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CAR T-Cell Therapy: A New Road to Treat Cancer

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Abstract

Accounts of cancer have been around for many centuries. The 'War on Cancer' has officially been going on since 1971 with the passing of the National Cancer Act. The aim of this was to understand more about the behavior and biology of cancer in addition to the development of efficacious drug therapies. Although such therapies have been discovered and developed, the precision of 'traditional chemotherapeutics' have shown not to be as precise as desired. For example, these drugs aim to kill cancer cells that are dividing at a higher rate than normal relative noncancerous cells. However, noncancerous cells that require to divide rapidly (gut, epidermis, keratinocytes of the scalp) are prone to these traditional chemotherapies and as such patients experience undesirable side effects. The aim of this paper is to address the development of a novel cellular-based cancer therapy called CAR T-cell therapy. The structure of such a therapy enables greater precision than traditional chemotherapies and potentially greater efficacy than standard cancer immunotherapy drugs. The structure, function, and cytotoxic mechanisms of CAR T-cells will be addressed along with landmark clinical trials that have lead to the US FDA approval of two CAR T-cell therapies. In addition to successes, consequences of this therapy will be discussed involving toxicities, economic costs, and hardships against solid tumors.

Keywords: CAR T-cell, cytotoxic mechanisms, CAR T-cell generations, CAR T-cell structure and function, tumor microenvironment, approved therapies

Introduction

Cancer has been noted since the ancient Egyptian times. The word 'cancer' comes from the Latin word 'crab.' More so, the crab is the zodiac sign of Cancer. Cancer is one of the most devastating and debilitating diseases in the modern era. Millions are diagnosed in the United States each year and it is the second most common cause of death behind heart disease. With such a devastating impact to those diagnosed and to their loved ones, relentless hours of research and funding have been spent to try and elucidate what cancer is, what causes cancer, what risk factors there are, how it can be most effectively treated, and finally how it can be cured. Throughout the years, it has been found that there are several risk factors that contribute to the development of cancer. These include, but are not limited to, tobacco smoking, excessive alcohol consumption, infectious diseases (Human Papilloma Virus, Hepatitis C, Hepatitis B), excessive sun exposure and/or tanning bed use, and physical inactivity.[1]

It's widely known and agreed upon that cancer is the result of an accumulation of several mutations that induce cell division to become unregulated. Therefore, traditional chemotherapeutic agents have aimed to exploit such cell cycle metabolic pathways of cancer cells to impede their aberrant division. Some of these drugs unfortunately do not have the ability to differentiate between a cancerous cell and a normal cell. For example, due to the fact cancer cells rapidly divide, traditional chemotherapeutic drugs work to target such rapidly dividing cells. Unfortunately, there are other tissues that can be healthy and divide rapidly such as gastrointestinal cells of the intestines, epidermal cells of the skin, and keratinocytes of the scalp. Due to this commonality, traditional chemotherapeutic drugs cause unfavorable side effects at the sights of these normal, healthy, rapidly dividing cells. Fore example, some side effects could include hair loss, diarrhea, and even fragility of the skin to name a few. Due to such undesirable

side effects, cancer researchers have sought to develop chemotherapeutics that are even more precise to target cancer cells vs. noncancerous cells.

Fortunately, there are some chemotherapeutic drugs on the market that do have the ability to target cancer cells more precisely. This is dependent upon the type of cancer the patient has been diagnosed with as well as the specific receptors those cancerous cells express. The cancerous cells may express a unique receptor that a normal cell does not, or the cancerous cells may express a certain receptor in a higher ratio compared to normal cells. Although targeted chemotherapy provides a better means to differentiate cancerous from noncancerous cells, it is not perfect and still comes with harsh side effects like traditional chemotherapy can cause. Traditional and targeted chemotherapeutic drug regimens have had their successes in many cancer patients but also have experienced failure in others. Again, there are a multitude of factors that play a role in the success of a chemotherapy regimen. For example, the type of cancer can give insight into how aggressive it is and therefore the prognosis. More so, the staging of the cancer at the time of diagnosis plays a crucial role into the success of treatment. Unfortunately, cancer can have the ability to become resistant to the chemotherapy given to the patient by way of upregulating drug efflux ABC transporters or by downregulating the expression of those proteins/receptors being targeted by the chemotherapy drugs, to name a few ways. Also used in conjunction with chemotherapy is radiation therapy and surgical excision. Advances in technology and research have allowed radiation therapy to provide a precise and specific route to treat the cancer. These other two forms of treatment can have their limits as well. Radiation therapy may not be ideally suited for those cancers that have spread considerably in the GI tract. In addition, surgical excision may not always provide a safe and operable way to reach a tumor for excision. It should be stated that no therapy or drug is perfect and always has a benefit-to-risk

ratio. Cancer treatment has advanced considerably through the years to prolong and even save the lives of those diagnosed with it.

