor (TCR). It is this innovative construct that allows for a CAR's potential recognition of numerous types of immune cancers. There are three different generations of CAR constructs. First-generation CARs are built by utilizing an activation domain (CD3ζ) as the only component, a simple design that effectively activated T-cells. However, the simplicity of the first-generation CAR model causes a limitation in sustained effector cell function and persistence. Second-generation CARs were then developed, which included a co-stimulatory signaling domain alongside CD3ζ such as CD28, 4–1BB, OX-40, or DAP10 [18,19]. Third-generation CARs further enhanced function and persistence by combining two co-stimulatory signals to the CD3ζ activation domain. Each CAR construct requires transduction into T-cells through lentivirus or gamma-retroviral vectors. Generally, gamma-retroviral vectors have an increased rate of transduction efficiency over lentiviral vectors, however the latter can result in complete response as well [20].

In addition to CAR activation and costimulatory domains, there are other features that can be engineered into T-cells to complement their functions. One complementation to CAR activity is the addition of costimulatory ligands. These extracellular ligands, when interacting with the CD19 antigen, enhance T-cell proliferation and increase number of cytokines secreted. A second supporting feature is the addition of chimeric costimulatory receptors. They imitate the costimulatory signals that are present in CARs, but they do not initiate signal activation in the T-cell. They instead provide costimulation that reinforces T-cell activity when a costimulatory ligand is not present [21].

CAR Target and the Immune Response

The surface marker CD19 is targeted for ALL treatment because the expression of this antigen is found expressly on B-cells (both healthy and oncogenic) and not in hematopoietic progenitor cells, so hematopoietic toxicity would not be expected. The antigen-binding domain of the infused CAR T-cell recognizes and targets CD19 through ligation. This stimulates a synapse formation that allows the

CAR signaling domain to initiate T-cell activation, which results in apoptosis of the target cell by either inducing cytotoxicity in the tumor cell or by releasing cytokines. Inducing cytotoxicity elicits the release and activation of perforin and granzymes, which induce cell death. Cytokine release involves cell-signaling cytokines that control the balance between humoral and other cellular immune responses. Cytokines can recruit other immune cells (e.g. macrophages) to suppress the tumor [16]. Different combinations of signaling domains and co-stimulatory receptors can improve CAR efficacy to produce a more efficient immune response.

Some cancers can resist CAR T-cell effector functions by manipulating immune response molecules such as macrophages, regulatory T-cells and interleukin factors. The stroma may also provide a nutritive environment for cancers and allow them to resist CAR therapy. However, T-cells redirected for universal cytokine-mediated killing (TRUCK) can be used to modify the stroma. When activated, CAR T-cells release IL-12, which activates an immune response in the target cell that attracts these TRUCKS to the tumor cell to initiate a flood of cytokines.

CAR Immune Toxicities

Though targeted therapy circumvents many of the complications associated with nontargeted treatments, there are some toxicities associated with CAR T-cell immunotherapy. In one clinical trial, some patients had severe symptoms such as fevers, hypotension, hypoxia, and neurological disturbances, while others experienced only minor toxicities or none at all. The cause of the toxicities was a surge of inflammatory cytokines associated with cytokine release syndrome (CRS). A positive correlation was found between the severity of the toxicity and the tumor burden of the patient [16].

Clinical Trials Utilizing CAR T-cells

CAR therapy can be used alone or in conjunction with other treatments. First generation CAR T-cells used to treat mice with B-cell malignancies had limited efficacy *in vivo*. The addition of a co-stimulatory domain increased efficacy *in vivo* by increasing persistence and function of effector cells. In a clinical trial involving B-cell chronic lymphocytic leukemia (CLL), CAR T-cells that contained a 4-1BB costimulatory domain were used. Even at low doses of infusion, the treatment resulted *in vivo* expansion of CAR T-cells and significant antitumor activity, as well as tumor lysis and B-cell aplasia. Another trial utilized CARs with a CD28 costimulatory domain and resulted in antitumor efficacy as well as evidence of tumor lysis and cytokine release. The advantage of using either of these costimulatory domains is still yet to be determined [22]. Treating patients with a combined regimen of CAR T-cells and chemotherapy drugs has showed great promise [16]. Successful results were obtained in a clinical trial involving CAR T-cell therapy along with chemotherapy conditioning in patients suffering from various B-cell malignancies, including diffuse large B-cell lymphoma (DLBCL). Prior to CAR T-cell infusion, patients were treated with cyclophosphamide and fludarabine, both of which are used to treat hematological malignancies and autoimmune diseases. Of 13 evaluable patients enrolled, eight patients achieved complete remissions and 4 achieved partial remissions. Some toxicities were observed including fever, hypotension, and other neurologic toxicities, but these were lessened three weeks after T-cell infusion [23].

