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CAR T Cell Therapy

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by

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Abstract

With chemotherapy and radiation being the most common forms of cancer treatment, some cancer cells are able to develop resistance and persist in the body. Because of this resistance, there is a need for a new cancer therapy that can attack these persistent cells. CAR T cell therapy utilizes the patient's own immune system to attack and potentially eliminate these cancer cells. These cells have successfully been used against B cell malignancies, however, not much success has been seen when used against solid tumors. Solid tumors pose many challenges and threats these T cells must overcome in order to produce any anti-tumor effect. Challenges researchers run into include finding a suitable antigen to target, successfully trafficking to and infiltrating the tumor, and overcoming the microenvironment of the tumor. Researchers are continuously trying to figure out ways to overcome these challenges and increase the success of CAR T cell therapy.

Introduction

In the United States, cancer is the second most common cause of death, estimating 610,000 deaths in 2018 (*Cancer Statistics*, 2018). There is a necessity for a new and improved cancer treatment option. The primary forms of treatment for most types of nonsurgical cancers are chemotherapy and radiation. One of the disadvantages of these therapies is that cancers have been known to develop resistance over time. This development of resistance has created a strong need for a new, effective form of treatment that decreases relapse rate. However, there has been little advancement in the development of a form of treatment that is capable of completely eliminating lingering malignant cells. A promising form of treatment utilizes the patient's own immune system, notably lymphocytes, to control and potentially eliminating malignant cells and the cancer all together (Figure 1) (Almåsbak, Aarvak, & Vemuri, 2016). Years ago, Gross and colleagues developed and demonstrated the idea of redirecting cytotoxic T lymphocytes to the tumor cells. They concluded their work with the statement, "Chimeric T cell receptors with antitumor specificity will enable testing feasibility of this approach in combating human tumors" (Gross, Waks, & Eshhar, 2006). Gross and colleagues laid the groundwork for a series of generations utilizing the immune system and cytotoxic T lymphocytes as a way to target tumor cells. First generation chimeric antigen receptors (CARs) were created by directly fusing a tumor targeting

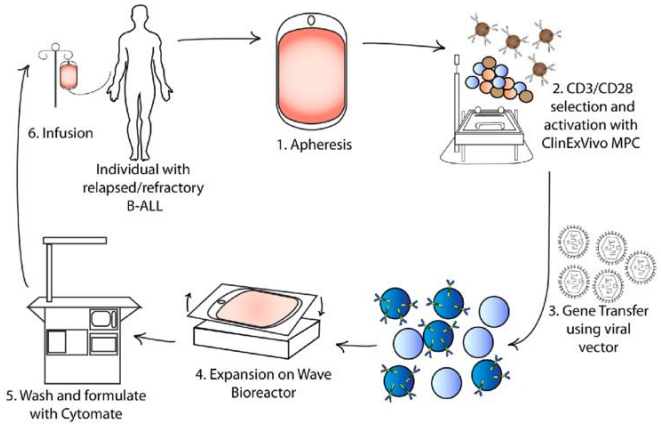

Figure 1. Production of CAR T cells in a GMP facility. Blood is taken from the patient (1) and is followed by T cell selection (2), and selection and activation of T cells (Davila, Bouhassira, et al., 2014).

antibody single chain variable fragment (scFv) to the signaling domain of the T cell receptor (TCR) signaling complex member CD3ζ (zeta). The first generation showed high target-cell specific killing *in vitro* and showed preclinical efficacies in mouse tumor models, however, when these T cells were used clinically in ovarian cancer, the results were not nearly as promising (Kershaw et al., 2006). There was no reduction of tumor burden in 14 patients due to a lack of specificity trafficking of the T cells to the tumor and short persistence of the T cells. Further research determined that first generation T cells were susceptible to activation induced cell death (AICD) in the absence of exogenous costimulation (Park & Brentjens, 2015). After years of continuous research, second generation CAR expressing T cells were developed.