Sometimes the best medicine is preventative medicine. As such, quality nutrition plays a significant role in the smooth, normal functioning of the human body. An important and vital system that sustains the well-being of the human body is the immune system. The immune system is comprised of diverse, vital cells combating not only foreign pathogens but also cancer. It is logical to deduce from this that cancer develops largely in part due to the immune system being defective in some manner. Therefore, cancer immunotherapy has been a hot area of research for many years. The well-known cells involved in detecting and destroying cancer cells directly are the $CD8^+$ cytotoxic T lymphocytes (CTLs), natural killer (NK) cells, dendritic cells, and macrophages. Of course, this list is by no means exhaustive. However, it has been an intensive goal to exploit the cytotoxic effects of these cells and stimulate or manipulate them to target cancer cells directly.

CAR T-Cell Overview

Fortunately, there has been recent advances and findings for a novel therapeutic approach to treat cancer, with the most promising patient outcomes being those diagnosed with nonsolid cancers such as lymphomas and leukemias. This therapy is unique in that it is not a drug but rather a genetic manipulation and engineering of the T-cells of a patient's immune system to precisely recognize and differentiate cancer cells from healthy cells. The T-cells are collected from the patient's blood and then separated from red blood cells and other components via leukophoresis. This novel, nondrug, cellular therapy is known as chimeric antigen receptor (CAR) T-Cell therapy. The term 'chimeric' derives from 'chimera' meaning mixture or combination. Thus, CAR implies the T-cell has been genetically altered to express a combinatory structure of an

antibody, a transmembrane domain, and the constant regions of a T-cell receptor (TCR) for the intracellular domain. The extracellular domain of a CAR consists of a single chain variable fragment (scFv). The scFv is connected to the transmembrane domain by a hinge domain. This scFv mimics the variable region of a typical antibody which can bind a specific epitope it recognizes. These scFv domains of a CAR are therefore engineered to be specific for a protein that is expressed by a cancer cell. Theoretically, the manipulation of scFvs can provide a highly specific means to target many different cancer types. Upon scFv binding a cancer cell antigen, the signal is transmitted to the intracellular domain of the CAR resulting in T-cell activation. The CAR confers a couple advantages due to its structure. First, the extracellular scFv domain provides direct specificity against cancer cells expressing antigens and enables the bypassing of antigen presentation via Major Histocompatibility Complex (MHC) to stimulate and activate T-cells. However, there is a disadvantage to the specificity the scFv domain confers. Not all cancers express a unique antigen that healthy cells do not. Some antigens expressed by tumors can be shared between healthy and cancerous cells. More optimistically, some antigens may be overexpressed in greater density in cancerous cells vs. healthy cells thereby favoring the eradication of cancerous cells relative to normal cells.

CAR T-Cell Structure, Function, and Evolution

The development of CAR T-cells has been an extensive process that has yielded promising outcomes in some clinical trials. There has been development of several generations of CAR T-cells that have been both researched intensively and/or tested clinically. The first CAR T-cells developed were known as first generation CAR T-cells. These consisted of an scFv extracellular domain attached to a hinge domain. This hinge domain is the link between the transmembrane domain and the scFv domain. The unique feature of the first-generation CAR T-cells is the

identity of the intracellular domain which is linked to the transmembrane domain as described above. The first-generation CAR T-cell consisted of the cluster of differentiation-3 zeta (CD3ζ). In normal T-cell activation, the TCR needs to bind to an antigen presented on MHCI or MHCII (dependent upon whether the T-cell is $CD4^+$ or $CD8^+$). Once bound, there needs to be cross-linking of TCRs to optimally activate the intracellular signaling cascade within the T-cell. The CD3ζ is an accessory costimulatory molecule associated with the TCR and upon TCR binding to a presented antigen on MHC, the cytoplasmic domain of CD3ζ becomes activated. The cytoplasmic domain of CD3ζ contains what are known as immunoreceptor tyrosine-based activation motifs (ITAMS). These motifs are tyrosine rich and will be phosphorylated by a protein called Lck. The subsequent phosphorylated tyrosines will become sites of docking for adaptor proteins as well as another well-known protein named zeta-associated protein of 70kD (ZAP-70). Ultimately two signal transduction pathways will ensue upon TCR binding to a presented antigen. One pathway is the Ras/Rac-MapK pathway and the other is the Ca^{2+}-NFAT pathway. The latter pathway results in the activation of the transcription factor NFAT which will stimulate the expression of IL-2 and its respective receptor (IL2-R). IL-2 is known as the T-cell growth factor. The former pathway will result in the activation of the transcription factor activating protein-1 (AP-1) which will also stimulate the expression of T-cell activating genes. Given the overview of normal TCR activation, it can be seen that CD3ζ is an important component of the cytoplasmic domain of a CAR T-cell helping with activation, persistence, and proliferation. However, it has been found in early clinical trials[3] as well as in other studies that the use of CD3ζ alone is not sufficient to induce CAR T-cell persistence and proliferation.[2] With the additional integration of another costimulatory molecule, CD28 or 4-1BB, comes a more robust proliferation and persistence of CAR T-cells. With this came the development of

