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Chimeric Antigen Receptor T Cell Therapy: A Review

Michael Knouse

Abstract

Chimeric Antigen receptor T cell (CAR-T cell) therapy is a novel adoptive immunotherapy where T lymphocytes are engineered with synthetic receptors known as chimeric antigen receptors (CAR). CARs are engineered and constructed specifically to reprogram a patient's T cells to target tumor cells. These CARs predominantly are used to treat hematological malignancies including Lymphoma and Multiple Myeloma. Specific targets often used include, CD19, CD20, CD30, and CD138. Although this novel therapy is promising it has its disadvantages. CAR T-cell therapy-associated toxicities, including cytokine release syndrome, on-target/off-tumor, and other neurologic toxicities have been observed and are being properly managed in clinic. In this review, the applications of CAR-T cells in different hematological malignancies, the anatomy and production of CARs, along with future directions are discussed. This technique could pave the way for future improvements on the effectiveness and persistence of adoptive immunologic therapies.

Introduction

Adoptive immunotherapy for cancer has a long, and checkered history. William Coley is widely attributed with the discovery and the first observation of the immune system having antitumor effects. Coley's observation of the regression of sarcoma following severe bacterial infections in the 1890s was the beginnings of adoptive immunotherapy (1). However, the pivotal finding that hematopoietic stem cell

transplantation using syngeneic donors was less effective at preventing relapse of leukemia compared with sibling donors provided the backbone for T-cell therapy (2). Over the past several years, there has been a spike in potentially beneficial therapies stemming from culturing, redirecting, and/or enhancing T-cells against certain tumor cell types (3). T-cell based adoptive immunotherapy, which is being developed to deal with malignancies, especially hematological cancers. Non-Hodgkin lymphoma and multiple myeloma are some of the most commonly treated with these techniques. However, solid tumors including, melanoma, breast cancer, and sarcoma have also shown susceptibility to adoptive immunotherapy. Three of the emerging therapies for treating these malignancies are: tumor infiltrating lymphocytes, T-cell receptor (TCR)-modified T-cells, and Chimeric antigen receptor T cells (CAR-T) (4). The first two techniques are inefficient due to the process of production, the poor success rate, and the dependence upon vaccination which limit development (5). However, CAR-T cell therapy is more sustainably efficient and has been over the past two decades. CAR-T cell therapy has been viewed with exceptional interest over the past two decades at an array of academic institutions. The redirection of the cell-mediated immune response toward tumor antigens by expressing chimeric antigen receptors is a hot topic amongst researchers.

CARS are recombinant receptors that typically target surface molecules (6). CAR is typically composed of two separate domains: an extracellular and intracellular domain. Typically, the extracellular domain is an antibody single-chain fragment (scFv), while the intracellular domain includes fused signaling domains from natural TCR complex and costimulatory domains (7). The extracellular domain links via a

spacer/hinge and transmembrane domains to the intracellular signaling domain that can include costimulatory domains and T-cell activation moieties (8). CARs recognize unprocessed antigens independently of their expression of major histocompatibility antigens, which is different from the physiologic T-cell receptors (TCRs). Hence, CAR T-cells are able to circumvent some of the major mechanisms by which tumors avoid MHC-restricted T-cell recognition (8). Another feature of CARs is their ability to bind to not only proteins but to carbohydrates (9), gangliosides (10), proteoglycan (11), and heavily glycosylated proteins (12) thereby expanding potential targets for binding. CARs engage the target via the single-chain variable fragment (scfv) derived from antibodies, although natural ligands have been used as well. However, the scfvs derived from murine immunoglobulins are the most common.

