REVIEW OF CAR T-CELL THERAPY AND ITS USE IN THE TREATMENT OF SOLID TUMOR CANCERS

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ABSTRACT

CAR T-cell immunotherapy has been rapidly developing over the last 2 decades. The expanding list of cancer targets provides more and more treatment opportunities. The purpose of this research review is to discuss CAR T-cell immunotherapy, to compare and contrast this therapy to the gold standards of cancer treatment, and to examine its developing use for solid tumor cancers.

Targeted immunotherapies, such as CAR T-cells, are a more recent addition to the treatment options for solid tumor cancers. While initially developed for use with hematologic malignancies, CAR T-cells have now successfully been used to treat a variety of solid tumor cancers (National Cancer Institute, 2022). The use of CAR T-cell immunotherapy for solid tumors is promising, with about 30 antigens being targeted in ongoing clinical trials (Newick et al., 2016). The current opportunities for treatment include lung cancer, kidney cancer, bone cancer, breast cancer, prostate cancer, and glioblastoma (Guzman et al., 2023).

The use of CAR T-cell immunotherapy for solid tumor cancers faces obstacles such as cell trafficking restrictions into the tumor site, a hostile tumor microenvironment, antigen heterogeneity, CAR T-cell exhaustion, and potential severe toxicities that can hamper clinical efficacy. With new and ongoing research of potential antigen targets and the development of counter strategies, such as combination therapies and additional modification of the CAR T-cells, being tested, there still significant potential therapeutic efficacy for the use of CAR T-cell immunotherapy for solid tumor cancers.

Keywords: CAR T-cells, immunotherapy, solid tumor

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I. INTRODUCTION

Cancer is one of medicine's most difficult problems. There are about 1.9 million new cases diagnosed each year in the United States, and about 600,000 yearly cancer related deaths (American Cancer Society, 2024), placing cancer as the second leading cause of death in the US. The economic burden of cancer treatment in the US was $21.09 billion in 2019, and the American Cancer Society estimates that it could balloon to as much as $246.6 billion by 2030 (National Cancer Institute, 2021). While there are many pharmacological and surgical solutions being developed and refined all the time, each treatment type comes with its own set of drawbacks, which perpetually leaves the door open for further advancements.

Cancer is characterized by abnormal, uncontrolled cell division. There are many different types of cancers borne of a wide variety of different cell types, with many ways of classifying and grouping the cancer types. Largely, cancers can be separated into solid tumors and non-solid (liquid) tumors. One example of liquid tumors are the leukemias, which are cancers of the body's blood forming tissues and develop in blood, bone marrow, and lymph. Solid tumors make up 90% of adult cancers and 40% of childhood cancers (American Cancer Society, 2024). Solid tumors are made up of abnormal cells of a single type that communicate through tight and gap junctions. Solid tumors form a "mass" as the cells multiply, and usually contain no fluid, pus, air, or other substances. While the treatment of solid tumors and liquid tumors have a lot of overlap, there are also many differences in the needs and availability of treatments for each. Solid tumor cancers include sarcomas, carcinomas, and lymphomas.

For years, the phrase "cure for cancer" has been synonymous with an elusive scientific breakthrough. While there is no so-called "cure" for cancer just yet, there are many effective options for the treatment of cancer available to medical professionals. Surgery, chemotherapy,

and radiation therapy are the most commonly used treatments in the US (American Cancer Society, 2024) and have been successfully used to treat cancer since the early 1900's. More recently, targeted therapies have joined the other mainstay treatments and become foundational to the way that cancer is treated (National Cancer Institute, 2022).

In order to treat cancer, it must first be defined and its mechanism understood. "The hallmarks of cancer" are an organizing principle that was developed to help streamline the complex nature of cancer so that it might be better understood. The hallmarks describe eight "capabilities" of cancer, and two "enabling capabilities". These hallmarks describe what cancer is able to do, and must do, in order to effectively replicate, evade our bodies defenses, and invade tissue (Hanahan & Weinberg, 2011).