what are called second generation CAR T-cells. The main difference between the second generation and the first is that the second generation has an additional costimulatory endodomain in the CAR (two total). CD28 and 4-1BB intracellular domains are what have been used in clinical trials and have shown promising results against hematological malignancies. When positive clinical results were observed using second generation CAR T-cells, further modification of CARs were engineered to consist of at least three or more total costimulatory endodomains. It is worth noting that these additional costimulatory molecules are linked in tandem with the CD3ζ domain. These became known as third generation CARs, fourth generation CARs, and so on. The additional costimulatory domains that have been added and have shown enhanced CAR T-cell proliferation, persistence, and activity are CD28, 4-1BB, OX40, CD27, and inducible T-cell co-stimulator (ICOS or CD278). As reported by Guedan e*t al.*, a third generation CAR T-cell with the intracellular domains CD3ζ, 4-1BB, and ICOS showed greater persistence and lethality towards tumors given that the ICOS domain is proximal to the transmembrane domain of the CAR.[4] It was shown from this same study that ICOS mediates the phosphoinositide-3 kinase (PI3K) signaling pathway resulting in increased expression of Akt. The endodomain ICOS plays a critical role in signal transduction in that it provides a docking site for PI3K signaling activation. An important well-known T-cell survival factor protein is Akt which can help with the persistence of T-cells to target cancer cells. Other exciting findings have been found via the use of ICOS as a costimulatory molecule in second generation CAR T-cells in mice against a human infused tumor. A study was conducted by Paulos e*t al.* and was reported that the use of CAR T-cells with ICOS-CD3ζ costimulatory intracellular domains, upon stimulation of the CAR T-cells, resulted in the differentiation, persistence, and antitumor activity of T-helper 17 cells (T_H17) compared to CAR constructs with

CD28- CD3ζ intracellular domains.[7] Additionally, findings from this study reported ICOS stimulation induced secretion of IL-21, IL-17, and IFN-γ. These secreted cytokines pose antitumor effects and boost the activity and persistence of CAR T-cell activity.

Fourth Generation CARs (TRUCKS)

Fourth generation CAR T-cells are a unique design maintaining the standard structure of the CAR but also contain the ability to release a transgenic protein upon CAR T-cell activation. This generation of CAR T-cells are also known as 'T-cells redirected for universal cytokine-mediated killing' (TRUCK). As the name implies, the transgenic protein is a cytokine that can be induced or released in a constant manner into the extracellular space which will then carry out antitumor effects. Commonly tested cytokines used thus far have been IL-12 and IL-18. It has been reported that the use of IL-12 secreting TRUCKs resulted in significant antitumor activity as well as even suppression of the tumor microenvironment and suppression of Tr_{eg} cells.[5] In addition, IL-18 secreting CAR T-cells (TRUCKs) showed similar results to IL-12 secreting CAR T-cells. It was found by Hu *et al.* that such IL-18 secreting CAR T-cells showed significant proliferation and antitumor activity.[6] The further innovation and refining of CAR T-cells provides a more potent way to attack cancer cells. It will be interesting to see future results from clinical trials in utilizing TRUCKs to combat cancer.