Of crucial importance for CAR design is the intracellular signaling modules, which are derived from lymphocyte signal-initiating molecules. These are known as first-generation CAR design that include specific chains of the TCR/CD3 complex and the high-affinity receptor for immunoglobulin E. These were shown to activate cellular responses to diseased cells (13). Tumor cells are able to induce specific tolerances of antigens based on MHC class I-restricted antigen presentation and a simultaneous lack of costimulatory ligands. The design and anatomy behind CAR attempts to provide the appropriate signals to activate effector T-cells. Each incorporation of simultaneous costimulatory signals improves the response and activation of the effector T-cells to handle the affected tissue. The adding of costimulatory signals is known as second-generation CAR. Lacking the signals creates a first-generation CAR. Altering the signaling molecules that are attached to CD3 define the structures. Those that posses

CD3 along with signaling endo-domains, such as, CD28, CD134, OX40 (CD134), or CD137 (4-1BB) are known as second and third generation CARs (Fig. 1). These structures imitate the costimulation signal when TCR combines with antigen presenting cells to complete the process of activation.

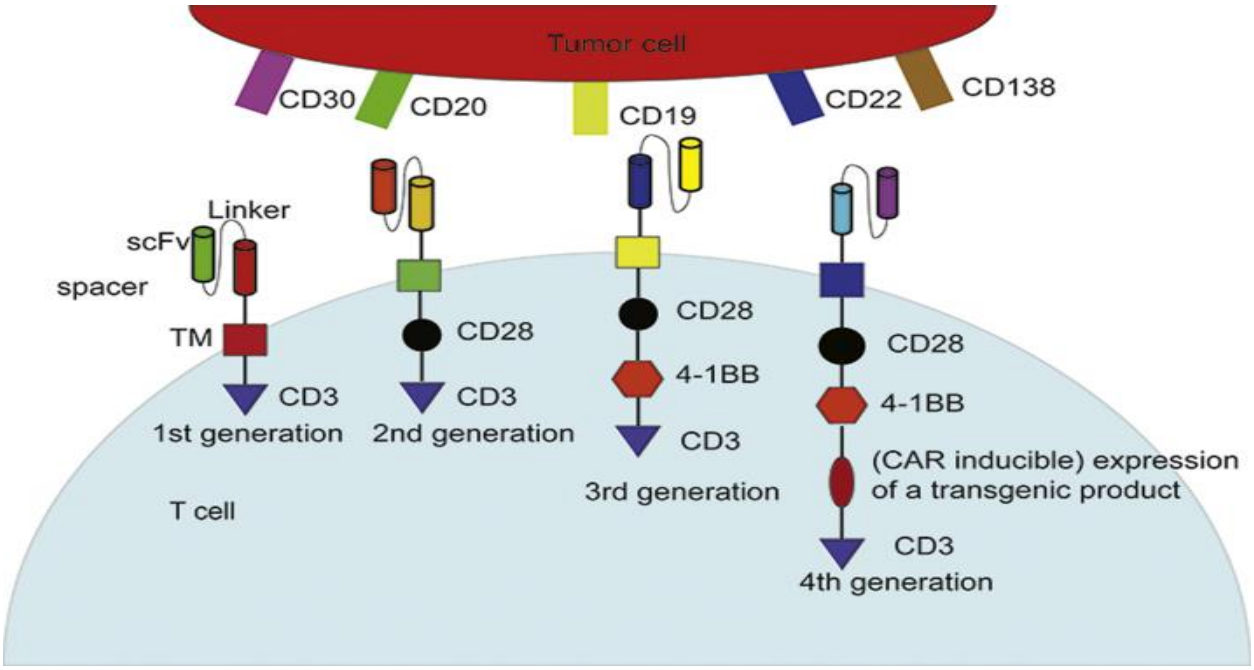

Figure 1

Illustration of basic structure of 4 generations of chimeric antigen receptor T cells (CAR-T cell) and common targets on tumor cells. The whole structure of CARs consisted of an antibody single-chain fragment (scFv, extracellular segment) specifically against a cell surface antigen as well as one or several fused signaling domain(s) from natural TCR complex and costimulatory molecules (intracellular segment). Different intracellular segments represent various CAR-T cell generations. scFv, single-chain fragment. TM, transmembrane region.