These hallmarks include the eight capabilities:

- Self-sufficient growth signals
- Insensitivity to anti-growth signals
- Evading apoptosis
- Limitless replicative potential
- Sustained angiogenesis
- Tissue invasion and metastasis
- Deregulated metabolism
- Evading the immune system

And the two enabling capabilities are:

- Genome instability
- Inflammation

The last two capabilities, as well as the two enabling characteristics, are a more recent addition to the hallmarks of cancer, having been proposed in 2011 to be added with the original 6 that were proposed in 2000 (Hanahan & Weinberg, 2011).

Being able to accomplish the phenotypes detailed in the hallmarks makes cancer cells virtually immortal, and sometimes beyond the natural healing abilities of which our bodies are capable. This transformation makes medical intervention a necessity to eliminate cancer. The hallmarks can work as targets for treatments, as the disruption of one or more of them can stop or slow cancer progression.

Added to the defining qualities of a cancer was its ability to evade the immune system. When functioning normally, the immune system detects and eliminates abnormal cells, which prevents or reduces the growth of many cancers. For example, immune cells called tumor-infiltrating lymphocytes (TILs) are oftentimes found in and around tumors (Yang et al., 2022). The secreted substances from these cells signal to the body that the immune system is responding properly to the tumor. Under normal conditions, TILs are lymphocytes that migrate to tumors and infiltrate them. TILs recognize tumor specific antigens, and use either direct or indirect cytotoxic activity to attack the tumor. TILs are constantly patrolling the body for tumors, and don't need to be directed toward the tumors to attack (Yang et al., 2022). Patients that have tumors containing TILs typically have better outcomes than those that do not (Yang et al., 2022).

Current available immunotherapies include immune checkpoint inhibitors, monoclonal antibodies directed at tumor cells, immune system modulators, and adoptive cell transfer. TILs are an example of adoptive cell transfer, which also includes chimeric antigen receptor (CAR) T-cell therapy. While TILs will attack tumors on their own, CAR T-cells are genetically modified to seek out specific tumors based on their antigens (Yang et al., 2022).

II. USE OF CAR T-CELLS FOR SOLID TUMORS

A. THE PROCESS OF CAR T-CELL IMMUNOTHERAPY

As stated, CAR T-cell therapy is classified as immunotherapy. Immunotherapies aim to either trigger, enhance, or block the immune response in order to achieve their medicinal goals. CAR T-cells are in the grouping of "activating" immunotherapies, as such they are designed to trigger mechanisms already used by our immune system and aim them at a cancer. Our bodies' immune systems are made up of white blood cells and organs and tissues of the lymph system; these built in systems are some of the best disease destroying mechanisms known to humankind, and immunotherapy was created to take advantage of this powerful system.

Under normal conditions, blood cells mature from stem cells, and then develop into either a myeloid or lymphoid precursor cell. The myeloid cells become the red blood cells and platelets, which are critical for the bodies' oxygen delivery system and wound repair systems. The myeloid stem cells can also develop into granulocytes and macrophages, which are the white blood cells (WBCs) that are active in the innate branch of the immune system. These WBCs will respond generally to defend the body against foreign invaders during bacterial, viral or parasitic infections. Lymphoid stem cells are the other maturation branch from blood stem cells; theu become lymphocytes, which then develop into either B lymphocytes or T lymphocytes. These cells are active in adaptive immunity, which adapts to fight specific foreign invaders once they have come in contact with them. B lymphocytes are responsible for humoral immunity, which uses secreted antibodies, complement proteins, and certain antimicrobial peptides to protect the body against diseases. T-lymphocytes (T-cells) are responsible for cell mediated immunity. T-cells can develop into a number of different subtypes, the relevant subtypes being helper T-cells, cytotoxic T-cells, or natural killer cells. In cell-mediated immunity, a macrophage or natural

killer cell, which are both activated to kill intracellular pathogens, will activate a helper T-cell to replicate by presenting it with an antigen from their ingested pathogens. Helper T-cells will replicate the signal to tell the cytotoxic T-cells to proliferate and kill foreign invaders. The molecules that helper T-cells use to signal the cytotoxic T-cells are called cytokines, which are small proteins that act as chemical messengers between cells. Cytokines carry signals that control the immune system and other cells in the body.

