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Emerging Treatment Options Utilizing Chimeric Antigen Receptor T-Cells

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Abstract

Malignant cells produce specific tumor antigen receptors (TAAs), which can be engineered to be recognized by a patient's own T-cells through the expression of chimeric antigen receptors (CARs). These CAR-T cells represent the latest product of adoptive cellular immunotherapy (ACI). In particular, the engineering of CAR-T cells has been most successful to date in the targeting of CD-19 B-cell associated hematologic malignancies. This review focuses on a general review of the engineering process of CAR-T, difference between the various generations, treatment options, and associated toxicities. To date, most of the literature regarding CAR-T cells is directed towards CD19 to treat B-cell malignancies, including chronic lymphocytic leukemia (CLL), B-cell acute lymphoblastic leukemia (ALL), and non-Hodgkin lymphomas.

Keywords: Chimeric antigen receptor T-cells; Tumor antigen receptors; Chronic lymphocytic leukemia; Acute lymphoblastic leukemia; non-Hodgkin lymphomas

Introduction

Oncologic pharmacotherapy throughout the decades has experienced a paradigm shift of sorts, which initially encompassed chemical blockers, and then moved toward biologic agents. However now, the future is promising great strides in the development of immunotherapies, and most recently, chimeric antigen receptor T-cells (CAR-T cells) which provide the next step toward personalized medicine.

Malignant cells produce specific tumor antigen receptors (TAAs), which can be engineered to be recognized by a patient's own T-cells through the expression of chimeric antigen receptors (CARs). These CAR-T cells represent the latest product of adoptive cellular immunotherapy (ACI). In particular, the engineering of CAR-T cells has been most successful to date in the targeting of CD-19 B-cell associated hematologic malignancies. This review focuses on a general review of the engineering process of CAR-T, various generations, the role in therapy of chronic lymphocytic leukemia (CLL), B-cell acute lymphoblastic leukemia (ALL), and non-Hodgkin lymphomas, as well as associated toxicities.

T-Cell Engineering

Previous ACI has centered on the identification of major histocompatibility complex (MHC) for tumor eradication [1]. CAR-T cells however, are chimeric as the name implies due to a synthesis of an antibody-antigen recognition constituent, along with the intracellular signaling portion of a TCR [2]. Due to CAR-T MHC independence, the lack of issues associated with variable efficacy due to mechanisms such as HLA expression, antigen binding affinity and induction of cell-mediated immune response makes it appealing in terms of its utilization [3].

CAR-T cell engineering begins when T-cells are cultured using an autologous blood sample. T-cells are then grown in a lab for 10-14 days during which transduction (through various methods discussed below), and expansion, occur. The cells are frozen, and finally re-administered to the patient. Currently this is a "blood bank" model as opposed to a central distribution model, which is not available due to the very individualized process as it currently stands. These re-administered CAR-T cells may target a variety of surface molecules, and additionally T cell subsets and progenitors, as well as natural killer cells, which can allow for recruitment of these cells toward an antigen in question [1]. This model proves very useful due to the avoidance of self-antigen tolerance. The question exists as to when it is appropriate to condition a patient to receiving this autologous transfer, which refers to whether or not the patient should first receive traditional chemotherapy and then the CAR-T cells, vice versa, or only the CAR-T cells.

Transduction of the CAR-T cells can occur through various approaches including those of both viral and non-viral methods. Currently, available transduction techniques include retroviral, lentiviral, and adeno-associated viral vectors, and non-viral methods including DNA-plasmid, transposon, and RNA-based gene practices. An in-depth evaluation of the engineering regarding each method is beyond the scope of this review; however, each will be discussed briefly.