More Diverse CAR Constructs: 5th Generation/Next-Generation CAR T-cells

Even though second-generation CAR T-cells have shown promising results in clinical trials against hematological malignancies, they are by no means one hundred percent effective. Furthermore, more success is trying to be replicated against solid tumors such as pancreatic, liver, colon cancer, etc. Nor do they convey toxicity free side effects from the therapy. Therefore,

even more intensive research has continued into the development and design of CAR constructs that can more effectively and precisely target the tumor and not healthy tissue as well as to reduce toxicity as much as possible. So far there have been successes on such developments and designs and several of them will be mentioned and include: Pooled CAR T-cell, Multi-CAR T-cell, Tandem CAR T-cell, Conditional CAR T-cell, iCARs, and Suicide Switch CAR T-cells. Pooled CAR T-cells are comprised of at least two or more CAR units each consisting of their own unique scFV domain expressed in different populations of CAR T-cells. More simply put, there are multiple CAR T-cells each expressing a unique CAR against a tumor antigen. Multi-CAR T-cells contain multiple unique CARs, each recognizing their tumor antigen via their scFv domains. As can be deduced from this construct, this can enhance the precision for targeting tumor cells rather than healthy cells which is known as 'on-target on-tumor' activity. The Tandem CAR T-cell has multiple, usually two, unique scFv domains linked together. Conditional CAR T-cells consist of two separated CAR constructs. One construct consists of the activation domain (CD3ζ) and the other consists of a costimulatory domain (CD28, 4-1BB, OX40 or ICOS, etc.). Each construct contains a unique scFv domain recognizing different tumor antigens. iCARs have been genetically manipulated to express a receptor. Once the respective ligand binds to this receptor, it will induce an inhibitory signal in the CAR T-cell. The ligand that binds this receptor is expressed by healthy tissue and therefore provides a 'safety' mechanism to prevent cytolytic activity against healthy cells. In a similarly related concept, suicide switch CAR T-cells can also be engineered to contain a suicide switch/gene, usually Caspase-9, that can be activated to abort the cytotoxicity of CAR T-cells via apoptosis.

Cytotoxic CAR T-cell Killing Mechanisms

The normal cytotoxicity of T-cells are exploited and phenotypically consistent with the killing action of CAR T-cells. There are several different pathways, working together, to induce cell death of cancer cells. The pathways that will be touched upon include: Perforin/Granzyme (P/G) release, Fas/Fas-Ligand interaction, and the release of cytokines into the interstitial space. The P/G release pathway involves the regulated exocytosis of cytotoxic granules into the extracellular medium which will act on the tumor cell's plasma membrane. The lytic enzymes released will perforate the cell membrane allowing the entry of granzymes. These granzymes promote activation of caspase enzymes in the tumor cell and subsequent induction of both caspase dependent and independent pathways.[8] The second pathway by which CAR T-cells mediate antitumor activity is the Fas/Fas Ligand pathway. CAR T-cells, upon activation, will upregulate the expression of Fas-Ligand at the cell surface increasing the probability of binding to the Fas receptor on the cancer cell. Once this pathway is activated, it will stimulate downstream signaling events leading to the formation of death-inducing signaling complex (DISC) and subsequent activation of capsase-3 leading to apoptosis of the tumor cell. Cytokine release directed by CAR T-cells was described above in 'Fourth Generation CARS (TRUCKs).' It may be worthy to note that cytokines of interest being studied involve not only the prior mentioned IL-12 and IL-18, but also the release of IFN-γ. IFN-γ is a well-known antitumor cytokine, surveilling the presence of cancer. However, it has been discovered in several studies that IFN-γ receptor is downregulated by cancer cells, a phenomenon known as tumor evasion owing to therapy resistance by tumors.

Hardships Against Solid Tumors

Much of the clinical success using CAR T-cell therapy has been against hematological malignancies, such as B-cell lymphomas and leukemias. Several factors are at play contributing

to such success and include the ease of the CAR T-cells traveling throughout the body to reach the tumor sites, a highly specific tumor antigen that is expressed by many hematological malignancies (CD19 and CD20), as well as less of a struggle engaging against the stroma of the tumor microenvironment. CD19 is an ideal tumor antigen to target because it is ubiquitously expressed by normal B-cells throughout all the stages of differentiation from the pro-B cell stage onward as well as on those B-cell malignancies. It is also expressed by follicular dendritic cells but is not expressed by other tissues or hematopoietic stem cells.[8] This helps to ensure more precise targeting by anti-$CD19^+$ CAR T-cells and thus reduce toxicity at other tissues. Furthermore, since CD19 is expressed on both healthy B-cells and cancerous B-cells, CAR T-cells will work to eliminate both resulting in what is called aplasia of healthy B-cells. Fortunately, this outcome can be easily managed via immunoglobulin replacement therapy. This entails the delivery of mainly IgG into the treated patient. Delivery can be by way of intravenous or subcutaneous routes. Unfortunately, many solid tumors such as liver cancer, pancreatic cancer, and colon cancer do not express unique tumor antigens like that seen in the B-cell malignancies. Thus, it's critical to identify tumor antigens of such cancers that express it at higher numbers relative to normal tissue as well as that tumor antigen ideally needs to be expressed in a local fashion rather than a systemic fashion. For example, CAR T-cells were designed to target ERBB2 in a patient with breast cancer. Unfortunately, the patient experienced a severe adverse event during the course of her treatment and died. It was later found that ERBB2 is widely expressed not only by breast tissue of the cancer cells but also on normal tissue of the lungs.[9]