CAR T-cell therapy works by increasing the cancer fighting capabilities of T-cells. The immune system monitors the body for foreign cells, which is how it views cancerous cells, by tracking proteins on the surface of the invading cells called antigens. The immune system relies on T-cells to track and kill foreign entities, including cancers.

T-cells, like all cells, have proteins on their surface called receptors. When these receptors sense antigens, they signal to the T- cell what types of cells or particles are present in the body. When these receptors sense foreign antigens, the T-cells attempt to catch and block the foreign substances. More than that, T-cells can kill the foreign substances and/or the cells that harbor the foreign substances. But antigens have their own form of protection because they can disguise themselves to hide from T-cells. CAR T-cell therapy ensures your T-cells aren't fooled by antigens in disguise.

As early as the 1990's, researchers demonstrated how the introduction of donor T-cell precursor cells into a patient with cancer can induce remission (Sun et al., 2024). In 1997 researchers did this with patients with chronic myeloid leukemia using a process called allogeneic stem cell transplantation (Kolb & Holler, 1997). Allogeneic stem cell transplantation involves extracting healthy stem cells from a donor's bone marrow surgically with a needle and then introducing the donated cells to the patient's body.

CAR T-cells are made by modifying a patient's existing T-cells *ex vivo* so that they express something called a chimeric antigen receptor (CAR). These CAR T-cells are essentially turned into a hybrid of B and T-cells, with a T-cell activating signal portion and an antibody-like antigen recognizing portion (Sun et al., 2024). The antibody portion of the CAR T-cell consists of a single-chain variable fragment derived from heavy and light chains. This redirects specificity and allows the T-cells to recognize the antigens present in the tumor cells without use of major histone compatibility complex proteins (Sun et al., 2024).