Gamma retroviral vectors assign a transgene in the place of a retroviral coding sequence of the viral genome, which by using the viral replication ability, allows for successful cell transfection with minimal likelihood of perpetuation of retroviral infection in a patient due to lack of a packaging signal [2,4]. Similarly, lentiviral vectors utilize the replacement of a viral genome (most commonly an HIV-1 modification) with a transgene; however, lentiviral vectors have the more attractive ability to infiltrate and integrate into antigenic cells without the necessity of cell division. Additionally, due to the lack of integration into promoter regions, the likelihood of mutagenesis is decreased [2,5,6]. These vectors require several distinct components to allow for the nuclear export of gag-pol mRNA and import of the pre-

integration viral complex [2,7,8]. These components include the *trans*-presentation of the *rev* gene and the central polypurine tract/central termination signal [2,7,8]. Adeno-associated viruses (AAV) are another attractive method for gene therapy due to being a single-stranded DNA parvovirus which is non-enveloped and devoid of replication potential, while still being capable of infiltrating non-dividing cells. Therefore unlike retroviruses which can potentially cause random insertion and possibly mutagenesis, AAV may be a more benign method. AAV also require the presence of another virus such as herpes simplex virus (HSV) to combine transgene capacity with genomic integration for a specific site of a host. However, rep protein interaction with the inverted terminal repeat (ITR) can allow for latency of the AAV when HSV is not present [2].

A comparatively less efficient engineering option exists with plasmid DNA-based vectors, which allows for transfection of plasmid DNA into T-cells. These T-cells unfortunately tend to endure a shorter life-span when carrying the plasmid. Due to the low frequency of genomic integration with plasmid DNA-based vectors, as well as a higher likelihood of insertional mutagenesis, this process is not as favored when compared to other engineering options [2]. Similarly, another non-viral transduction option exists with transposon-based gene transfer. This method allows for the transfer of genes between various chromosomes. Currently, the two varieties of transponson-based gene transfer include Sleeping Beauty (SB), and PiggyBac (PB). The latter may show more potential in humans due to the capacity to deliver larger transgenes. Conversely, the former may allow for more stability in regard to chromosomal integration [2,9].

An interesting substitute for viral and DNA-based non-viral gene methods is *in vitro*-transcribed messenger RNA (mRNA)-mediated gene expression. The attraction of this method is due to the ease of the engineering process, effective transduction *in vivo*, and low likelihood of *in vivo* mutagenesis [2,10,11]. However, this process' appeal may be offset by the 7-10 day time span of administration to titrate to antitumor effect. Yet some may argue that this extended interval of administration may prove to be safer for the patient [2].

Ultimate efficacy of CAR-T cells depends on their ability to identify and bind to the malignant antigen, activate the T-cell, defeat the immunosuppressive microenvironment of the tumor to avoid inactivation, proliferate and mount a response, and ultimately lead to the formation of memory cells in order to prevent future malignancies [12]. However, understanding the core design of CAR-T cells helps appreciate their varying potential for success *in vivo*.

A standard CAR-T cell structure comprises an extracellular antigen recognition domain made of single-chain variable fragment (scFv) from heavy- and light-chain variable regions of a TAA-specific monoclonal antibody [13]. The scFv domain's role is to target specific surface markers of the individualized tumor cells, without the necessity of MHC presentation or patient halotype. There are additional ligand proteins including those for vascular endothelial growth factor receptor (VEGF), as well as natural killer cell receptor G2D (NKG2D) receptors, which may be utilized in place of scFv [14,15]. Regardless, this extracellular antigen recognition domain is connected to a transmembrane, as well as intracellular signaling domains within the T-cell receptor [13]. Depending on the design there may or may not be spacer regions, which when present have the duty of safeguarding the position of the TAA-CAR connection, and can cause variance among the signal strength of complex [2]. Additionally, the transmembrane domain provides critical functionality in CAR response to the TAA in the role T-cell signaling. The transmembrane domain results from proteins such as CD3, CD4, CD8, and CD28 [2,14]. Some CAR-T designs incorporate co-stimulatory signal domains as seen in 2nd and 3rd generations, giving the advantage by allowing for further production of cytokines and prolonged survival of these chimeric T-cells. Advancements in the engineering of CAR-T cells have allowed for the development of four generations to date [2]. Due to differences in design including antigen selection, strength of the attraction, differences in hinge region size, as well as type of signaling domains, there is much variability in terms of potential success [12].

