Review Article

CAR-T Cell Therapy for Malignant Melanoma: Current Landscape and Future Directions

Aryan Chavda

George S. Middleton High School, 4801 N 22nd St, Tampa, FL, 33610, United States

ABSTRACT

Chimeric Antigen Receptor (CAR) T cells are modified T cells that have been genetically engineered to produce chimeric antigen receptors. Chimeric antigen receptors allow the T cells to identify and destroy specific cancer cells more efficiently, thus making CAR-T cell therapy a transformative immunotherapy. CAR-T cells have been tested and used as a treatment for leukemia and lymphomas and have shown high success and remission rates for decades. In this review, the modification and use of this therapy for solid cancers, specifically malignant melanomas, as well as its effectiveness, is explored. Studies work on discovering the most effective modifications of CAR-T cells, including inhibiting certain growth factors and testing numerous generations of the intracellular signaling domain. CAR-T cells are shown to be partially effective in certain studies and have been attributed to reduced recurrence rates in patients. The heterogeneity of melanoma cancers, antigen loss, and toxicities associated with the therapy limit the viability and effectiveness of the current treatment model. As such, the effectiveness, feasibility, and safety of CAR-T cell therapies in melanoma treatment is inconclusive as of now. More research is needed in overcoming the limitations of this therapy in order to determine its effectiveness as a treatment for melanoma cancers. Additionally, future research should focus on testing combinations of modifications of CAR-T cells, instead of testing the modification of only one component, in order to accelerate the understanding of whether this therapy is effective for treating melanomas, and if so, how it could best be used to increase efficiency while combating toxicities and limitations.

Keywords: Cancer; melanoma; CAR-T cell; tumor; antigen(s)

INTRODUCTION

Melanoma is one of the most common and lethal types of skin cancer (1). It is a cancer of melanin producing cells called melanocytes. Like other cancers, malignant melanoma is also staged based on tumor size, lymph node involvement and spread to distal sites per 2009 American Joint Committee on Cancer (2). Metastatic melanoma involves cancer that has spread to other distal sites throughout the body. Currently, there are various treatments available for the treatment of malignant melanoma including chemotherapy, radiation therapy, targeted therapy and immunotherapy (3).

CARs are engineered proteins that allow T cells, a

Corresponding author: Aryan Chavda, E-mail: aryankchavda@gmail.com.

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type of immune cell, to recognize and attack cancer cells. They are designed by combining elements from different sources to create a receptor that can specifically bind to certain antigens on the surface of cancer cells. By engineering T cells to express these CARs, researchers can enhance the ability of the immune system to identify and destroy cancer cells more effectively. CAR-T cell therapy has shown remarkable success in certain blood cancers and is being explored for other malignancies and diseases (4). This article reviews recent advances in CAR-T Cell Therapy for Malignant Melanoma, as well as current challenges and future directions to overcome challenges and improve outcomes for patients.

CAR-T cell therapy is a major advancement in the field of cancer treatment. The first CAR-T cell therapy was approved in 2017 for refractory or relapsed acute lymphocytic leukemia in pediatric and young adults (5). In addition to leukemia, it is also useful in the treatment of solid tumors and other diseases like systemic lupus erythematosus. Although there are many proposed uses of CAR-T cell therapy to date, five CAR-T cell therapies have been approved by the FDA.

History of Car-T Cell Therapy

In 1928, Raymond Pearl, an American biologist, observed that patients with tuberculosis have significantly lower incidence of cancers (6). This discovery leads to use of Bacillus Calmette-Guérin (BCG) -an attenuated live bacterial vaccine - to treat superficial bladder tumors (7). When introduced into the bladder, it stimulates the immune system to recognize and attack cancer cells. BCG therapy not only targets existing cancer cells but also helps prevent the recurrence of bladder tumors by training the immune system to recognize and attack potential future cancer cells.