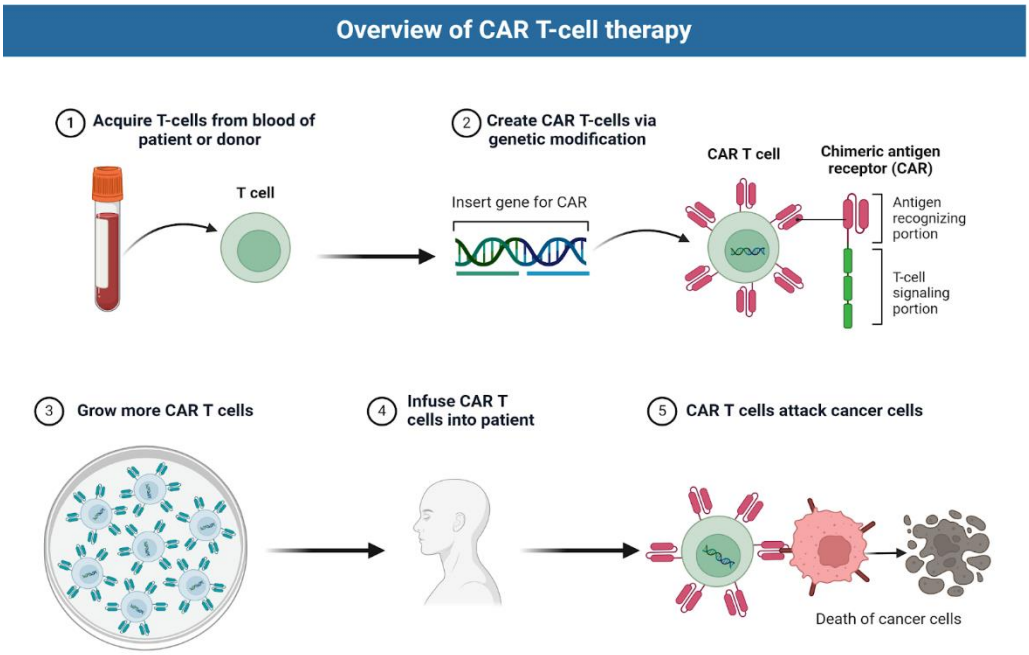

Figure 1. Created with biorender.com

The majority of advancements in the structure of CAR T-cells have been in modifying their intracellular T-cell activating portion. First generation CAR T-cells only included a CD3 domain, while second generation and beyond have added costimulatory molecule (CM) domains in order to improve activation and enhance effectiveness. These advancements have led to CAR T-cells being known as a "living drug" (Jackson et al., 2016). In its early days, CAR T-cells were used primarily on liquid tumors (Jackson et al., 2016). The improvements in the third and fourth

generations of the treatment has expanded the possibilities of use for many solid tumors as well, with the use of additional costimulatory domains and genes for cytokine secretion that have allowed for a larger variety of target antigens (Rohit et al., 2021).

Below is a table to summarize the 5 generations of CAR T-cells.

Table 1. A summary of CAR T-cell generations.

	Features	Limitations
1st Generation	• one signaling domain • capable of T-cell activation only	• Inadequate T-cell proliferation • Cytokine release • T-cell persistence • Antitumor activity
2nd Generation	• Provides two signals via CD3ζ and CM • Improved T-cell activation, proliferation, and persistence over gen 1	• T-Cell persistence • Relapse
3rd Generation	• Multiple signaling via CD3ζ and 2 CMs • Improved safety profiles, proliferation, perseverance, and anti-tumor functions	• Higher incidence of severe side effects (e.g., CRS and faster T-cell exhaustion)
4th Generation	• Primary CD3ζ, costimulatory signals • Expression of transgenic proteins • Potentiates T-cell expansion, persistence, and anti-tumor capacity by overcoming tumor antigen loss by cytokine activation at the tumor site and modulating the tumor milieu • Reduced toxicity	• Poor cancer-killing potential in solid tumors • Side effects such as on-target off-tumor activation of TRUCK T-cells and release of the transgenic cytokine in healthy tissues • Double modification of T-cells
5th Generation	• Improved T-cell activation, proliferation, solid tumor infiltration, and persistence by cytokine-inducing JAK/STAT signaling • Improved safety profile	• Limitations infiltrating and trafficking into solid tumors when compared to blood cancers • Associated with some side effects

Table 1 (cont'd)

5th Generation (cont'd)	• Wider therapeutic window • Creates a more favorable tumor milieu and reestablishes the immune system after infusion	

(Asmamaw Dejenie et al., 2022)

The first step in preparing CAR T-cell treatment is collecting T-cells from the patient. These cells from the patient are called "autologous T-cells". The patient undergoes apheresis, a process where blood is removed from the body and passed through a machine that separates the blood cells and blood products from the blood plasma. Apheresis is followed by leukapheresis, which allows for the further filtering of the blood cells according to size. Leukapheresis allows for the separation of the white blood cells (leukocytes) and the isolation of the T lymphocytes that are to be used in the treatment. Unused portions of the blood are then reintroduced into the patient (Wang et al., 2023).

The separated T-cells are then sent to a lab for genetic engineering. Genetic engineering of the T-cells is accomplished by inserting genes by way of an inactive virus/non-virus vector, which will produce the CARs on the surface of the patient's T-cells. The modified T-cells are then allowed to grow for 2-3 weeks after which they are frozen (Wang et al., 2023). Lastly, the modified T-cells are injected back into the patient via a single infusion. Patients receive chemotherapy before this last step to deplete their own lymphocytes. This treatment reduces the population of the less helpful native population of T-cells and gives the newly introduced CAR T-cells room to expand (Subklewe et al., 2019).

