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# Combining CAR T-Cell and Immune Checkpoint Inhibitor Therapy, A Promising, Yet Unproven Immuno-Oncology Approach

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## ABSTRACT

Chimeric antigen receptor (CAR) T-cell therapy is a method that extracts T cells from the patient's blood and virally introduces a genetically engineered T cell receptor targeting a specific cancer antigen and subsequently readministering these genetically engineered CAR T cells to the patient. These T cells are then better at identifying the tumors and attaching to these tumor cells resulting in a stronger cytotoxic immune response. The development of CAR T cells has been a huge success as an immunotherapy, especially for the targeting of non-solid tumors. Since their original inception in 1987, there are now six independent FDA approved CAR T cell therapies targeting a variety of blood cancers, with the first being approved in 2017. As a relatively new treatment, there is a continuous effort in improving the safety and efficacy of CAR T cell therapies. As mentioned previously, CAR T cells have undoubtedly been successful in the treatment of non-solid tumors, however their efficacy towards treatment of solid tumors has been limited. Additionally, the safety and long-term effects of CAR T cell treatments is still a concern. Combination therapy utilizing CAR-T cells and immune checkpoint inhibitors is being explored to potentially mitigate some of the limitations associated with CAR-T cells.

## Introduction

Chimeric Antigen Receptor T cell Therapy, also known as CAR-T cell Therapy, is a form of immunotherapy in which a genetically engineered T cell receptor targeting a specific cancer antigen is virally introduced to a patient's T cells ex vivo and then readministered to the patient in order to improve the body's ability at fighting cancer cells. This process begins with the initial procedure of collecting the T-cells from the patient's blood. This is accomplished by utilizing leukapheresis to remove the white blood cells from the patient's body. This is conducted using two IV lines: one that removes blood so the white blood cells can be separated out, and the other that puts the blood back into the body. After the white cells are drawn out, the T cells are separated from the white cells. These initial T cells are then altered by adding a gene for the specific Chimeric Antigen Receptor which are subsequently grown and multiplied in a lab for several weeks. Once this process comes to an end, the final step of reintroducing the CAR T-cells into the patient's body occurs and the procedure is complete.

### Structure

The structure of CAR-T cells is composed of the antigen binding domain, the hinge region, the transmembrane domain, and the intracellular T cell signaling domain. The antigen binding domain is what grants target antigen specificity, which consequently determines the affinity of the CAR-T cell therapies and further aids in antigen recognition. It utilizes its encounters with potential target molecules to discern which of these cells express a matching molecule and subsequently targets the CAR-T cells to these molecules (1). The hinge region, also

known as a spacer, is located between the antigen binding domain and the cell's outer membrane and is responsible for mitigating spatial restrictions between the CAR and its target antigen by improving the flexibility of the scFv receptor head. This is vital because in order to allow for immunological synapse formation, the length of the spacer is very important in ensuring enough intercellular distance is created (2). The transmembrane domain's purpose is to ensure the CAR is firmly affixed to the T cell membrane (1). However, it does also play a small role in CAR-T cell function as studies have shown that it has proven to affect CAR expression level, stability, signaling, and synapse formation (2). Lastly, the intracellular signaling domain is in charge of transmitting a signal to the T cell and launching the signaling cycle. In addition to intracellular signals, costimulatory signals are utilized to sustain this cycle, typically controlling the metabolic cycles, apoptosis, activation-induced cell death, and the pathway for T cell development (3).