In the 1980s, researchers found that T cells might be genetically altered to produce novel receptors that would enable them to better detect particular antigens on cancer cells. Dr Yoshikazu Kurosawa, a Japanese Immunologist, first proposed the concept for CAR-T cells. He combined the T cell receptor (TCR) derived constant regions and antibody-derived variable regions (8). This led to the idea of employing T cells to target cancer. Later, Dr. Zelig Eshhar and his colleagues in Israel created the first CAR. They modified a receptor to cause T cells to focus on a particular cancer antigen (9). His study demonstrated that T cells get activated when TNP antigens bind to chimeric TCRs on the cell surface. This reaction leads to IL-r production and kills target cells (9).

The goal of research in the 1990s was to increase T cell persistence and activation. Dr. Carl June of the University of Pennsylvania demonstrated that CAR-T cells might result in full remission in patients with chronic lymphocytic leukemia, which attracted a lot of attention (10). His findings sparked more interest and study into CAR-T cell treatment for leukemia and other cancers (10).

In 2017, the FDA authorized the first use of CAR-T cell therapy for treating pediatric and young adult patients with acute lymphoblastic leukemia who had relapsed or were resistant to treatment.

CAR-T CELL STRUCTURE AND FUNCTION

Structure of Chimeric Antigen Receptor T-Cells

The CAR-T cell's ability to operate properly is attributed to four primary parts: the antigen binding domain, the hinge region, the transmembrane domain, and the intracellular signaling domain.

The antigen binding domain of CAR-T cell detects and binds to a specific antigen. It is made up of two different monoclonal antibodies which form a single chain variable fragment. These monoclonal antibodies are variable light and variable heavy antibodies. This variable fragment recognizes antigens on cancer cells and then T cells get activated. This activated T cell will eliminate cancer cells (11). The ability of variable fragments to bind and recognize cancer cell antigen depends on various things like target antigen density, location of antigen and interaction between variable light and heavy chains (12, 13).

The hinge regions on the extracellular portion of a cell have an important function of helping CAR-T cells detect target antigen. It is located on the extracellular region and provides flexibility and length to reach the target epitope (14, 15). There are long and short hinges useful for distal and proximal epitopes respectively (16). Commonly known hinge regions are CD8, CD28, IgG1 and IgG4.

Another important cell component in binding the CAR to the T cell membrane is the transmembrane domain. The combined action of the transmembrane domain and hinge regions helps cytokine production and causes cell death from the CAR-T cell (17). Transmembrane domains are commonly made from proteins such as CD3, CD4, CD8, and CD28 (18). This transmembrane domain is important in providing stability, synapse formation and level of depression for T cells. When the proximal intracellular domain is used with its corresponding

transmembrane domain, it optimizes the signaling of the CAR-T cells.

The CAR sends a signal to the intracellular signaling domain. Intracellular signaling domain then amplifies this signal and relays it to the nucleus to elicit a T cell reaction. This is a very important step in the activation of CAR-T cells and could be a rate limiting factor too. The first generation of CARs had limited signaling efficacy which limited their efficacy (19). This was corrected in the second generation of CAR which provided enhanced efficacy in relaying signals to the nucleus and showed an increased response rate in patients (20). To improve responsiveness, researchers added a second domain to the next generation of CAR-T cells.

Four Generations of CARs

CARs have four parts including an antibody binding domain, a hinge region, a transmembrane domain, and an intracellular signaling domain (21). Extracellular domains recognize antigens in the absence of presentation by the major histocompatibility complex (MHC).

The first CAR-T cell was developed in 1993. However, it lacked specificity and effectiveness in recognizing specific antigen. Due to this, newer generations of CAR-T cells were developed to overcome these limitations (22). Currently we have the 5th generation of CAR-T cells. With each generation, the intracellular domain was improved and facilitated a stronger response from the immune cells. The first generation of CAR-T cells had one intracellular domain. Second-generation CARs included one co-stimulatory molecule (CM) 1 (23). Third-generation CARs had two do-stimulatory molecules.

First generation CARs contained one CD3 chain intracellular domain, similar to the T cell receptor. They were unable to produce IL-2 (interleukin-2) and had a weak response. In addition, this generation of T cells has low cell proliferation and short in vivo lifespan (24).