Another area of progress with CAR T-cell treatment is the use of "allogeneic T-cells". In this process, T-cells from a donor are used instead of the patient's own. This overcomes some of the disadvantages of autologous T-cells in regards to the time of production, the cost, and

manufacturing delays (Martínez Bedoya et al., 2021). Another benefit of donor T-cells is that it alleviates dependance on the patient's T-cells that may have reduced function or supply due to previous cancer treatments (Martínez Bedoya et al., 2021).

B. POTENTIAL BENEFITS AND NEGATIVES OF CAR T-CELL IMMUNOTHERAPY

Unlike most other forms of cancer treatment, CAR T-cells are customized to the individual patient. The cancer is being fought by the patient's own T-cells, which are modified to attack the antigens that are specific to the patient's cancer tumor. The patient's body also makes more of the CAR T-cells because the CAR T-cells replicate inside the patient after initial injection via clonal expansion.

The leading benefits of CAR T-cell immunotherapies are the short treatment time that is required to complete the therapy course. CAR T-cell treatment also boasts a more rapid recovery than traditional cancer treatments, and scores higher in long term remission, with a better reported quality of life (NIH, 2022).

In one trial where CAR T-cells were being directed towards HER2-expressing glioblastoma (GBM) tumors, 7 out of 16 patients had stabilized their disease for up to 29 months (Daei Sorkhabi et al., 2023). The average survival time was 24.5 months from time of diagnosis. Using traditional glioblastoma treatments (surgery, followed by radiation therapy with or without chemo), the average survival of a patient is 12-15 months (Marra et al., 2019). This is a very significant increase in the lifespan of GBM patients, who's median survival is under 12 months (Mohammed et al., 2022). This shows how monumental this treatment could be for the treatment of certain solid tumor cancers if some of the current treatment barriers can be overcome.

At the moment, CAR T-cell therapy is primarily being used only after a patient has failed several lines of more traditional treatments, but there is a push to use CAR T-cells as first line in some cases (NIH, 2022). One study compared 3 trials using CAR T-cells after patients received a single treatment of chemotherapy for non-Hodgkin lymphoma, versus patients receiving only the standard treatment approach. In two of the three trials, patients that had received the CAR T-cell therapy lived longer without progression of the disease than patients who underwent the standard treatment (NIH, 2022). One of those trials showed patients given CAR T-cell treatment for large B-cell lymphoma had a median event-free survival of 10.1 months versus only 2.3 months with standard of care treatment (Kamdar et al., 2022).

While the side effects associated with CAR T-cell immunotherapy are typically less severe than those of conventional cancer treatments like surgical excision, chemotherapy, and radiotherapy, that does not mean they are nonexistent (NIH, 2022). One of the most concerning side effects is the cytokine release syndrome (CRS), also known as “cytokine storm”. When immune cells attack an infection, they release cytokines. Cytokines are small proteins that are important for cell signaling. When too many cytokines are released, they can wreak havoc on the body leading to fever, chills, hypotension, tachycardia, breathing difficulty and lowered oxygen. If a patient is suspected of having CRS, vitals will need to be closely monitored, including ferritin levels and C-reactive protein. They may need to be given oxygen for hypoxia and fluid for hypotension.

On-target, off-target toxicity is also a side effect experienced by some patients who take CAR T-cell therapy. On-target, off-target toxicity is when antigens targeted by the CAR T-cells are also expressed on the nearby healthy cells. This side effect can be life threatening when these healthy cells are essential, such as in the heart, lungs, or liver. When using allogeneic CAR T-

cells, a disease known as graft vs. host disease (GVHD) may be an issue, where the immune system attacks foreign donated tissues and cells. Anaphylaxis is another potential side effect, as well as tumor lysis syndrome (fragments of destroyed tumor in the bloodstream).

Pricing can also be an issue when considering whether CAR T-cell therapy is the right fit for a patient. Experts estimate that CAR T-cell therapy can cost between $500,000 and $1,000,000 (Choi et al., 2022). For comparison, the average monthly cost for chemo is about $1,000 to $12,000 depending on the drugs, and about $48,000 total (Choi et al., 2022). Private insurance may or may not pay for CAR T-cell therapy, and Medicaid only covers it depending on the state. Even if insurance does cover it, that does not mean no out of pocket expenses.