In addition to solid tumors not expressing unique antigens associated with the tumor, CAR T-cells have more of a difficult time traveling to and infiltrating the tumor. The CAR T-cells have

to traverse the circulatory system and exit at the location of the tumor. In addition, once at the tumor location, the CAR T-cells have to overcome and penetrate a hostile environment known as the tumor microenvironment. This tumor microenvironment contains immunosuppressive cytokines, immunosuppressive cells (T_{regs} and T_H17 cells), the extracellular matrix, and other cells consisting of the tumor stroma. Methodologies on how to administer CAR T-cells to combat solid tumors are being tested or have been previously tested. Such methodologies involved the delivery of the CAR T-cells into the region of where the tumor is located as opposed to the intravenous administration of CAR T-cells. A benefit to local delivery involves using less total counts of CAR T-cells to mount an antitumor response and thus poses less of a threat to toxic effects for the patient. As stated previously, tumors secrete a host of biological molecules that induce immunosuppression of CAR T-cells. Some of these molecules that will be discussed include TGF-β, IL-4, IL-10, PGE_2, and sialomucins. In addition, tumors induce the recruitment of T regulatory cells, T_H17 cells, myeloid-derived suppressor cells (MDSCs), and cancer associated fibroblasts (CAFs).

TGF-β is a well know soluble inhibitor of T-cell activation. TGF-β are one of the many soluble cytokines responsible for the failed persistence and cytotoxicity of CAR T-cell therapy. It has been reported in a study that TGF-β binds its receptor on the T-cell in a mouse model and acts to induce the suppression of genes that express proteins pertinent to a T-cell's cytotoxicity. These proteins include: Perforin, Granzyme A (GzmA), GzmB, IFN-γ, and FasL[10]. Interestingly, from this same study, it was found that once the TGF-β was removed, the T-cells regained their ability to attack tumor cells.

IL-10 is another immunosuppressive cytokine contributing to decreased efficacy of CAR T-cell therapy. It has been shown to induce downregulation of TAP 1/2 which is involved in the

processing of antigen presentation onto MHC. Therefore, this cytokine negatively impacts the functionality of antigen presentation by professional antigen presenting cells like dendritic cells and macrophages. PGE_2 was reported to be manufactured at high levels by an overexpressed COX-2 enzyme in a human derived glioma cell line. This resulted in the stimulation of mature dendritic cells to secrete IL-10. This resulted in the mature dendritic cells to induce $CD4^+$ T cells to adopt a $T_{reg}1$ phenotype characterized by the secretion of IL-10 and TGF-β, potent inhibitors of T-cell cytotoxicity.[11]

Sialomucins, specifically Muc1, are overexpressed in many solid tumors like breast and colon cancers. In a study conducted by Agrawal e*t al.*, tumor secreted Muc1 was found to impede T-cell proliferation. Interestingly though, this inhibited proliferation could be reversed by addition of IL-2 as well as administration of anti-CD28 monoclonal antibody.[12] CAFs are abundantly present in the stroma of the tumor microenvironment and express the surface protein fibroblast activation protein (FAP). To increase the efficacy of CAR T-cells against solid tumors would be to engineer separate pools of CAR T-cells. One pool to target against the FAP expressed on CAFs and the other pool to target against a tumor specific antigen. Another highly expressed tumor antigen expressed is vascular endothelial growth factor-2 (VEGF-2) which helps enable an increased vascular supply for the tumor to support rapid growth and subsequently possible metastasis. Therefore, the development of CAR T-cells to target against VEGF-2, CAFs, and the tumor associated antigen can increase the potency against the tumor and its associated microenvironment. Dendritic cells are a vital type of antigen presenting cells that play a pivotal role in tumor immunosurveillance. The tumor contributes to a microenvironment that impedes the maturation of dendritic cells. The tumor is also capable of upregulating the expression of FAS-L and PD-L1 at the tumor plasma membrane to suppress immune cells. Cancer cells induce

a high lactate and low tryptophan type of microenvironment that induces immunosuppression. CTLA-4 is expressed at the membrane of activated T-cells and engagement with CD80 and CD86 with antigen presenting cells induces an inhibitory signal to the T-cells.