To overcome the shortfall of first-generation CARs, second generation CARs have additional cytoplasmic domains like CD28, 4-1BB or OX-40 (11). This dual signaling makes second generation CARs stronger and more effective than first generation CARs. This co-stimulatory signal improves in-vivo life span and cell proliferation.

The success of two domains inspired researchers to add multiple co-stimulatory signaling domains in the CARs to develop a third generation of CAR T cells using CD3, CD28, OX40 and others. This not only increases life span but also triggers rapid tumor elimination (25).

The fourth and fifth generation CARs are based on second generation CARs. These CARs have a protein, such as interleukin 12 (IL-12) that is expressed by T cells upon activation. Activation of these CARs promotes the production and secretion of the desired cytokine to promote killing of the tumor cells. T cells transduced with these fourth-generation CARs are referred to as T cells redirected for universal cytokine-mediated killing (TRUCKs) (26).

TRUCK CAR-T cells have two transgenic cytokines - one for CAR cell structure and a second one for producing cytokines. These cytokines significantly increase the efficacy of these CAR-T cells compared to second generation CARs. This is also very useful in reducing the systemic toxicity of CAR-T cell therapy associated with the first and second generations of CAR-T cell therapy. The fifth generation of CAR-T cells being researched are also called Next generation CARs. There are several different approaches being deployed for the Next generation CARs such as the addition of the IL-2 receptor which activates JAK/STAT pathways and the development of switch receptors which incorporate drug-dependent on and off switching of the CAR's activity (27).

Further advancements are being made to make CAR-T cells safer, which are called Smart T cells. These 'Smart' CAR-T cells incorporate additional genetic modifications that enhance their ability to distinguish between cancer cells and healthy cells. This characteristic helps ensure precision, enhances effectiveness, prolongs life span, and minimizes side effects. There are several Smart CARs being tested, including 'EchoBack' CAR-T cells, 'Inducible' CAR-T cells, and Dual CAR-T cells (27).

CAR-T CELL THERAPY FOR TREATMENT OF MALIGNANT MELANOMA

CAR-T Cell Therapy for Malignant Melanoma

After CAR-T cell therapy was demonstrated to be a successful treatment in leukemia, it has been studied and tested on other malignancies such as lymphoma and multiple myeloma. Each kind of CAR-T cell therapy is made to fight a specific kind of cancer antigen. So, a CAR-T cell therapy made for one type of cancer will not work against another type of cancer.

Metastatic melanoma is the most aggressive type of skin cancer. It has been difficult to treat with conventional chemotherapy and radiation and has a survival rate of 10%. Because of this, newer immunotherapies and targeted therapies are being evaluated and implemented to treat metastatic melanoma.

The treatment options for metastatic melanoma, which has spread beyond the skin, may include surgery, radiation therapy, chemotherapy, immunotherapy, and targeted therapy.

Mechanism of Melanoma Cancer Spread

Stage IV melanoma has distal tumors spread beyond the skin. This spread of malignant tumor cells is mainly done by lymphatics (28,29). However, hematogenous spread via blood vessels has also been observed in some patients. There are several mechanisms through which melanoma cells spread, such as angiogenesis, leukocyte fusion, embolization, intravasation, and cancer stem cells.

Angiogenesis

Ultraviolet radiation causes damage to skin cell genomes, especially in melanocytes. This leads to the uncontrolled growth of these melanocytes. This exponential growth increases the consumption of oxygen and nutrients. To fulfill this increasing demand, additional vasculature needs to be developed (30). Thus, to increase the blood supply, the tissue begins to produce a spectrum of growth factors which trigger the process of angiogenesis. Angiogenesis is a process by which new blood vessels are formed from pre-existing blood vessels, and is one of the most important factors involved in tumor progression and metastasis (30, 31). There are several angiogenesis growth factors identified like vascular endothelial growth factors (VEGFR), Angiogenin, Transforming growth factor, and Epidermal growth factor (31). In addition, there are cytokines such as Interleukin 1, 6, and 8, which also play an important role in helping tumors spread beyond their original tumor site. Proteases and protease inhibitors, oncogenes, and endogenous modulators such as Angiopoietin-1 also play an important role in angiogenesis.