All of these generate CAR-T cell specificity for a certain type of cancer which leads to their elimination. Because of the monoclonal antibody against tumor antigen offers novel T cell specificity for certain types of cancer cells and bypasses the established antigen-presenting process, an important strength of this method is that the recognition

is independent of the MHC (7). Nevertheless, a novel generation of CARs has proven attractive to scientists. However, recently, studies have been performed on optimizing CAR design. Fourth generation CAR T-cells contain a cytokine killing mechanism, they are known as TRUCKs or T-cell redirected for universal cytokine killing. The cytokine, usually pro-inflammatory cytokine, may be constitutively produced or induced once the T cell is activated. The mechanism is to deposit inflammatory cytokines in the targeted tumor site, which attracts an innate immune response toward those cancers cells that are invisible to CAR T-cells (14). These properties have demonstrated the ability to decrease tumor growth and development within hematological carcinomas and are likely to translate to immunotherapy of solid tumors.

In general, the process of manufacturing and delivery of CAR-T involves the following and is complicated. (fig 2). Firstly, T cells from peripheral blood are collected by leukapheresis, followed by apheresis without adding colony stimulating factor. Colony stimulating factor can cause disruption in the proliferation of the T cells, along with their responsiveness (15). Antibody coated beads serving as artificial dendritic cells are used to activate the isolated T cells. The genetically reprogrammed T cells are then transfected ex vivo with a CAR viral or non-viral vector, where a section of genome DNA is inserted. T-cell ex vivo expansion and purification are the subsequent and key steps, determining the efficacy of this novel adoptive immunotherapy (15). Finally, test of cell quality and sterility are needed, which take around 2-4 weeks to complete (16). Before T-cell infusion, the patient receives a preparative lymphodepleting regimen and then the CAR T-cell infusion occurs.