Enhancing the cytotoxicity and persistence of CAR T-cells against solid tumors would seem to require additional immunotherapy medicines. Next generation CAR constructs could be utilized to combat both the immunosuppressive tumor microenvironment and the tumor itself. Separate pools of CAR T-cells could effectively combat solid tumors. CAR T-cell Pool A could be engineered to target a tumor specific antigen and Pool B could be targeted against those soluble immunosuppressive cytokines. Binding of such a CAR (expressed by Pool B) to an immunosuppressive cytokine can turn a normally biologically inhibitory signal into a stimulatory signal causing the CAR T-cells to become active. In addition, monoclonal antibodies could be used adjunctively to inhibit immunosuppressive cytokines and/or inhibit the activation of those cytokines' respective receptors.

Solid Tumor Trials

According to Schmidts *et al.*, there are well over 80 clinical trials testing CAR T-cells against solid cancers as of 2017.[13] The cancers that are being targeted against include neuroblastoma, glioblastoma, epithelial cancers, hepatic cancer, and prostate cancer. In 2011, a study was carried out using CAR T-cells directed against $GD2^{+}$ neuroblastoma cells. Although the results from this study were not as successful as therapies against hematological malignancies, promising results were recorded and insights were learned. Three of the eleven patients treated who had active disease experienced complete remission[14] and an average long term persistence of the CAR T-cells for over 40 weeks. A study has been completed involving the use of CAR T-cells against EphA2 gliomas (NCT02575261). However, no results have been posted yet. With time, it will be

interesting to see the development and use of newly designed CAR T-cells against solid tumors and the use of adjunct immunotherapy to help maximize efficacy against solid malignancies.

Landmark Hematological Malignancy Trials

There have been a few landmark clinical trials reporting exciting results concerning the efficacy of CAR T-cell therapy against hematological malignancies. These trials include the ELIANA trial, ZUMA-1 trial, JULIET trial, and the TRANSCEND trial. The phase 2 ELIANA clinical trial provided successful results and ultimately led the U.S. Food and Drug Administration to approve tisagenlecleucel-T. This therapy will be elaborated on in the next section but will be noted here that it is used to treat B-cell acute lymphoblastic leukemia. ELIANA results reported an 81% overall response rate (ORR) and a 60% complete remission (CR) rate. More to this is the 80% 6-month relapse-free survival (RFS) rate that was noted.[17] This trial contained 75 children and young adults. The toxicities of this study were frequent with 73% of the patients experiencing severe adverse events (grade 3 or higher) and 47% developing severe CRS. To note, a severe adverse event that is grade 3 is severe and/or medically significant but is not immediately life-threatening. Grade 4 indicates life-threatening signs/symptoms requiring immediate medical attention and grade 5 indicates death related to the severe adverse event.

The phase 2 ZUMA-1 trial results reported led to the U.S. FDA to approve a second CAR T-cell therapy, axicabtagene ciloleucel. This is used to treat aggressive lymphomas that are refractive. More specifics of this therapy are described in the next section. Data from this trial came from a patient sample size of 101. There was an 83% ORR along with a 58% CR rate. With a follow-up of nearly 27 months, 39% had ongoing responses. Relative to tisagenlecleucel therapy, a higher incidence of severe neurotoxicity was reported at 32%. However, a less reported incidence of CRS was reported at 11%.[17]

The JULIET trial assessed a therapy that was later approved by the FDA to treat relapsed and refractory lymphoma. Of the 93 patients treated who had been diagnosed with diffuse large B-cell lymphoma (DLBCL), 40% showed a CR. Of all those cases a duration of 29.3 months was reported upon follow-up. Neurotoxicity was reported at 12% (grade 3 or higher) and CRS at 22% (grade 3 or higher).[17]

The TRANSCEND trial is looking at a potential therapy that could be approved by the FDA in the future. The therapeutic product, meant to treat DLBCL, is called lisocabtagene maraleucel and showed promising results in the TRANSCEND trial. An 80% ORR, 55% CR rate has been reported. Furthermore, 50% continued to be in remission at 6 months. What is interesting to note is the low rate of severe adverse events that have been reported. Just one patient had CRS (grade 3 or higher) and neurotoxicity was reported at 12% (grade 3 and 4).[17] From these exciting results, a clinical trial is continuing to be studied and is expected to be complete in August, 2022 (NCT03744676).