Embolization

Tumor spread by embolization occurs when cancerous cells break off from a primary tumor, enter the bloodstream, and travel to distant sites in the body where they can lodge and form secondary tumors, essentially "floating" through the circulatory system like small emboli (32). This most commonly happens when a tumor directly invades a blood vessel and fragments detach into the bloodstream.

Certain prothrombotic agents are shown to be expressed on metastatic melanoma cells like protease-activated thrombin receptor (PAR-1), thrombin, and platelet-specific receptor glycoprotein Ib-IX (gpIb-IX) (33). Increased expression of these prothrombotic agents plays an important role in malignant melanoma metastasis (34). Therefore, targeting these prothrombotic agents with monotherapy or in conjunction with CAR-T cells may increase the effectiveness of CAR-T cell therapy. PAR-1 cleaves to produce TR47, which is shown to decrease metastasis of malignant melanoma (35). So, TR47 targeting CAR-T cells could also be an effective treatment for melanoma metastasis.

Leukocyte-Cancer Cell Fusion Hypothesis

The leukocyte-cancer cell fusion hypothesis proposes that cancer cells fuse with bone marrow-derived cells, such as macrophages, to form hybrid cells that can initiate metastasis. This hypothesis was proposed by John Pawelek in 1992 (36). The fusion of a cancer cell with a leukocyte creates a hybrid cell that can migrate and divide uncontrollably. The hybrid cell acquires genetic and functional properties from both cells. The hypothesis has been confirmed in animal models and in patients with melanoma and renal cell carcinoma. The fusion of cancer cells with leukocytes may be a major cause of metastasis. Understanding the mechanisms of this process could lead to new therapeutic targets.

The metastatic hybrid cells increase the expression of macrophage markers, including SPARC, SNAIL, MET, MITF, CD14, CD68, CD163, CD204 and CD206. Therefore, targeting antigens in CAR-T cells that are specific to surface proteins of MTFs (macrophage-tumor fusion cells) may improve CAR-T cell performance in metastatic melanoma by eliminating leukocyte-cancer fusion cells.

Cancer Stem Cells

Cancer stem cells (CSCs) are a small group of cells within a tumor that have properties similar to normal stem cells like self-renewal, multi-lineage differentiation, and multi-lineage differentiation. CSCs promote neovascularization and angiogenesis. Melanoma stem cells can differentiate into endothelial-like cells and promote neovascularization (37). Melanoma stem cells have CD133 and CD20 expressed on them. So, targeting CD133 and CD20 decreases tumor growth and specifically reduces metastasis of melanoma cancer cells, making them good targets for CAR-T cells in the treatment of malignant melanoma.

Chemotactic Molecules

Chemotactic molecules play an important role in tumor metastasis by helping in the movement and

migration of cancer cells (38). They act as a signal to guide cancer cells through the metastasis cascade. A few examples of chemotactic molecules are growth factors, cytokines, and extracellular matrix components such as the epidermal growth factor (EGF) and interleukins. These molecules bind to specific receptors on the surface of cancer cells, triggering intracellular signaling pathways to help metastasis.

Once tumor cells reach distal sites such as the liver, lung, and bone, these chemotactic molecules also help in creating microenvironments at distal sites for tumor establishment. Chemotactic molecules interact with distal organ cells, which can enhance cancer cell survival and proliferation after they arrive at a distal site (27). There is a feedback loop between tumor microenvironments and chemotactic molecules in which both stimulate each other to enhance metastasis.

Deeper understanding of these chemotactic molecules and their signaling pathways can help in creating CAR-T cells designed to target specific antigens to inhibit this signal pathway and decrease melanoma metastasis.