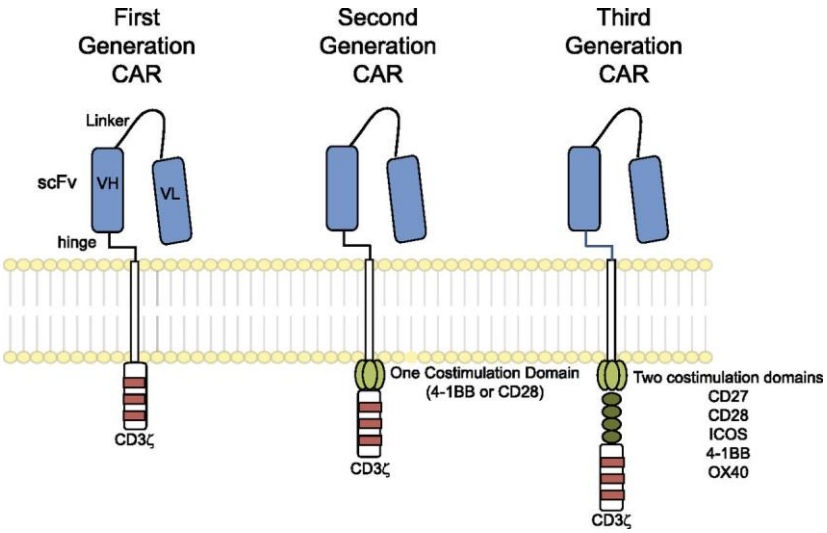

Figure 2. The various CAR T generations developed. First generation CARs have a single chain variable fragment. Second generation CARs differ from first generation CARs by the presence of a single costimulatory domain. Third generation CARs take second generation CARs one step further by having two costimulatory domains (Maus, Grupp, Porter, & June, 2014).

According to Essand and Loskog, second generation CARs were constructed to provide signaling through CD3ζ chain and CD28 costimulatory molecule by placing the signaling domains in series as a single gene multidomain product (Essand & Loskog, 2012) (Finney, Lawson, Bebbington, & Weir, 1998). This design was able to mediate 20 times more IL-2 production upon stimulation with solid-phase antigen when compared with first generation CARs. It was discovered that second generation CARs with the costimulatory signals, from B7

and tumor necrosis factor receptor (TNFR), in series with CD3ζ, promote self-sufficient clonal expansion and enhanced effector function in resting human T cells. In order to create the most effective second generation CAR T cells, studies were done to compare various signaling domains. Various studies reported increased persistence, tumor localization, and antitumor activity of CAR T cells with a 4-IBB signaling domain when compared with CARs with CD28 signaling domain (Essand & Loskog, 2012).

As a way to try and improve second generation CARs, third generation CARs were constructed containing a CD3ζ chain, CD27, CD28, ICOS, and OX-40 or 4-IBB signaling domain (Figure 2). Ideally, these receptors would provide a full complement of activation, proliferation, and survival signals for enhanced antitumor activity; however, using third generation CARs has been somewhat disadvantageous. Despite the promising preclinical results, there were concerns of triggering lethal cytokine storms within patients treated. Another concern of the third generation CARs, is the reduction of the signal threshold to a level at which the activation of grafted T cells can occur without triggering antigens (Essand & Loskog, 2012).

With these advances in CAR T cell technology and the many studies that have been performed using all three generations of CAR, these cells have been successful or on the road to success when treating blood borne and solid tumors. For B-cell malignancies, CAR T cells are expected to be a mainstream therapy, especially for refractory or relapsed B-cell malignancies (Almåsbak et al., 2016). By changing the antigen T cells target, CARs can be used for many different types of cancers, both hematologic and solid. Though treatment of hematologic cancers with CAR T cells has been more successful, treating solid cancers has posed difficult challenges that researchers are trying to overcome.

Review

CAR T cells have changed the way various cancers are treated. The targeting of tumor cells begins when a CAR on a T cell binds to its antigen on the tumor, leading to signaling and activation. Activation can lead to the killing of tumor cells through the release of granzyme and perforin or by the activation of other immune system components by CD4+ T cell cytokine release. The use of T cells can lead to the formation of memory T cells, developing long-lived T cells that are specific to that specific tumor type (Figure 3) (Davila, Bouhassira, et al., 2014).