## Current FDA-Approved CAR T-Cell Therapies

Currently, there are many CAR T-cell therapies approved by the US Food and Drug Administration (FDA) that typically center around treating various types of lymphoma, leukemia, and multiple myeloma. Some of these therapies include Tisagenlecleucel (Kymriah), Axicabtagene ciloleucel (Yescarta), Brexucabtagene autoleucel (Tecartus), Lisocabtagene maraleucel (Breyanzi), Idecabtagene vicleucel (Abecma), and Ciltacabtegene autoleucel (Carvykti). KYMRIAH is a genetically engineered autologous T cell immunotherapy that is aimed against CD19 for the treatment of individuals with B-cell precursor acute lymphoblastic leukemia who are under the age of 25 and are in second or later relapse (4). Adult patients with large B-cell lymphoma who are unresponsive to first-line chemoimmunotherapy or who relapse within a year after receiving it are treated with another approved CAR T-cell therapy: YESCARTA (axicabtagene) (5). Another therapy, TECARTUS is a CD19 modified T cell immunotherapy utilized to treat adult patients with relapsed or refractory mantle cell lymphoma (MCL) (6). BREYANZI is used for treatment of adult patients who have large B-cell lymphoma including diffuse large B-cell lymphoma, high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, and follicular lymphoma grade 3B (7). ABECMA was approved to treat adult patients with relapsed or refractory multiple myeloma after four or more prior lines of therapy, including an immunomodulatory agent, a proteasome inhibitor, and an anti-CD38 monoclonal antibody. The approval of this treatment was the first cell-based gene therapy for multiple myeloma that was approved by the FDA (8). The FDA later approved the CARVYKTI therapy for treating adult patients with relapsed or refractory multiple myeloma after four or more prior lines of therapy, including a proteasome inhibitor (PI), an immunomodulatory agent (IMiD), and an anti-CD38 monoclonal antibody (9). Currently, many other CAR T-cell therapies are now being tested and studied in order to make this therapy more widely available for other cancers as well and take a step closer in potentially treating these cancers.

**Table 1.** FDA-approved CAR-T cell Therapies

| FDA clinically approved therapies | Target | Year Approved | Targeted Cancers | Other Notes |
|---|---|---|---|---|
| KYMRIAH (Tisagenlecleucel) | • CD19 | • August 2017 (For patients younger than 25) | • B-cell precursor acute lymphoblastic leukemia | • Utilizes CAR with 4-1BB costimulatory domain |

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|---|---|---|---|---|
| YESCARTA (Axicabtagene ciloleucel) | • CD19 | • October 2017 (For ages 21-80) | • Large B-cell lymphoma | • Used for patients unresponsive to chemotherapy |
| TECARTUS (Brexucabtagene autoleucel) | • CD19 | • July 2020 (For ages 26 and above) | • Treats relapsed or refractory mantle cell lymphoma | |
| BREYANZI (Lisocabtagene maraleucel) | • CD19 | • June 2020 (For adult patients) | • Treats large B-cell lymphoma<br>○ Diffuse large B-cell lymphoma<br>○ High-grade B-cell lymphoma<br>○ Primary mediastinal large B-cell lymphoma<br>○ Follicular lymphoma grade 3B | • Used for patients not eligible for hematopoietic stem cell transplantation |
| ABECMA (Idecabtagene vicleucel) | • CD38 | • March 2021 (For adult patients) | • Relapsed or refractory multiple myeloma | • First FDA approved cell-based gene therapy for multiple myeloma<br>• Used after 4 or more other therapies have been attempted including..<br>○ immunomodulatory agent<br>○ A proteasome inhibitor<br>○ An anti-CD38 monoclonal antibody |

| | | | | |
|---|---|---|---|---|
| CARVYKTI (Ciltacabtegene autoleucel) | • CD38 | • February 2022 (For adult patients) | • Relapsed or refractory multiple myeloma | • Used after 4 or more other therapies have been attempted including..<br>○ immunomodulatory agent<br>○ a proteasome inhibitor<br>○ an anti-CD38 monoclonal antibody |