Tumor Antigen Targets for CAR-T Cells in Malignant Melanoma

There are a few tumor antigen targets identified and used for the treatment of malignant melanoma with CAR-T cell therapy. They include vascular endothelial growth factor receptor 2 (VEGFR-2), glycolipid 2, and melanoma-associated antigen. In recent research, TYRP1 and TYRP2 have been targeted on tumor cells and have shown promising results in preclinical tests (39, 40). TYRP1 is a melanoma differentiation antigen involved in the melanin biosynthesis pathway. It is overexpressed in malignant melanoma and so it is efficient as a target for CAR-T cells. Approximately 30% of cutaneous melanoma patients have high TYRP1 overexpression. Thus, targeting TYRP1 and TYPR2 could result in higher remission rates and less side effects in metastatic melanoma patients.

Another important antigen is Interleukin-13 Receptor Subunit Alpha-2 (IL13Rα2). This antigen is specific to melanoma and found in other malignancies too. However, this overexpression can be measured and, if present, can be targeted by CAR-T cells (40).

Side Effects of CAR-T Cell Therapy in Malignant Melanoma

CAR-T cell therapy does have its own challenges and side effects. Some of the normal tissue cells also express tumor-associated antigens, so they are recognized and attacked by CAR-T cells (41). Despite tumor specific antigens on CAR-T cells, they can cross-react with normal tissue cell antigens and cause damage to normal tissue (42). Additionally, tumor lysis syndrome and acute anaphylaxis may occur with CAR-T cell therapy (10).

Cytokine release syndrome (CRS) is the most prominent and well-described toxicity of CAR-T cells. CRS leads to fever, hypoxia, acute renal insufficiency, neurological symptoms, and hypotension.

Challenges of CAR-T Cells as Malignant Melanoma Immunotherapy

There are several challenges and limitations of CAR-T cell therapy as a cancer immunotherapy. The tumor microenvironment (TME), insufficient infiltration and penetration of the T cells, heterogeneity of tumor antigens, antigen selection, host organ toxicity, and T-cell exhaustion are the main challenges clinical trials face as of now.

The TME in malignant melanoma consists of tumor associated macrophages, regulatory T cells, and myeloid derived suppressor cells (43). Cytokines, such as TGF-B, produced by these cells deactivate CAR-T cells and hinder its function. Prolonged exposure to tumor antigens and hostile TMEs can also lead to T-Cell exhaustion and limited tumor infiltration.

There is significant tumor antigen heterogeneity in antigen expression within the same tumor and between two melanomas. This makes it difficult for CAR-T cells to recognize and kill melanoma cells. This results in reduced effectiveness and increased recurrence of malignant melanoma.

Solutions to CAR-T Cell Therapy Challenges and Limitations

There are several solutions proposed to overcome challenges in CAR-T cell therapy. For example, host organ toxicity can be reduced by improving the specific recognition of tumor cells and selecting safer target antigens. Targeting VEGFR2 seems to be safe in patients, but more research should be performed to improve its effectiveness in clinics (44).

Using TAAs as target antigens for CAR-T cells may help in improving antigen selection and reducing toxicity (45). Another approach researchers are using is to have CAR-T cells target more than one site on the tumor surface simultaneously. This increases the effectiveness of the treatment and makes it more specific to cancer cells in order to reduce toxicity. These cells are called 'AND-gate' CAR-T cells.

The TME includes several immunosuppressive molecules and inhibitory cells (46). The TME suppresses CAR-T cell proliferation and activity. Recently scientists have modified CAR-T cells to overcome this hostile TME by overproducing interleukin (47).

Metastatic melanoma is difficult to treat with any single chemotherapy or immunotherapy including CAR-T cells. Therefore, a combination of different therapies has been suggested to control this disease. One of the most effective combination therapies is using an oncolytic virus (OV) in addition to CAR-T cells. OVs have several benefits including the enhancement of immune cell infiltration, reversal of tumor immunosuppression, and secretion of TNF. Talimogene laherparepvec is the first and only FDA approved oncolytic virus used in the treatment of malignant melanoma (48). Several studies have shown potential benefits of combining OV and CAR-T cell therapy. This research has also furthered interest in studying other viral vectors such as adenovirus, echovirus, and reovirus.