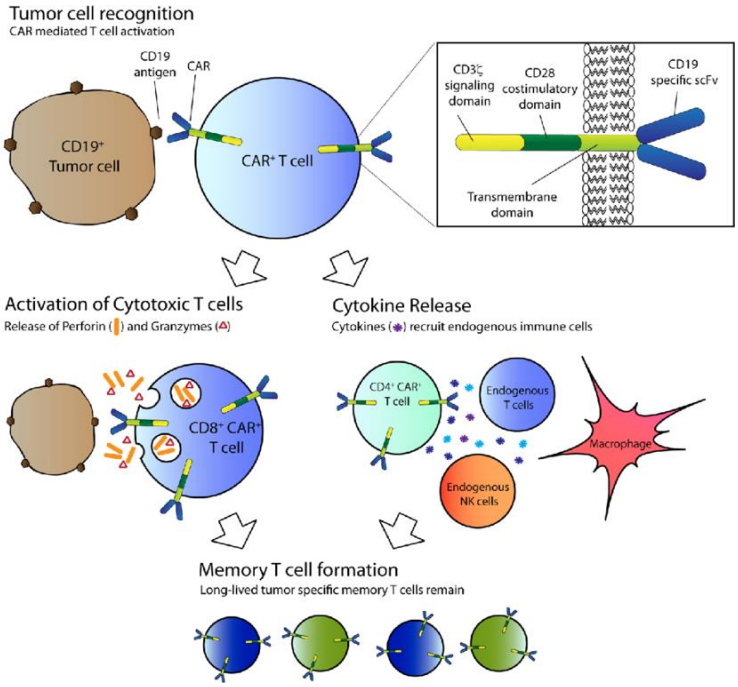

Figure 3. The recognition of tumor cells can lead to two possible outcomes: activation of cytotoxic T cells or cytokine release recruiting other immune cells (Davila, Bouhassira, et al., 2014).

Treatment of B cell malignancies with CAR T cells

Using the mechanism described above, CAR T cells have shown much promise and are expected to be the mainstream treatment. B cell malignancies include B cell acute lymphoblastic leukemia, B cell non-Hodgkin's lymphoma, chronic lymphocytic leukemia, and Hodgkin's lymphoma. Many of the studies conducted (65%) that involved T cell therapy were directed towards hematological malignancies. Of those studies, CD19 was the most common antigen targeted in B cell cancers, used over 80% of the time. There are studies to investigate other potential target antigens, such as CD20, CD22, CD30, ROR1, κ light chain, CD123, CD33,

CD133, CD138, and B-cell maturation antigen (Berger et al., 2014) (Dotti, Gottschalk, Savoldo, & Brenner, 2014) (Carpenter et al., 2013). The most successful outcomes have come by targeting CD19, CD20, or CD30, however, the most advancement has been seen by using CD19-specific CAR T cells for B cell acute lymphoblastic leukemia with complete remission rates of 70-94% (Z. Wang, Wu, Liu, & Han, 2017). One of the first successful studies of the use of CD19 was conducted at the National Cancer Institute in 2010. Follicular lymphoma patients were treated with this form of CAR T cell therapy, developing partial remission after receiving chemotherapy and an infusion of retrovirally transduced T cells that expressed a second generation CD19 CAR with a CD28 costimulation domain (Kochenderfer et al., 2010). Shortly after this study showed success, a group at the University of Pennsylvania showed antileukemia efficacy in T cells transduced with a lentiviral vector carrying a CD19 CAR with a 4-IBB costimulation domain (Kalos, Levine, et al., 2011) (Kalos, June, Levine, Bagg, & Porter, 2011). Of the three end-stage advanced chronic lymphocytic leukemia patients treated, two developed complete remission and the other developed a partial response. This study exhibited the expansion of CAR T cells *in vivo* correlated with clinical responses (Almåsbak et al., 2016).