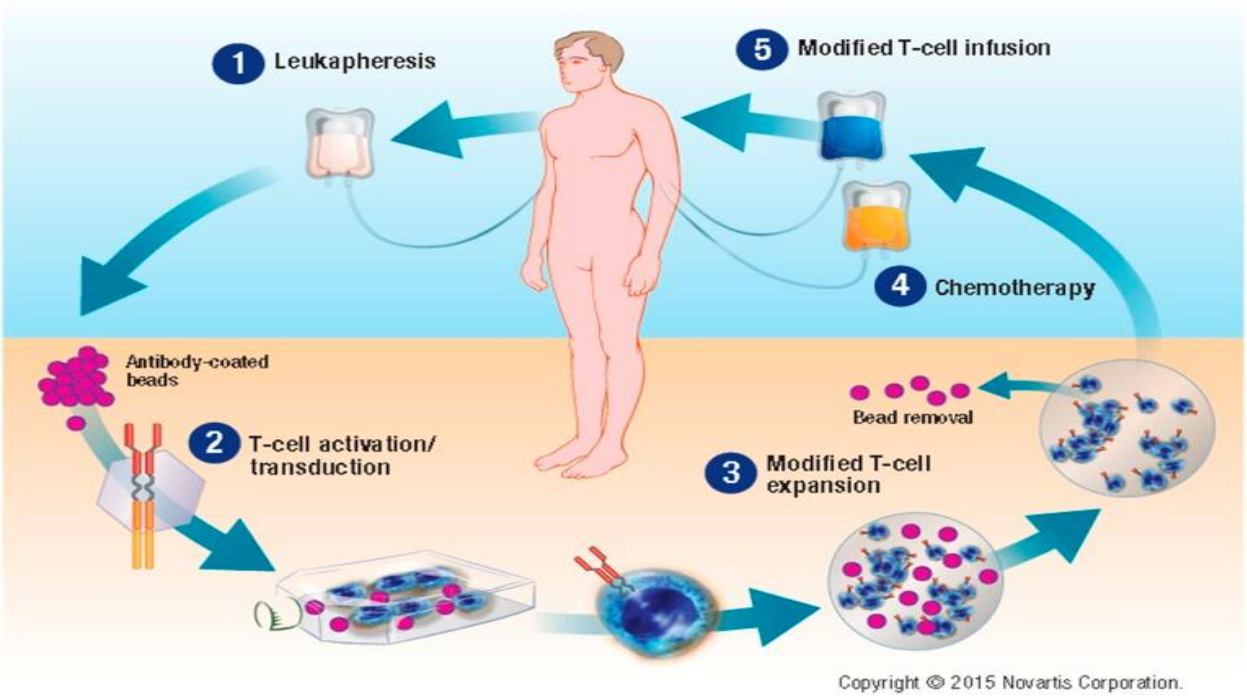

Figure 2

Flow chart of the whole procedure of the chimeric antigen receptor T cell (CAR-T cell) production

Patients with disease deterioration after primary and secondary therapies for lymphoma have been found to have poor prognosis even after improvements through cytotoxic chemotherapy regimens and monoclonal antibody therapies (17). However, CAR-T cell therapies are among the latest advanced immunotherapies for relapse or chemotherapy-refractory B-cell non-Hodgkin lymphoma (18).

There are several types of CARs modified on the surface of T cells. The anti-CD19 CAR-T cell is the earliest and the most traditional, however, CD20 and CD30 are potential targets along with many others for multiple B cell malignancies. For lymphoma, the first generation of CAR-T cells were not effective in preventing proliferation,

persistence and homing compared to the second and third generations (17). Two different studies have reported preclinical results showing superior proliferative and antitumor activities of the second and third generation T cells, with the CD28 or 4-1BB cytoplasmic signaling domains, both *in vitro* and *in vivo (19)*. Despite a positive effect of CD28 as an early signal in improving cell expansion and persistence, some trials have suggested that using CAR containing 4-1BB as a late costimulatory signal yields more remarkable expansion and anti-tumor activity in indolent B cell malignancies (20). For example, CTL019 therapy has shown great effect in some patients with advanced relapse/refractory (r/r) follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL). Among 8 eligible patients, 4 people had responses at different levels: 3 complete remissions (CR rate is 13%) and 1 partial remission (PR rate is 4%), with a 50% 3-month overall response rate. Four patients with DLBCL had progressive disease before or at initial response assessment, and there was no treatment-related mortality (21).

Besides CD19 other surface markers are also essential. CD20, a trans-membrane protein presents in more than 90% of B-cell lymphomas, as well as being a well-established target for non-Hodgkin Lymphoma treatments (22). Currently, first–generation anti-CD20 CAR-T cell therapy has been used in several studies. In one clinical trial study 7 participants suffering from lymphomas were treated. Two patients in this study achieved CR, 1 subject got a PR while the disease in another 4 patients was stable (23). To test whether the second-generation of CAR-T treatment is effective in DLBCL patients, a study in 2014 using anti-CD20 CAR-T cell with 4-1BB reported a promising effect of this novel treatment (22). To test whether the second-generation of

CAR-T treatment is effective in DLBCL patients, a study in 2014 using anti-CD20 CAR-T cell with 4-1BB reported a promising effect of this novel treatment (22). In this study, 7 patients with refractory advanced $CD20^+$ DLBCL were recruited. Among them, 5 patients were hampered with bulky tumors and the other 2 were not. Except for 1 subject, the other 6 patients received preconditioning chemotherapy for disease control or the tumor was debulked before anti-CD20 CAR-T cell infusion. One of the two patients without a bulky tumor burden achieved a 14-month long lasting and ongoing CR without preconditioning regimen, and another gained a 6-month tumor regression. 3 of 5 with bulky tumors got 3- to 6-month tumor regression (22).