CLINICAL AND PRECLINICAL TRIALS OF CAR-T CELL THERAPY FOR MALIGNANT MELANOMA

Preclinical Studies Using CAR-T Cell Therapy in Metastatic Melanoma

Several preclinical studies have laid the foundation for further studies that may progress to clinical trials (49). The main findings, hypotheses, and future questions of these preclinical trials are summarized in this section.

The chondroitin sulfate proteoglycan 4 (CSPG4) is an antigen that is overexpressed in various cancer histologies including melanoma, triple-negative breast cancer, glioblastoma, mesothelioma, and sarcoma. It plays a crucial role in melanoma cell proliferation, migration, invasion, and metastasis (50). Anti-CSPG4 CAR-transduced T cells recognize and kill these glioblastoma cancer stem cells. In-vitro data indicates an improvement in the functionality of CAR-T cells by using co-electroporated T cells with messenger RNA (mRNA) encoding a CAR specific for chondroitin sulphate proteoglycan 4 (CSPG4) and small-interfering RNAs to downregulate PD-1 (siPD-1) and CTLA-4 (siCTLA-4).

Some tumors express more than one antigen at the same time. These can be tackled with more than one chemotherapy or immunotherapy. However, if one immunotherapy can target multiple targets, it will be more effective and feasible. Simon et al. engineered T cells expressing anti-CSPG4 CAR and anti-gp100 at the same time (51). While these T cells carried out activity against both antigens, it shows no suppression of receptors. This activity against the two targets at the same time may enhance the effectiveness of CAR-T cells and reduce T-cell exhaustion.

One way to control tumor metastasis is to inhibit vascular endothelial growth factor receptor-2 (VEGFR-2). Chinnasamy et al. used CAR-T cells to target VEGFR-2 to reduce tumor metastasis (52, 53). Inoo et al. utilized CAR-coding mRNA to create anti-VEGFR-2 CAR-T cells. This study proved to be 100% effective without affecting host T cell activity and phenotype (54).

However, these CAR-T cells will kill tumor cells but only mildly reduce its metastasis. Another study tried to overcome this shortfall by combining two TCRs: one to target melanoma tumor cells and another to target VEGFR-2. Yang et al. reported using innovative tandem CAR-T cells that target CD70 and B7-H3 simultaneously (55).

All of these studies have shown promising results of various CAR-T cell treatment regimens against malignant melanoma. However, further research is ongoing to design more effective CAR-T cells with minimum interaction with host cells in order to reduce side effects.

Clinical Studies of the Treatment of Malignant Melanoma using CAR-T Cells

After getting data from preclinical trials that support CAR-T cell therapy's efficacy, clinical trials started in 2010 to treat malignant melanoma using CAR-T cells. Only two studies have been completed and only one has published data. More than six studies are still ongoing and awaiting results. All these trials are phase I or II non-randomized trials, or single-arm trials, which will warrant further investigation if successful (Table 1).

Study Design

Patients are selected based on specific criteria in each study. Once patients meet all the requirements of the particular study, they will undergo leukapheresis which only removes white blood cells from the patient. The patient's T cells are then modified and grown in the laboratory for a specific target antigen. Once done, they are infused back into the patient. Usually, patients stay in the hospital for three to four weeks after the infusion. Then, they are followed in the clinic for long-term monitoring (56).

Study Results

One of the initial studies done by the National Institute of Health (NIH) was a phase I and II trial started in 2010, and the last result was published in 2019. There were 24 patients selected. All 24 patients had metastatic melanoma. CAR-T cells were targeting VEGFR-2 antigen in this study (57). CAR-T cells were used with IL-2. They used different numbers of CAR-T cells in different cycles in this study. 24 patients had adverse events and 5 of them had serious adverse events. 23 out of 24 patents showed progression of disease. Unfortunately, the study was terminated due to no objective response to the CAR-T cell therapy.

Two separate studies have used anti-GD-2 CAR-T cells from samples from melanoma patients. CAR-T cells showed significant cancer cell killing. One study added the C7R gene to the CAR-T cells to increase their survival and provide a constant cytokine supply (58). This study has been completed and is currently awaiting publication. Researchers are hopeful to get durable responses from these two clinical trials that are targeting GD2.