Current FDA-Approved Treatments Against Hematological Malignancies

In 2017 the U.S. Food and Drug Administration (FDA) approved the use of two unique designs of CAR T-cells to treat hematological malignancies due to prior clinical trial successes. The therapies approved are named Kymriah® and Yescarta®. Tisagenlecleucel (Kymriah®) is meant to treat pediatric patients who are younger than 25 years-old and have B-cell precursor acute lymphoblastic leukemia (ALL) that is either refractory or in a second or later relapse. In addition, it can treat adult patients with R/R large B-cell lymphoma after two or more lines of systemic therapy including diffuse large B-cell lymphoma (DLBCL) not specified otherwise and high

grade B-cell lymphoma and DLBCL arising from follicular lymphoma.[15] Axicabtagene ciloleucel (Yescarta®) is meant to treat adult patients with relapsed/refractory large B-cell lymphoma after two or more lines of systemic therapy, including diffuse large B-cell lymphoma (DLBCL) not specified otherwise, primary mediastinal large B-cell lymphoma, high grade B-cell lymphoma, and DLBCL arising from follicular lymphoma[16].

The structure of Kymriah® CAR T-cells follows a second-generation construct. The scFV region of the CAR is targeted against the tumor associated antigen CD19. The intracellular domains consist of 4-1BB and CD3ζ. As stated earlier, the CD3ζ is responsible for T-cell activation and cytolytic toxicity towards its target cell. 4-1BB is responsible for T-cell proliferation and persistence. Yescarta® CAR T-cells also follow a second-generation construct. The intracellular domains also consist of CD3ζ but differ in the identity of the second intracellular domain which is CD28.

Toxicities

Provided that there has been promise in the use of cellular based cancer therapy, there has been some noted toxicities associated with the administration of such therapy. The two main toxicities that have been noted are known as cytokine release syndrome (CRS) and immune effector-cell associated neurotoxicity syndrome (ICANS) or just generally termed neurotoxicity. Some other toxicities that have been observed has been touched in this report earlier relating to on-target off-tumor toxicity. This is seen when the CAR T-cell recognizes the tumor associated antigen it is directed against but unfortunately it is at other tissue locations also expressing such an antigen. CRS culminates due to the large number of CAR T-cells becoming activated once engaged with their cancer cell target. This on-target engagement results in the release of cytokines with the

concentrations of those cytokines determining the severity of toxicity. As in an immune response to a mild cold or flu, the release of cytokines in CRS can induce mild symptoms like fever, fatigue, joint pain, etc. However, there has been severe adverse reactions because of CRS. These symptoms follow what is seen in a severe immunological reaction to foreign pathogens. In high enough concentrations, cytokines can induce circulatory shock and multi-organ system failure which is lethal to the patient. Fortunately, the symptoms of CRS can be reversible by administering the patient with drugs that act to antagonize proinflammatory cytokines such as IL-6. Administering drugs that block IL-6 receptors or IL-6 itself has shown to quickly reverse the symptoms of CRS. There has been no elucidation yet on the mechanisms that induce ICANS. Symptoms of ICANS include confusion, cerebral edema, and seizures to name a few. It has been observed that ICANS can occur with CRS but no clear direct association has been proven yet. Given the potential toxic severity that can be experienced by patients infused with CAR T-cells, there is interventional treatment that can be administered to save the patient's life. Treatment involves the use of drugs that antagonize proinflammatory cytokines (IL-1, IL-6, etc.) and their respective receptors. In addition, glucocorticoids can also be used to treat toxic symptoms given that they are potent anti-inflammatory compounds.

Economic Costs of CAR T-cell Therapy

The concept of utilizing CAR T-cells to specifically attack tumors has shown promising results against hematological malignancies. The ability to translate these promising results against solid tumors has shown to be difficult with less success than against hematological malignancies. Compounding this drawback against solid malignancies is the economic burden this treatment imposes on those patients receiving this form of cellular based treatment. Unfortunately, the cost for each patient to be treated with Yescarta® CAR T-cell therapy is $373,000 and $475,000 to be

treated with Kymriah®.[18] An important note to add to this is that these dollar amounts do not include hospital costs, doctor visits, or additional immunotherapy that may be administered to treat CRS, for example. Table 1 (below) is a representation of the expensive costs that go into the treatment of pancreatic cancer. The cohort under study included men ≥66 years-old and the costs seen come from Medicare payments. As can be deduced from this information is that accrued costs incurred on patients treated by FDA approved CAR T-cell therapy can surmount to well over $500,000. This could most likely be even more expensive if one considers those cancer patients who are years younger and therefore are likely to experience more visits to the hospital or their physician's office for check-ups.