While efficacy remains a highly important function of CAR-T cells, safety is an aspect that must also be addressed. Unique adverse effects will be addressed later in this review; however, in terms of engineering there are approaches that are being investigated to decrease the possibility of toxicities. One such toxicity includes cytokine release syndrome (CRS). This occurs when the proliferating CAR-T cells continue to release cytokines uncontrollably due to lack of an apoptotic control mechanism. Currently, CRS can be treated in patients using corticosteroids or anti-IL-6 receptor antibodies [16,17]. Additionally, concern exists for long lasting B-cell depletion due to this perpetual proliferation. The ideas of apoptosis mediated *via* suicide genes, as well as a lessened proliferation of the CAR-T cells through transient mRNA CAR have been studied [18,19]. However, there is current development as seen in the study by Ma, in the engineering of "switch molecules", which allow for modulation of the CAR-T cell response. This design could allow for control of the CAR-T cell response through titratable doses, or even terminate the response through removal of the switch in order to reduce aforementioned toxicities *in vivo* [20]. An advantage of switch molecules over apoptotic suicide genes is the allowance for controlled therapy, versus the immediate premature death of the CAR-T cells, resulting in the termination of treatment [21]. Switch molecules may also prove useful in targeting heterogeneous tumors expressing two or more antigens such as CD19, and CD22, in addition to tumor escape variants [20].

CAR T-Cell Generations

The 1st generation CAR-T cells provided the foundational engineering in the construct of future generations through their design of scFv antigen-binding epitope signaling an intracellular moiety without the production of much IL-2. Unfortunately, the proliferative ability of these models *in vivo* was bleak, and it was determined that there was a need for co-stimulation to successfully activate T-cells, which led to the birth of later CAR-T cell generations [2,22].

The 2nd generation models became more complex in design with two intracellular signaling domains, as opposed to just one in the 1st generation model. These intracellular domains consist of an endodomain from co-stimulatory models, namely CD28 among others, as well as an intracellular region of CD3ζ [2]. The fusion of these domains together allows for abundant propagation of the CAR-T cells without the need for HLA mediated stimulation. Additionally, the CD28 domain may play a protective role for the T-cell itself by preventing antigen mediated death of the activated T-cells which in turn leads to longer proliferation, and therefore a potent antitumor effect [2].

The 3rd generation models have been developed with two co-stimulatory signals, potentially providing additional superior signaling as compared to the 2nd generation models. The signaling domains of these 3rd generation models are composed of CD28, CD3ζ, and OX40 or 4-1BB. This superior signaling is believed to be especially true of 3rd

generation models with coupling of the co-stimulatory site to CD3ζ, by leading to more successful proliferation.

It is the 4th generation models, also known as T-Cells Redirected for Universal Cytokine Killing (TRUCK), that are showing the most promise in the evolution of the CAR-T cell constructs. These TRUCKs are integral in endogenous recruitment of cytokines, namely IL-12 which then further cascade recruitment of additional cells to target the antigenic tumor cells. Depending on the engineering design of the TRUCK, cytokines can either be secreted through the control of a constantly active promoter, or upon the binding of the antigen and CAR [2,23,24].

Role in Therapy

When developing a new molecular entity for the treatment of cancer, it is ideal to target a tumor by finding an antigen specifically expressed on the tumor cells but not on normal cells [25]. This can result from altered expression, translocation producing a fusion protein, or mutation leading to altered configuration or antibody binding. CD19 is an attractive target since it is a B-cell surface protein expressed throughout B-cell development. Therefore, antibodies against CD19 inhibit tumor cell growth while leaving healthy cells largely untouched. To date, most of the literature regarding CAR-T cells is directed towards CD19 to treat B-cell malignancies, including CLL, B-cell ALL, and non-Hodgkin lymphomas [25].