Pre-infusion chemotherapy utilizes tumor infiltrating lymphocytes (TILs). TILs require adoptive cell transfer therapy in which the patient's tumor lymphocytes are isolated. First, lymphodepletion is carried out *in vivo*, and then the TILs are administered. However, TILs are restricted in their actions because their HLA is specific to certain types of lymphocytes from immune malignancies [24]. The cells are clear of the tumor and reinfused into the patient. Patients at the University of Pennsylvania were treated with pre-cell infusion therapy with TILs along with CAR T-cells, with encouraging results [18]. Another form of combination therapy was directed against acute myelogenous leukemia (AML). In this set of clinical trials, patients were treated with fludarabine and cytarabine (chemotherapy re-induction drugs) and second generation CAR T-cells in which their receptor was modified to recognize not CD19, but LeY instead, an antigen on the surface of AML cells. CAR T-cell activity was seen in one patient along with some toxicity, while another patient had stable disease as a result of treatment.

Before clinical trials can be attempted, forms of adoptive T cell therapies are studied by targeting receptors on mouse models utilizing human tumor xenografts. Though the receptors in mouse models and the patients are not identical, the mouse trials serve to provide information on antigen-specific toxicities in the xenograft samples. T-cell therapy for colorectal cancer can be examined in a mouse model using a CEA antigen (a target in colorectal cancer), and toxicities can be monitored by measuring the antigen expression levels in the tissues of the mice [25]. Whether or not the mice developed signs of colitis, the mouse model system became indicator that showed that CAR T-cells could distinguish between cells with different concentrations of antigens. Based on these studies, specific target antigens can be identified that are expressed in large amounts by tumors and in smaller amounts in normal tissues.

A major concern of using CAR T-cells as a form of immunotherapy is the immune toxicity that accompanies it. One clinical trial assessed the efficiency and safety of CAR T-cells in twenty-one patients with relapsed or refractory acute lymphoblastic leukemia. Patients were treated in a dose-escalation trial of anti-CD19 CAR T-cell doses. After patients were given the prescribed doses of CAR T-cells, those who experienced toxicities were able to recover completely, as all toxicities that resulted from infusion were reversible [26]. Toxicity can also be mitigated using anti-IL-6 antibody therapy. Because toxicities are transient and manageable, CAR T-cell immunotherapy is a viable option for patients with relapsed acute lymphoblastic leukemia.

Mechanisms of Resistance

The emergence of CD19-negative escape variants has been reported in a number of clinical trials involving anti-CD19 CAR T-cell treatment for patients with ALL. In one trial, two children with relapsed and refractory B-ALL both achieved complete remissions, with one child relapsing at 2 months with ALL blasts that no longer expressed CD19 [27]. In a larger clinical trial, 3 of 30 patients relapsed with CD19-negative blasts following treatment with anti-CD19 CAR T-cells [28]. More recently, a dose-escalation trial of 20 B-ALL patients resulted in 2 patients who sustained relapse with CD19-negative disease [26]. The mechanism by which CD19 is downregulated in response to CAR T-cell selective pressure has yet to be determined. This form of resistance may be able to be avoided by including additional targets, such as CD20 or CD22.

Some B-cell cancers resist treatment by up-regulating the B-cell lymphoma (Bcl-2) family of anti-apoptotic proteins, thus affecting the apoptosis pathway and making the cancer resistant to various forms of therapy. There are small molecule inhibitors of Bcl-2 (ABT-737) that can re-induce a functional pathway for apoptosis in cancer cells. Caspase was used as an indicator of apoptosis in children with pre B-cell ALL to show that combining CAR T-cells with the small molecular inhibitor ABT-737 had enhanced efficacy. CAR T-cells alone had a higher rate of apoptosis than ABT-737 alone, but the combined form of therapy was far more effective [29].

Resistance *via* alternative B-cell differentiation has been observed in one case of CLL/SLL treated with anti-CD19 CAR T-cells. The patient experienced relapse with CD19-negative disease as well as CD19-negative plasmablastic lymphoma (PBL) that arose from alternative differentiation of leukemic stem cells that evaded treatment [30]. These cancer stem cells are thought to have a multi-drug resistance, because their ATP-binding cassette transporters can act as a pump to expel cytotoxic drugs [31]. This form of resistance could potentially arise in ALL as well, so targeting antigens that are highly expressed on leukemic stem cells may help to lower the risk of resistance.