Treatment of solid tumors with CAR T cells

Much success has been shown with blood-borne tumors, but little has been seen with solid tumors. There has been a steady rise in the number of clinical trails that focus on solid tumors. These clinical trials target carcinoembryonic antigen, diganglioside GD2, mesothelin, interleukin 13 receptor α (IL13Rα), human epidermal growth factor receptor 2 (HER2), fibroblast activation protein, and L1 cell adhesion molecule (Gill, Maus, & Porter, 2016) (Fousek & Ahmed, 2015). Despite these trials, little success has been achieved. The two more successful

trials have used GD2 CARs to target neuroblastoma, 3 of 11 patients develop complete remission, and HER2 CARs for sarcoma, 4 of 17 patients showing stable disease (Louis et al., 2011) (Ahmed et al., 2015). The reasons for such little success are yet to be discovered and pose to have many factors. When compared to blood-borne malignancies, solid tumor landscapes contain many barriers (Newick, Moon, & Albelda, 2016). In order for CAR T cells to impact solid tumors, they must successfully travel to the site of the tumor regardless of potential T-cell chemokine receptor-tumor-deprived chemokine mismatches. Also, CAR T cells must infiltrate the stromal elements of the tumors in order to elicit tumor-associated antigens-specific cytotoxicity, despite antigen loss of heterogeneity. Even if the CAR T cells make it to the tumor and are able to infiltrate it, the T cells must overcome the environment, the presence of suppressive soluble factors and cytokines, suppressive immune cells, and T-cell intrinsic negative regulatory mechanisms and overexpression of inhibitory molecules. Within the environment of the tumor, there is oxidative stress, the T cells would have to overcome nutritional depletion, and the T cells would experience a low pH and hypoxia (Newick et al., 2016) (Knochelmann et al., 2018).

Difficulties faced when treating solid tumors with CAR T cells

One of the obstacles that must be overcome when using CAR T cell therapy against solid tumors is determining which tumor-associated antigen is optimal and would be the most effective. In blood-borne malignancies, CD19 is consistently expressed, leading to effective treatment with CD19 CAR T cells. However, with solid tumors, identifying specific antigens to target has posed a challenge. Currently, 30 different tumor-associated antigens have been identified. Of the 30 recognized and evaluated, neoantigens were particularly attractive to use for

CAR T cell therapy because they are only expressed on tumor cells (Restifo, Dudley, & Rosenberg, 2012). With further research, neoantigens were discovered to be a product of tumor-specific mutations and are highly individualized, not making them an ideal target of CAR T cell therapy. Despite tumor-specific mutations of neoantigens, many generalized neoepitopes have been identified (Newick et al., 2016).

Instead of using only tumor-specific antigens, antigens expressed only during developmental growth have been potential targets of CAR T cell therapy. An example of an antigen expressed during development and is restricted in normal adult tissues is CEA. This has been a target for mouse studies, showing evidence of tumor eradication by CEA-CAR T cells (Chmielewski et al., 2012). Targeting this antigen has caused serious side effects, specifically transient colitis, in three metastatic colon cancer patients (Parkhurst et al., 2011). Other types of antigens have been researched and are currently being tested in mouse studies and clinical trials (Newick et al., 2016).

As soon as an antigen has been identified, generated, and infused into a cancer patient, a major obstacle that is immediately encountered is the ability of the CAR T cells to get to the tumor and successfully infiltrate it. This process is dependent on the expression of adhesion receptors on T cells and the tumor endothelium, and a "pairing" between the chemokine receptors on the CAR and the chemokines tumors secrete. However, there is often a mismatch of chemokine/chemokine receptor, with tumors producing very small amounts of ligands that enhance CD8+ recruitment (Harlin et al., 2009). Often times, tumors that express less chemokines evade host surveillance by impairing the recruitment of effector T cells and their ability to infiltrate the solid tumor. Some chemotherapy drugs are able to enhance the recruitment of CD8+ T cells and reduce tumor growth by inducing CXCR3-ligand and CCL5

(Hong et al., 2011). Studies have shown that two groups enhance the infiltration of T cells and augment antitumor activity of the CAR T cells, these groups are GD2 in neuroblastoma and CCR2 on CAR T cells (Craddock et al., 2010). One of the main struggles scientists face is the individuality of each form of cancer (Knochelmann et al., 2018). In order to create effective CAR T cells and traffic them to the correct areas, where the solid tumors are located, the unique chemokine profiles have to be determined and understood.