C. CAR T-CELL IMMUNOTHERAPY COMPARED TO ALTERNATIVES

While the mainstay gold standards for the treatment of solid cell cancers can result in successful outcomes, every available therapy has drawbacks. During modern radiation therapy, doctors are able to create a 3D model of the tumor and surrounding area in order to target the cancer and spare the normal tissue. Radiation therapy is able to cause the death of a large portion of the cancer cells within a tumor. This treatment is relatively safe and is useful for shrinking tumors before surgical excision, or possibly even eradicating smaller tumors (Debela et al., 2021). Radiation therapy kills cancer cells or slows their growth at high doses by damaging the DNA beyond repair. The radiation exposure will directly kill the cancer cells or weaken them, which stimulates the immune system and allows the cancer cells to then be broken down and cleared by the cells of the immune system (Debela et al., 2021)

While peripheral organ preservation is significant with radiation therapy, close proximity of a tumor to surrounding tissues can cause them to be damaged by the treatment. Radiation therapy can kill healthy cells that are not accurately represented in the 3D model, potentially

disrupting wound healing. Radiation therapy can also be an inconvenience to the patient as the treatment can be delivered daily, up to 5 times per week, and up to 2 months (Debela et al., 2021).

Chemotherapy is typically considered the treatment of cancers using chemical substances, but today can involve the use of small molecules and biologics. The benefits of chemotherapy include its ability to act systemically. This systemic activity allows for the treatment of cancers throughout the whole body, including cancers that are bloodborne or have spread to multiple areas. Chemotherapy can be used alongside radiation therapy, which can boost the effects beyond what either treatment would do on their own. Some specific patient tailoring can be done, as there are more than a hundred chemo drugs available for patients and doctors to choose from (NCI, 2022). Chemotherapy can also be a good option if a partial or total organ removal would negatively impact the patient's life, as there is a possibility it might eliminate or limit the need for surgical resection. An example of this would be using chemotherapy to treat a woman's uterine cancer instead of treating the cancer with a complete hysterectomy, as she may not have completed her family and still wants to carry a child.

For nearly as long as cancer treatment has existed, the first line treatment for most solid tumors was surgical resection. The surgery process involves patients being taken to an operating room, being put under anesthesia, and the tumor being removed by a surgeon. This is either a complete removal of the solid tumor, or a partial removal, called debulking, in cases where total excision cannot be achieved. There are many benefits to this treatment option, including removing a large volume of the tumor that relieves mass effect, removal of all cancer cells in a small area, and the treatment may take as little as a single session while the patient is unconscious. Another benefit of surgery is the ability to send the removed tissue sample to a

pathologist, who is then able to check the margins of the sample to confirm whether or not all the cancer cells were removed. Looking at the top 10 solid tumor cancers in the US, only about 8.85% of surgeries return positive margins results, meaning there was still cancer found in the periphery of the sample and therefore likely still present in the patient (Orosco et al., 2018).

The drawbacks of surgical treatment are the inability to kill microscopically, some cancerous cells may be left behind on the margins, some patients cannot tolerate anesthesia, some damage to nearby structures may occur, surgical complications that are not site specific, inability to remove cancer from other parts of the body (metastasis), and removal of organs that may decrease the patient's quality of life.

III. CURRENT LIMITATIONS AND POTENTIAL ADVANCEMENTS OF CAR T-CELLS

A. SOME DRAWBACKS TO CAR T-CELLS

As of today, one of the biggest hurdles for the use of CAR T-cells against solid tumor cancers is finding the right antigenic target. The issue here is tumor heterogeneity, which means all the cells in the tumor might not have the antigen that the CAR T-cells are programmed to attack. While research in this area is ongoing, there has still been difficulty in finding antigens that target the unwanted cancer cells but leave the healthy cells unharmed.