Approaches to Improve CAR T cell-based immunotherapy

Although CAR T cells therapy has shown promising results so far, its clinical efficacy is not fully optimized, in part, due to on target/off tumor effect and cytokine release syndrome (CRS) [32,33]. Since tumor associated antigens (TAAs) are expressed on both cancerous and healthy cells, CAR T cells also target healthy cells, causing serious adverse events (SAEs) [34]. Several strategies have been developed to avoid the non-selective effects of CAR T cell therapy, including suicide gene, inhibitory CAR, and exogenous molecules as safety switch to control CAR T cell activity [33].

In suicide gene approach, a genetically encoded molecule that can induce apoptosis is used for selective destruction of adoptively transferred cells [35]. The ideal suicide gene should ensure irreversible selective elimination cells responsible for unwanted toxicity. Additionally, an ideal suicide gene should have adequate bio-availability and bio-distribution profiles [32,35]. Currently, several suicide gene technologies have become available. Based on their mechanisms of action, these technologies can be classified into three categories: metabolic, dimerization inducing, and therapeutic monoclonal antibody-mediated (mAb).

In gene-directed enzyme prodrug therapy (GDEPT), which utilizes metabolic mechanisms, a nontoxic drug is converted to a toxic compound in gene-modified cells [36]. A notable example is the Herpes simplex thymidine kinase (HSV-TK)/ganciclovir (GCV) suicide system [37]. In this system, the nucleotide analogs acyclovir and GCV are phosphorylated by HSV-TK, and their phosphorylated forms are incorporated into DNA by DNA polymerase, resulting in chain termination and cell death [38]. This allows for elimination of alloreactive cells when the system is used in allogeneic settings; however, since the origin of HSV-TK is viral, the HSV-TK/GCV system may cause the immune system to reject genetically modified T cells [20,32,37,39,40].

With the advance of genome-editing technology, the dimerization inducing mechanism of suicide gene using inducible caspase 9 (iCasp9) has become more efficacious. iCasp9 gene contains the intracellular portion of human pro-apoptotic caspase 9 protein fused to a drug-binding domain derived from human FK506 protein [41]. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 genome editing technology enables the synthesis of CD19 CAR T cells that can be directed to the T cell receptor α constant (TRAC) locus in mouse model of acute lymphoblastic lymphoma (ALL), resulting in uniform CAR T cell expression in human peripheral blood T cells and enhancement of T cells potency [42]. Genome editing technologies also allows for the synthesis of iCasp9, which is used together with the chemical induction of dimerization (CID) drug AP1903 [43]. Upon intravenous administration, AP1903 induces the cross-linking of the drug-binding domain of the fused protein to dimerize caspase-9 and activate executioner caspase- 3, leading to apoptosis [43]. Compared to HSV-TK/GCV suicide system, iCasp9 is non-immunogenic and allows for rapid elimination of CAR T cells; however, this elimination is not complete [34].

In addition to iCasp9, approaches using therapeutic mAb-mediated mechanism can be utilized to avoid immunogenic responses. Anti-CD20 is the commonly used mAb. CAR T cells are engineered to express CD20 receptors, and after treatment, anti-CD20 molecules are administered to eliminate unwanted or excess CAR T cells [44-46]. Nevertheless, CD20 receptors are also expressed by healthy B cells, so this approach can cause on-target toxicity [32]. Besides CD20, CAR T cells can be designed to express or not express certain receptors to enhance their potency. CAR T cells therapy has been reported to be unsuccessful partly due to tumor-induced immunosuppressive mechanisms, one of which is the production of adenosine [47]. In tumor microenvironment, adenosine is observed to be at immunosuppressive concentrations [48]. In mouse model expressing HER2+self-antigen tumor, targeting of A2AR (a type of adenosine receptor) using A2AR antagonists or short hairpin RNA (shRNA) greatly improved the efficacy of CAR T cell therapy, particularly when the therapy was combined with other checkpoint inhibitors such as program death (PD) -1 [47].

Conclusion

Chimeric antigen receptors have great potential for ALL treatment. Their variable domains and receptors allow for a wide range of specificity in target recognition, which plays a key role in their success. However, the high-level of specificity of CARs detracts from their usage on different kinds of cancers that can occur throughout the body. To counteract this setback, TRUCKs have been developed to allow the full potential of CARs to be utilized. Thus far, the majority of CAR trials have targeted immune malignancies. Despite promising results in clinical trials, some recurring toxicities occur in many patients, such as cytokine release syndrome. Further studies can be performed in order to better understand how to avoid such side effects. More specific targeting can still be accomplished, making treatment even safer and more effective. While trials conducted using this form of antibody-mediated treatment have shown significant efficacy, there is still much room for improvement as CARs continue to be modified [18].

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