In a Phase I/IIA study, a total of 30 patients were given CTL019 T-cell therapy to assess the safety and feasibility [26]. Both pediatric and adult patients were included in the study with 25 patients between the ages of 5-22 and 5 patients between the ages of 26-60. All patients had relapsed or refractory $CD19^+$ cancers with 26 patients having B-cell ALL in the first to fourth relapse, 3 with primary refractory B-cell disease, and 1 patient with T-cell ALL. Eighteen patients had relapsed after allogenic stem cell transplant and 3 patients were refractory to blinatumomab. CTL019 lentiviral vector was traduced with autologous T-cells and administered to the patients at doses of 0.76×10^6 to 20.6×10^6 CTL019 cells per kilogram of body weight. After 1 month, 27 of 30 patients (90%) had morphologic complete remission with 2 of the 3 patients previously refractory to blinatumomab having a complete remission. By means of multiparametric flow cytometry, minimal residual disease (MRD) was negative in 22 patients and positive in 3 patients with level of 0.1%. All 25 patients were negative for MRD at 3 months (0.09% and 0.22%, respectively). Two patients did not receive flow cytometry to determine MRD. Of the 27 patients with complete remission, 19 remained in remission with 15 patients not receiving further treatment and 4 withdrawing from the study to receive another medication. Median follow-up time was 7 months (range 1-24 months). Event-free survival rate at 6 months was 67% (95% CI, 51-88) and overall survival was 78% (95% CI, 65-96). CTL019 cells were detectable in the blood by means of flow cytometry for up to 11 months after administration of treatment suggesting continued effector function.

In a Phase I trial, 16 adult patients with B-ALL either in first complete remission or relapsed or refractory disease were treated with 19-28z CAR T cell [27]. The median age of enrollment was 50 years. Patients underwent leukaphersis followed by cyclophosphamide conditioning chemotherapy then infusion of 19-28z CAR T cell dosed at 3×10^6 CAR T cells/kg. Overall complete response rate was 88% with MRD negative or complete molecular response of 75% by flow cytometry. The peak CAR-T cells in the bone marrow were found to be at 1-2 weeks after infusion and decreased to low or undetectable by 2-3 months.

Anti-CD19 CAR-T cells were administered in 9 patients with diffuse large B-cell lymphoma (DLBCL), 2 patients with indolent lymphoma, and 4 patients with CLL [28]. All patients received chemotherapy with cyclophosphamide and fludarabine followed by anti-CD19 CAR-T cells dosed at 1×10^6 to 5×10^6 CAR-positive T cells/kg. Thirteen total patients (7 patients with DLBCL and 6 patients with indolent B-cell malignancies) were evaluated due to one patient being lost to follow-up and one patient dying 16 days after receiving treatment. Out of the 7 patients with DLBCL, 4 patients obtained a CR, 2 patients obtained a PR, and 1 patient obtained a SD. All 6 patients with indolent B-cell malignancies received a PR or CR and 3 of the 4 patients with CLL are in ongoing CR confirmed by multicolor flow cytometry of the bone marrow. The longest duration of CR in this trial is 23 months to date. The number of CAR-positive blood cells peaked between 7 and 17 days after treatment and rapidly decreased afterwards measured by qPCR.