The surrounding environment poses a unique challenge for treating solid tumors. On a larger scale, physical barriers can block the CAR T-cells from reaching their intended targets. A major example of a physical barrier that can block CAR T-cells is the dysregulated tumor vasculature and dense fibrogenic extracellular matrix (ECM) (Kankeu Fonkoua et al., 2022). On a micro scale, immune-suppressing molecules that the cancer cells secrete can damage the CAR T-cells and render them useless. Immunosuppressive cytokines (interleukin-10, IL-4, and transforming growth factor β), tumor-associated macrophages, and regulatory T-cells hinder effective antitumor function of CAR T-cells (Kankeu Fonkoua et al., 2022). Competing for metabolic fuels (glucose and O2) can also hinder CAR T-cell function (Kankeu Fonkoua et al., 2022).

While the ability for “personalization” is one of the benefits of CAR T-cell therapies, a problem arises in this regard when it comes to solid tumors. Solid tumors can vary quite a bit on the molecular level for each patient, and sometimes even in the same patient. This can further hinder the process of finding a suitable target antigen, as there may be little to no suitable target

antigens on some tumor cells. If there are no suitable antigens the CAR T-cells cannot do their job as intended.

B. LOOKING TO THE FUTURE

CAR T-cells have many areas of development that will make up the next generation of CAR T-cells. Once these features are able to be implemented, they will overcome some of the barriers to the use of CAR T-cells for solid tumor cancers, as well overall improving their performance as a treatment.

Overcoming CAR T-cell toxicity is a high priority for the next generation of CAR T-cells, as safety is paramount when developing new medicines. The majority of CAR T-cell therapies are currently autologous small-scale treatments for patients that suffer from B-cell malignancies–liquid tumor cancers. One of the reasons CAR T-cell therapies are not used more for solid tumor cancers is due to the safety concerns about the potential development of a GVHD in allogeneic therapies, and therefore allogeneic therapies have been less effective than autologous ones. One way this problem is being addressed is through the use of something called a suicide gene switch.

Suicide genes are genetically encoded elements that are integrated into CAR T-cells to allow for the elimination of the introduced T-cells in cases where the patient experiences toxicities. They are activated by the introduction of a pharmaceutical agent that triggers the inserted genes to kill the CAR T-cells with up to 90% efficiency (Moghanloo et al., 2021). A drawback to this process is expense.

Bispecific T-cell engagers are another genetically encoded switch that can be added to CAR T-cells. These can allow for the switching of antigenic targets with already inserted CAR

T-cells. This will help overcome the problem of tumor heterogeneity when it comes to using this treatment for solid tumor cancers (Moghanloo et al., 2021).

Combinational target-antigens are another method being used to overcome tumor heterogeneity. With this addition, once the CAR T-cells have been bound to one antigen they will then activate to bind to an additional target antigen; binding to both antigens is necessary for the modified T-cells to have full effect (Moghanloo et al., 2021).

IV. CONCLUSION

CAR T-cell immunotherapy has its place in the current line-up of solid tumor cancer treatments, with its own set of benefits and drawbacks. The “living drug” has immense potential when it comes to the treatment of solid tumor cancers. The benefits that CAR T-cell immunotherapy has brought to hematological malignancy, such as its customizability, convenience, and adaptability would be a welcome addition to the treatment of solid tumor cancers. And while there is great progress that has been made, and many promising trials and advancements on the horizon, there are still many hurdles that must be overcome for using CAR T-cells for solid tumor cells.

Two of the major barriers CAR T-cells present when it comes to their use with solid tumor cancers are toxicity and tumor heterogeneity. Some of the new developments in CAR T-cell therapy such as suicide gene switches and bispecific T-cell engagers show a lot of promise that these obstacles can be overcome.

With the treatment of any disease, the more options the better. What works for one patient might not be the best fit for another. CAR T-cells are a welcome addition to the lineup of cancer treatments with the improvements and differences they provide.

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