There are several more studies currently undergoing in various stages to determine the effectiveness of CAR-T cell therapy for melanoma patients. Overall, current evidence from clinical studies is very limited in CAR-T cell therapy for melanoma and metastatic melanoma. However, the completion of the ongoing clinical trials might decipher the unknowns in this field and pave the path for possible larger clinical trials.

DISCUSSION

Early detection of metastatic melanoma is strongly correlated to higher remission rates, but this type of cancer is difficult to detect early. The depth of the cancer is also a factor in the remission rates for patients as cancerous cell growth deeper in the tissue leads to a higher chance of metastasis and thus leads to lower survival rates for patients, even with treatment.

Currently, CAR-T cell therapy is being used to treat melanoma by identifying antigens such as VEGFR-2, glycolipid 2, and melanoma-associated antigen. TYPR1 and TYPR2 have been proven to also be suitable antigen markers for melanoma. Current studies focused on CAR-T cell therapy for melanoma are limited, and most are ongoing. Initial results do show mild success. However, as of now, CAR-T cells for the treatment of melanoma are not efficient due to several challenges.

One of the limitations is that the microenvironment of melanoma tumors often contains immunosuppressive cytokines that recognize and inhibit T cells, which includes modified CAR-T cells. Once this occurs, the CAR-T cells will not be able to destroy cancer cells,

Table 1. The results and progress of multiple studies from various research groups are shown in the table (58)

Study #	Year of Study	Study Design	Study Result
NCT01218867	2010	Phase I / II	40.1% stable disease 95.8% Progression of disease
NCT02107963	2014	Phase I - Children and young adult	Completed but not published
NCT03060356	2016	Phase I - 77 patients	Terminated due funding issue
NCT02830724	2017	Phase I/II -	Suspended. No result published
NCT03649529	2018	Early Phase I	Ongoing
NCT03638206	2018	Phase I/II - 73 patients	Ongoing
NCT03635632	2019	Phase I - 94 pt	Ongoing
NCT03893019	2019	Early Phase I- unresectable melanoma	Ongoing
NCT04119024	2019	Phase I - Phase IIC & IV melanoma	Ongoing
NCT04483778	2020	Phase I - Children and young adult	Ongoing

While most studies are currently ongoing or have not yet been published, one study's result is currently shown. In it, it showed that some patients did experience a termination in the metastasis of their cancer at some point during the trials. However, a large majority of patients also showed progression of the disease during treatment at some point during the clinical trial.

thus reducing the efficacy of the CAR-T cells against melanoma tumors. Elevated immunosuppressive cytokine activity is also a major cause of symptoms such as fever and fatigue that decrease the safety and quality of life for patients undergoing CAR-T cell therapy.

Another major limitation is the heterogeneity of melanoma tumors. There is a variety of antigens that melanoma cancer cells could express, making the process of CAR-T cell therapy more difficult to implement as a patient specific process. This also reduces the success rate of this therapy because antigen-receptor binding and recognition of cancer cells is highly specific. Therefore, the cancer cells must be extracted to determine the antigen present. However, this can only be done if the cancer cells express an antigen that is already known and mapped. Furthermore, even after this process is completed, there is still a chance that the wrong antigen was determined, meaning that the wrong receptor was modified to the CAR-T cell, and as a result the CAR-T cells will not recognize and destroy the cancer cells. This makes the process of creating and using CAR-T cells for melanomas a lengthy process with much room for error and inaccuracy.

T-cell exhaustion has been another major limitation for using CAR-T cell therapy to treat metastatic melanoma. Once successfully created and inserted into the body at the tumor site, CAR-T cells are constantly activated and destroying cancer cells. This has been linked to decreased efficiency, accuracy, and functionality because of adverse effects such as increased inhibitory receptor expression (59).