Multiple Myeloma (MM) is a bone marrow derived malignancy leading to anemia, immunosuppression, hypercalcemia, and renal insufficiency (24). Despite multiple techniques to slow and eradicate, this disease remains incurable. However, the fact that myeloma can subside as a result of the graft-*versus*-myeloma effect in allogeneic stem cell transplantation provides insight into the role of T-cell-based immunotherapy (25). These techniques use many biomarkers to eliminate the malignancies from circulation. One of the most popular, as mentioned above is CD19. However, CD19 is minimally expressed on the surface. This lower expression does not allow the anti-CD19 CAR-T cells in bind and perform well to kill the malignant cells (26). It is evident then that more specific targets should be explored to treat MM. CD138 and B-cell maturation antigen (BCMA, CD269) have been identified as molecules that show promising immunotherapeutic potential in treatment of MM. By virtue of its expression in nearly all MM patients, CD138 is used as a primary diagnostic marker (27). The result of a clinical trial using second-generation of anti-CD138 CAR-T cell treatment of 5 refractory MM

subjects showed that, after 7-month follow-up treatment, the conditions of at least 4 patients were stable and one patient with advanced plasma cell leukemia had a reduction of myeloma cells in peripheral blood (28). The results of this clinical trial show CAR-T cell therapy for MM is tolerable and has anti-tumor immunity when using anti-CD138. BCMA also has potential in treatment of MM. This technique uses anti-BCMA to cripple the survival of long-lived plasma cells within MM patients. In a clinical trial, patients were administered BCMA/CD269 CAR-T cells in a dose-escalation. 6 patients were treated at the lowest 2 dose levels, low anti-myeloma activity or toxicity was noticed. Additionally, at the third dose level 1 patient obtained partial remission. Furthermore, two chemotherapy-resistant patients were treated on the fourth dose level with anti-BCMA CAR-T and achieved complete remission lasting for 17 weeks before relapse (29). Similar ongoing studies have shown therapeutic outcomes as well. Many new approaches to treat MM have progressed and been achieved in vitro. Hopefully, many more trials will usher in a new era of MM immunotherapy.

CAR-T cell as an immunotherapeutic effect has its advantages and disadvantages. In contrast with common adaptive immune cells, CAR-T cells have unique specificity and can eliminate cancer cells containing the unique and corresponding antigen. To some extent this technique will avoid killing healthy tissues. Furthermore, CAR-T cells have MHC-unrestricted activity, they can circumvent some major mechanisms by which tumors avoid MHC-restricted T cell recognition. For example, the down regulation of human leukocyte antigen class I molecules (30). In addition, the flexibility of the intracellular signaling domains permits the cell to counteract the down-regulation of co-stimulatory molecules. Also, the potential that

CAR-T cells can bind to multiple different antigen forms such as, carbohydrates, lipids, and proteins leads to many different potential combinations (31).

When treating hematological malignancies using CAR-T cells there are many advantages and promising results, however, there are some disadvantages as well. Neurologic toxicity is a serious potential toxicity from CAR-T cell therapy and it has been observed in some clinical trials. Some symptoms include, delirium, dysphasia, akinetic mutism, and seizures have been reported (32). Endothelial dysfunction, including vascular instability, capillary leak, blood-brain barrier disruption and disseminated intravascular coagulation are clinical evidence of neurotoxicity (33). Some of the prime molecules that have been identified as inducing acute neurotoxicity are, IL6, IFN-γ and TNF.

Similarly, another disadvantage of CAR-T cell therapy is cytokine release syndrome which is caused by release of the same proinflammatory cytokines mentioned above. Cytokine release syndrome (CRS) which manifests in high fevers, hypotension, and hypoxia potential organ failure is related to the release and production of IL6, IFN-γ and TNF, secondary to the production of CAR T-cell activation (34). Systemic corticosteroids are used to treat the hyper-proliferative effects of CRS. However, the downside is the rapid ablation of these T cells with decrease the efficacy of CD19 CAR-T cells, which could trigger a relapse (35). Thus, a new way of treatment has to be found. Tocilizumab, an anti-IL-6 receptor antagonist is being used to weaken the effects of CRS.