Once the CAR T cells have made it to the solid tumors, another obstacle is run into, the microenvironment of the tumor. Normally, the microenvironment of the tumor is hostile, both physically and metabolically. A couple of physical barriers the T cells must overcome are stroma and high tissue pressure that prevents extravasation. To overcome these barriers, some studies used FAP-CAR T cells to reduce the number of tumor fibroblasts. Other studies used specific CAR T cells that secrete an enzyme that degrades the matrix. These two methods, FAP-CAR T cells and enzyme secreted CAR T cells, have only been tested in animal models, they have yet to be tested on humans (L.-C. S. Wang et al., 2014) (Caruana et al., 2015).

Despite the hostility of the physical environment of the tumor, the metabolic landscape poses a threat to the viability of CAR T cells. Tumor microenvironments are, often times, rich in suppressor cytokines, such as TGF-β and IL-4, and inhibitory molecules, such as PD-L1. PD-L1 helps the tumor escape the host immune system. In order to overcome this, researchers have added domains to the CAR T cells. These domains either limit suppressive signaling or convert the suppressive signals into activating signals (Foster et al., 2008). Recently, studies have used a chimeric cytokine receptor to bind IL-4. When IL-4 binds the receptor, the therapeutic IL-7 signal pathway phosphorylates STAT5 and polarizes the cell toward an inflammatory Th1 response (Leen et al., 2014). Another similar study was done using a PD-1/CD28 chimeric

switch receptor. This switch receptor was designed to convert an exhaustive stimulus into a costimulatory signal, enhancing cytokine production and efficacy (Liu et al., 2016) (Knochelmann et al., 2018).

Though the suppressive cytokines are a major hurdle that must be overcome in order for the CAR T cells to survive the tumor microenvironment, other microenvironment issues include hypoxia and nutrient starvation. Because of hypoxia and nutrient starvation, the generation of lactate is increased, leading to acidosis within the tumor (Fischer et al., 2007). The deficiency in nutrients, specifically tryptophan, arginine, and lysine, can activate the integrated stress response within the T cells. The activation of the integrated stress response can cause shutdown of translation and stimulate an autophagy response in effector T cells as a way to generate a source of nutrients (Howie, Waldmann, & Cobbold, 2014) (Newick et al., 2016).

Adverse effects of CAR T cells

CAR T cells have provided a way to attack refractory or relapsed malignancies, but there are some adverse effects of using CARs. These adverse effects can be immediate or delayed, mild or severe, or persist for a short or long period of time. One of most prevalent adverse effects is the onset of immune activation, known as cytokine release syndrome (Lee et al., 2014). This results in the increase in inflammatory cytokines, specifically IL-10 and IL-6. Clinical features include high fever, malaise, fatigue, myalgia, nausea, anorexia, tachycardia, capillary leak, and many more. The severity of CRS is determined by the burden of the disease at the time of infusion; patients with high tumor burden experience more severe CRS. Researchers have been studying the use of IL-6R blockade as a treatment for CRS while looking at its effect on proliferation, persistence, and antitumor effect (Maude, Barrett, Teachey, & Grupp, 2014).

Another adverse effect reported with the use of CAR T cell therapy is on-target/off-tumor recognition. This often occurs because the epitope targeted by the CAR T cells is often shared with normal cells in other tissues (Curran, Pegram, & Brentjens, 2012). The severity of this on-target/off-tumor ranges from manageable lineage depletion to severe toxicity possibly leading to death. This is often seen in various organ systems: gastrointestinal, pulmonary, and hematologic. On-target/off-tumor recognition was seen in one trial utilizing a carboxyanhydrase-IX-specific CAR T cell for renal cell carcinoma. This utilization of these specific CARs led to the development of cholestasis because of carboxyanhydrase-IX expression on bile duct epithelium (Lamers et al., 2006). An example of manageable lineage depletion was seen when CD19-specific CAR T cells were used. The CD19-specific CARs targeted normal B cells resulting in B-cell aplasia, which required an infusion of pooled immunoglobulin as prophylaxis from infectious complications (Kochenderfer et al., 2010). On the other hand, on-target/off-tumor recognition can be lethal. An example of this was seen in a patient treated with CAR T cells specific for the cancer-associated antigen HER-2/neu. This patient quickly developed respiratory failure and multi-organ dysfunction, ultimately leading to death. The death of the patient was attributed to reactivity against pulmonary tissue expression of HER-2/neu and the high dose of infused CAR T cells (Morgan et al., 2010).