More testing needs to be done to find optimal ways to avoid these limitations or improve the CAR-T cells so that the effects of these limitations are minimal. Once this occurs, the efficacy and safety of CAR-T cell therapy for treating metastatic melanoma will greatly improve. Furthermore, once these obstacles have been overcome, further research into CAR-T cell therapy and research on its use with other co-therapies can be completed.

FUTURE DIRECTION

To optimize CAR-T cell therapy for metastatic melanoma, it is important that research continues, especially research that involves testing multiple independent variables at once, such as the target antigen and the generation of the CAR. While most research on CAR-T cells isolates and manipulates only one variable, such as the antigen or intracellular signaling domain, changing multiple variables at once would allow us to observe which combination of variables and modifications to the T cell work best to recognize and destroy cancer cells. This would remove many limitations to research that occur by only being able to observe the effects of one variable at a time, which may not provide the most efficient and safe version of the CAR-T cell.

CAR-T cell therapy with multi-antigen targeting is currently being explored. Instead of the traditional process of modifying T-cells to express one receptor, this process would involve inserting the genes for multiple receptors into T-cells. As a result, multiple receptors would be expressed, allowing the T-cells to recognize a wider variety of possible antigens on the surface of cancer cells, which would lead to the T-cells recognizing and destroying cancer cells more frequently. This would also resolve a major limitation of current CAR-T cells, which is that the chosen receptor of CAR-T cells may not match the antigens of many of the cancer cells, reducing the efficiency of the therapy.

Another area of research related to cell-based therapy is testing other cells to target cancer cells like natural killer (NK) cells, T lymphocytes, or dendritic cells. Recently, the FDA has approved the first therapy called AMTAGVI (Lifileucel). This is a Tumor Infiltrating Lymphocyte. AMTAGVI involves collecting T-cells that have infiltrated or destroyed cancer cells, which are usually collected at the tumor site, amplifying these cells in a lab, and then re-introducing these T-cells back into the body to improve the body's ability to destroy cancer cells (60).

CONCLUSION

CAR-T cell therapy utilizes chimeric antigen receptors and patient-specific antigen markers to better target cancer cells. CAR-T cell therapy is usually paired with lymphodepletion for increased efficiency and to avoid an excess number of T cells in the body. This combination therapy has been successful in cancers like lymphoma and leukemia. Now, CAR-T cell therapy is being researched for its safety and efficiency in other, solid tumors such as metastatic melanoma. The CARs being tested are mainly specific for GD2, TYRP1, TYRP2, and GP100 antigens, which are common antigen markers in metastatic melanoma. There are a few clinical studies completed and published that show some minor to moderate recession of the tumor. However, in general, CAR-T cell's safety and efficacy for metastatic melanoma is still inconclusive. Another major unknown is how much activation of the CAR-T cells is best for the

balance between CAR-T cell efficiency and cytotoxicities, such as cytokine release syndrome. In other words, each generation of CARs allows for more activation of the T-cell than the previous generation, but it also comes with more cytotoxicities that can increase symptoms and risks to other organs. Testing which generation of CARs allows for optimal efficiency and cytotoxicities should remain a major focus in research centered around CAR-T cell therapy for metastatic melanoma. Another issue is tumor microenvironments, which can inhibit CAR-T cells, cause T-cell exhaustion due to overactivation, and even degrade T-cells due to pH levels. CAR-T cell inhibition, specifically, has been a major challenge in successful CAR-T cell therapy for metastatic melanoma, as well as for other cancer types too. Therefore, future CAR-T cell therapy trials and research should focus on overcoming these challenges, specifically T-cell inhibition from immunosuppressive cytokines released from cancer cells. One possible solution to this has been combinatory therapy with CAR-T cells and immune checkpoint inhibitors, which prevent cytokines from inhibiting T-cell activity. Another avenue of research that should be explored further are CAR-T cells equipped with more than one type of receptor, which allows the T-cells to target and kill more cancer cells by recognizing more antigens. Overall, CAR-T cell therapy for metastatic melanoma has shown moderate efficiency but needs more research in order to overcome TME challenges, find optimal combinations or modifications of the CARs, and establish results of CAR-T cells being used simultaneously with other therapies.

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