Conclusions and Future Directions

Chimeric antigen receptor (CAR) T-cells represent a cellular based cancer therapy generated by genetically engineering a receptor and getting it to be expressed by a cancer patient's own T-cells. This is done by collecting a sample of blood from the patient and isolating their T-cells, a process called leukophoresis. Delivery of the CAR is usually done by way of retroviral or lentiviral vectors. It should be noted that this is not the only way of delivering the CAR gene. For example, delivery can be done via injection of a plasmid. The CAR construct can be manipulated to recognize and bind to TSAs. It especially important to target a TSA that is uniquely expressed by a tumor cell or is expressed in a more relative abundance compared to normal healthy cells. This helps to maximize the safety of this cell-based therapy by limiting 'on-target/off-tumor' effects and thus toxicity to the recipient. The most success of CAR T-cell therapy has been against hematological malignancies. This is due to these types of cancers being 'soluble' and thus the delivery of this therapy is efficacious compared to solid tumors. Further, some lymphomas and leukemias express TSAs (CD19 and CD20) that are not expressed at other tissue

sites. Treatment against solid tumors has proven difficult due to a suppressive TME consisting of both immunosuppressant cells (CAFs, T_h17) and immunosuppressant cytokines (IL-4, IL-10). However, refining the development and further experimentation with next-generation CAR T-cells could provide promising solutions to treat solid tumors in the future. Other downfalls of CAR T-cell therapy include the CRS and neurotoxicity as well as the expensive costs to manufacture CAR T-cells. The toxicity of this therapy can be managed appropriately with closely monitoring the recipient throughout the treatment and administering the appropriate medication to negative toxic levels of cytokines in the body. Despite the downfalls, CAR T-cell therapy is revolutionizing a way to treat cancer. It has shown to be efficacious against hematological malignancies leading to the US FDA to approve two types of therapies. With more experiments and clinical trials it also has the potential to be just as effective against solid tumors, too.

Figure 1

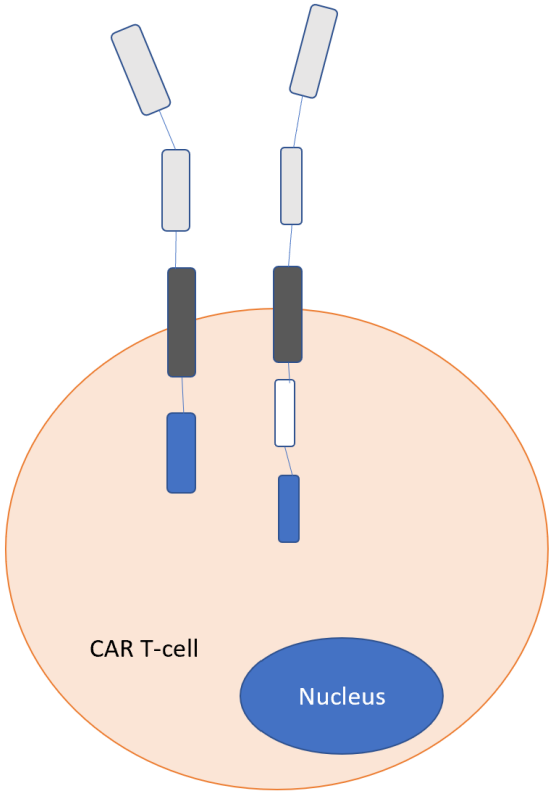

Figure 1 represents the general structure of a CAR. The CAR with four domains (rectangles) represents the first-generation CAR. The light gray domains represent the scFV domains. The charcoal color domains represent the transmembrane domain. The blue domains represent CD3ζ. The white domain signifies the structure of a second-generation CAR. This can consist of either 4-1BB, CD28, OX40, ICOS, etc.

Table 1

https://ascopubs.org/doi/abs/10.1200/jco.2011.29.15_suppl.6015

	All stages		Localized		Regional		Distant	
	(N = 11,914)		(N = 1,048)		(N = 3,887)		(N = 6,979)	
	Mean $	% Total	Mean $	% Cost	Mean $	% Cost	Mean $	% Cost
Total	59,300	100	73,900	100	80,800	100	45,100	100
Procedures	14,100	24	20,000	27	25,400	32	6,900	15
Chemo/RT	8,200	14	8,500	12	10,200	13	7,100	16
Inpatient	19,500	33	22,900	31	22,100	27	17,600	39
Hospice	4,000	7	5,100	7	5,100	6	3,300	7
Other	13,400	23	17,400	24	17,900	22	10,300	23

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