Unique Adverse Effects

Cytokine release syndrome (CRS)

CRS after administration of CAR-T cell can range in severity from mild to severe and life-threatening [26]. CRS is defined as a systemic inflammation response that is produced by elevated levels of cytokines and these elevations are associated with T-cell activation and proliferation. Mild severity symptoms can be classified as high temperatures and mylagias [26]. Whereas, severe CRS produces vascular leakage, hypotension, respiratory and renal insufficiency, cytopenias, neurological changes and coagulopathy [26,27]. Davila, et al. created criteria for diagnosis of non-severe CRS versus severe CRS based on three factors: presence of fevers (38°C) for 3 or more days, elevation of characteristic cytokines, and presence of clinical toxicities [27]. Fevers usually start approximately 24 hours after administration and can last several days. The elevation of seven cytokines was identified with correlation to pretreatment tumor burden. In Davila, et al. trial, patients that required intensive interventions for CRS treatment had a 75-fold increase over pretreatment baseline levels in 2 of the 7 identified cytokines. Patients with non-severe CRS tolerated treatment and only required routine observation and monitoring. The average hospital stay for non-severe CRS patients was 15.1 days (SD, 18.8, range 4-61 days). Severe CRS patients required closer observation and were more likely to need medical and pharmacologic interventions. These patients length of stay in the hospital was on average 56.7 days (SD, 28.6, range 20-104).

Although the toxicities associated with CRS are concerning, to date all cases have been fully reversible [27]. It is essential to recognize the signs and symptoms of CRS; however, premature intervention that is not necessary may diminish the T-cell persistence or efficacy. The treatment for severe CRS includes tocilizumab, an IL-6 receptor blocking antibody, vasoactive pressors, mechanical ventilation, antiepileptics, and antipyretics [26,27].

In the Maude, et al. trial, all of the 30 patients experienced CRS with 22 patients (73%) experiencing mild to moderate severity needing hospitalization and 8 patients (27%) with severe issues requiring intensive care with respiratory support and vasopressor support for hypotension [26]. All patients had increased C-reactive protein and ferritin levels. Patients with severe symptoms had higher peak levels of interleukin-6, C-reactive protein, ferritin, interferon-γ, and soluble

interleukin-2 receptor. Severe CRS occurred one day after treatment; whereas, moderate CRS occurred 4 days after treatment. Tociliziumab was used in 9 patients which resulted in rapid defervescence and blood pressure stabilization over 1 to 3 days. Six of these patients required a short course of glucocorticoids and 4 patients had to receive a second dose of tocilizumab. Two of the 9 patients, has relapses of CRS. All 30 patients did recover with complete reversal of symptoms and all laboratory values returned to normal.

Neurotoxcity

Neurotoxicity associated with CAR-T cell treatment can range from aphasia, tremor, myoclonus, gait abnormalities, apraxia, obtundation, encephalopathy or confusion [26,28]. Encephalopathy usually occurs in a small number of patients and usually after development of CRS [26]. The exact cause of neurotoxicity is unclear but it may be due to CAR recognition of CNS antigens or may be due to unusual cytokines or cytokine concentrations in CSF. The symptoms are usually self-limiting and last around 2 to 3 days, with resolution over an additional 2 to 3 days. Full symptom recovery can be expected without further invention or long-term consequences.

In the Maude, et al. trial, 13 patients (43%) experienced neurotoxicity issues ranging from delirium during high temperatures to global encephalopathy with at least one of the following: aphasia, confusion, delirium, and hallucinations [26]. One of the patients with encephalopathy experienced two seizures; however, it is unclear if that was related to electrolyte abnormalities. In 6 patients, delayed encephalopathy occurred after resolution of high temperatures. This encephalopathy was independent from the severity of CRS and whether the patient received tocilizumab.

Conclusion

There are numerous advantages of using CAR-T cells in the treatment of cancer disease states. As seen in the trial results is an impressive response potential with utilizing CAR-T cells. Due to CAR-T cell MHC independence, it can be used regardless of HLA expression. The cells can be derived from peripheral blood to ease access. The target of the CAR-T cell is known and the tumor expression of target can be confirmed. The CAR-T cells may bypass some inhibitory mechanisms that other treatment agents may encounter. A few of the disadvantages include high potential for on target, off tumor autoimmune toxicity and a possible higher probability for CRS.

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