Recent technological advances in T cell engineering with retroviral and plasmid vectors allow the generation of high numbers of tumor targeting T cells by introducing tumor specific T cell receptors (TCR) or CARs. CARs exhibit high-affinity major histocompatibility complex (MHC) independent recognition of any surface antigen (Almåsbak et al., 2016). The use of retroviral vectors has produced adverse effects within patients, from the retroviral vector insertion near the LMO-2 oncogene. The risk of transgene insertion into differentiated T cells

leading to induced malignant transformation has been a concern of researchers. However, there have been no reported cases of transformation after the infusion of genetically modified T cells. This can be due to the fact that the LMO-2 oncogene is silent in T cells, therefore, making it an undesirable location of retroviral integration. Overall, the risk of insertional oncogenesis following gene transfer into T cells is low, but there is still a risk it may occur (Scholler et al., 2012).

Managing toxicity of CAR T cell therapy

Of the adverse effects cited above, there are a few more worth mentioning. Following CAR T cell therapy, there is a risk of neurological toxicity, anaphylaxis, and off-target antigen recognition (Bonifant, Jackson, Brentjens, & Curran, 2016). Though there are many risks to be concerned about, there are many measures being taken to manage toxicity and these adverse effects, especially in hematologic malignancies. The most common toxicity developed by patients after the administration of CD19-specific T-cells is the uncontrolled immune activation in the form of cytokine release syndrome. Tocilizumab had demonstrated IL-6R blockade, resulting in the reversal of cytokine release syndrome symptoms. Corticosteroids have also been used and have demonstrated immunosuppressive qualities. However, the prolonged use of corticosteroids has resulted in the diminished persistence and efficacy of CAR T cells (Davila, Riviere, et al., 2014).

As this technology has progressed and developed through the years, the integration of suicide genes has allowed for the selective depletion of CAR T cells, helping with the wide range of toxicities that can develop (Marin et al., 2012). Another way to selectively deplete CAR T cells is to program these cells to express a CD20 and EGFR cell-surface antigens with the

triggering of cell death via the infusion of the associated monoclonal antibody. The expression of these cell-surface antigens allows for the selection and tracking of the genetically modified T cells (Philip et al., 2014).

CAR T cell therapy in dogs

The development and use of CAR T cells in humans has sparked interest in using this therapy in canines. Canine cancers closely parallel those in humans through the biology, behavior, and genetic aspects. In dogs, Non-Hodgkin's Lymphoma is the most common cancer and has posed many difficulties when treating. Combinations of chemotherapeutic agents have shown success by leading to remission in about 75% of dogs, however, most dogs relapse within six to nine months. Dr. Nicola Mason, a professor at eh University of Pennsylvania's School of Veterinary Medicine, and Dr. Avery Posey, an instructor at the University of Pennsylvania's School of Medicine, have developed CAR T cell therapy for canine use. In 2018, a first-in-dog clinical trial took place at the University of Pennsylvania School of Veterinary medicine. Patients had relapsed, refractory B cell lymphoma or leukemia. Much success was seen in the preliminary results, but further research must be done to improve the persistence and function of these CAR T cells. With the use of these canine models, veterinary oncologists and researchers hope to establish better therapeutic platforms that will improve the effectiveness in both blood-borne cancers and solid tumors and reduce side effects in both humans and canine patients (Lee & Mason, 2018).

Conclusion

CAR T cell therapy is a cancer treatment option that will someday become mainstream for many types of cancers. In order for this to be achieved, the safety and efficacy of this treatment has to be improved, and with further research, it will be. Thus far, CAR T cells have demonstrated efficacy and success in various hematological cancers. Clinical data seems promising in solid tumors, including neuroblastoma and tumors overexpressing mesothelin, HER2 and EGFR. However, utilizing CAR T cells for solid tumors has posed quite a challenge and has not shown much success. As research continues, more types of cancers will be targeted and the safety and efficacy will be increased. In order for this treatment to be effective, it must work at the cellular level and it has to be affordable. As this treatment becomes more mainstream and generates success, the industrialization of the development of these cells will become more important.

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