The last major concern for CAR T-cell is known as on-target/off-toxicity. Although CAR T-cells have relative specificity it has been observed that these cells can still affect

different levels of tissue and cause damage, especially in the lymphatic tissues. This includes B cell aplasia in anti-CD19/CD20 CAR-T cell treatment. Also, antigen escape, typically CD19-negative relapse of B cell malignancy, may challenge the success rate of CARs in blood cancer (36).

To broaden the application of the T-cell therapy, is desirable to develop techniques that increase the efficiency and promote safety at each step in the process. CAR T-cell therapy is an emerging and powerful therapy that is likely to be incorporated into the mainstream oncologic treatment. Although challenges remain in the manufacturing of the single-patient products, in which the raw materials are derived from the patient's own cells, technologies for isolating, culturing, and improving the efficacy are rapidly increasing. In the longer term the development of universal T-cell products from allogenic donors is in the works. However, it remains to be seen whether universal T-cell products are as potent and durable as those collected from the patients own blood stream.

In addition to manufacturing improvements, research is ongoing to identify, validate, and target lineage-specific antigens or tumor-associated antigens in other cancers. Theoretically, CAR T cells can result in deleterious effects by damaging healthy organs and structures when tumor-associated antigens are expressed on normal cells. Thus, the potential on-target but off-tumor effects must be investigated for each candidate antigen. Additionally, the benefit of therapy versus this potential cost must be considered for each disease before considering CAR technology. Preclinical models are currently investigating CARs coexpressed with other genes to improve various characteristics of the modified T cell, such as safety, persistence, and effector

functions. For example, current research involving engineering CAR T cells to also express the NKG2D receptor coupled to CD3-zeta (37). This receptor engages several stress ligands that are known to be upregulated on tumor cells, and evidence suggests that the genetically reprogrammed T cells also target inhibitory regulatory T cells (Tregs) for destruction. Additionally, CAR T cells can be further modified with additional transgenes that can express specific cytokines to promote the recruitment of tumor-associated macrophages, thereby enhancing both the antigen-presenting and tumor lytic activity of T cells (38). To date, this is only one of many different directions that CAR T-cell therapy can move in. However, many other inquiries still need to be made into the negative side effects of use.

Furthermore, research testing the preclinical function and safety of these techniques has largely been explored in murine models. While preclinical human xenograft mouse models in immune compromised mice played an important role in establishing proof-of-principle of the CAR T cell approach, they are limited in their clinical relevance and predictive value. Specifically, injected tumors in immune compromised mice may not fully recapitulate the immunosuppressive tumor microenvironment. Given the rapid and ongoing advances in CAR T cell technology in the laboratory, it now becomes necessary to identify and develop methodologies that will allow us to evaluate CAR T cell therapy in dogs with spontaneous cancers. This approach will enable us to determine and optimize the safety of novel targets and the therapeutic effectiveness of redirected T cells. This would accelerate the translation of the safest and most promising CAR therapies into the human clinic.

Pet dogs share a very close relationship and living environment with humans and develop cancers with similar biology and outcomes (39). Additionally, companion dogs with spontaneous cancers are being increasingly recognized as a relevant and potentially predictive preclinical model of human disease and could be effectively used to test the safety and efficacy of next generation CAR T cell therapies (40). In particular, canine cancer patients lend themselves far better than murine models for the evaluation of immunotherapies, including assessment of preconditioning regimes, engraftment, cellular trafficking into malignant lesions, transferred cell persistence, immune memory development, and effectiveness in preventing relapse (41).

The field of cancer immunotherapy has rapidly developed over the past decade. Such genetic modifications allow us to evolve an expanding field. These therapies have shown promise for the future of treating and potentially curing patients with advanced hematological malignancies.

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