## Limitations

However, despite the many approved CAR-T cell therapies that are now being widely used, this treatment does have some limitations. Besides top level drawbacks such as a high cost and the timely process, there are a plethora of other limitations of this therapy that adversely affect the body. One common side effect is antigen escape in which a tumor develops immunity against the CARs that target only one specific type of antigen (1). Even though initially the treatment would be effective, the tumor cells eventually start to experience complete or partial loss of the antigen the CAR is targeting, a phenomenon known as antigen escape. In order to fix this, new CAR-T cell therapies are now starting to target multiple antigens using either dual or tandem CARs (10). Another limitation is called on-target off-tumor (1). This occurs because sometimes the tumor antigen that the CAR targets is also present on normal cells resulting in the CAR attaching to these antigens as well, harming the body's own healthy cells. In order to overcome this, multi-targeted CAR T cells are being developed in order to have a better on-target/off-target specificity, resulting in decreased side effects than single-targeted CAR-T cells (11). A third limitation is an immunosuppressive microenvironment which results when cells such as myeloid-derived suppressor cells (MDSCs) that drive immunosuppression infiltrate the tumor resulting in the augmented production of tumor assisting cytokines, chemokines, and growth factors (1). Checkpoints can also be utilized to boost antitumor immunity. Tumor-associated macrophages (TAMs) play a major role in the immunosuppressive environment as they express inhibitory immune checkpoint ligands and suppress immune cells through their production of multiple anti-inflammatory cytokines and enzymes that deplete the amino acids that are essential for T cells to function (1). To address this challenge, one technique being used is the combination of CAR T cells with pro-inflammatory cytokines. In order to avoid drastic consequences due to the excessive administration of cytokines, the cytokine gene is being incorporated into the CAR T-cell construct (12). All these limitations and potential solutions demonstrate steps scientists are taking in order to help CAR-T cells be more widely available and useful as an effective treatment for cancer patients around the world. Although there are these significant challenges and shortcomings of CAR T cell therapies there is potentially groundbreaking work being done to address these issues, specifically solutions to combat against the immunosuppressive microenvironment present in solid tumors.

### *Current Research to Overcome these Caveats*

Immune checkpoint inhibitors are an effective therapy that helps overcome the immunosuppressive microenvironment in tumors. These inhibitors prevent the tumor from being able to overcome the immune system's checkpoints, preventing the T cells from identifying and killing the tumor cells. In normal conditions, these immune checkpoint inhibitors maintain balance between proinflammatory and anti-inflammatory signaling throughout the body. Because malignant cells promote an immunosuppressive state favoring immune evasion

and tumor growth, this delicate balance is disrupted. Through the recruitment of T regulatory cells (Tregs), the downregulation of tumor antigen expression, the induction of T cell tolerance, and the production of immunosuppressive cytokines, a highly immunosuppressive tumor microenvironment is created (13). As shown in figure 1, immune checkpoint inhibitors block the effects of this immunosuppressive tumor environment. One common checkpoint protein, programmed cell death receptor-1 ligand (PD-L1), is located on the tumor cells and binds to programmed cell death receptor-1 (PD-1) on T cells, resulting in negative costimulatory signal being transmitted that sends an "off" signal to the T cells. This ensures that the immune response does not occur and that the tumor is not destroyed. However, these immune checkpoint inhibitors would block this binding of PD-L1 to PD-1 helps the T cells be able to kill the tumor cells. Some other similar proteins, cytotoxic T lymphocyte-associated molecule-4 (CTLA-4) and B7, act in a similar fashion as PD-L1 and PD-1. The binding of these two checkpoint proteins prevents the immune response, so by blocking CTLA-4, the T cell is able to successfully kill the tumor cell (14). Ipilimumab, an anti-CTLA-4 monoclonal antibody, was the first FDA-approved immune checkpoint inhibitor and was used for patients with advanced melanoma. Later, other ICIs were created such as nivolumab, pembrolizumab, cemiplimab, atezolizumab, avelumab, and durvalumab. Other treatments utilizing these immune checkpoint inhibitors (ICIs) are currently being tested through clinical trials and even though many ICI therapies have proven to perform well in combating various tumors, others have shown to only have short-lived results with the tumor eventually recurring in the patient (15).

## Methods

This research paper is a retrospective analysis that collects various clinical trial information from sources such as PubMed and analyzes this pre-existing information to highlight the ongoing work being done to combine CAR T cell and immune checkpoint inhibitor therapies in the clinic. Using these sources and data available, we generated graphical representations focusing on the current trend in CAR T, ICI, and combination therapies. Graphics and figures were generated using BioRender and GraphPad Prism9 software. Primary sources for the data included Rossetti et al., 2022 and ClinicalTrials.gov.

## Results

Currently, clinical trials are aimed towards combining multiple ICIs with other agents that result in the death of the tumor by enhancing anti-tumor effects through the enhancement of antigen presentation. By reinvigorating a worn-out immune response, by itself, checkpoint blockade (CPB) treatment can have long-lasting therapeutic effects. However, because there isn't an immune infiltrate that is responsive to the tumor, response rates are still low. Chimeric antigen receptor (CAR) T cells might deliver the required immunological infiltration of the tumor as well as a highly targeted antitumor immune response. Early trials have shown that CD19-targeting CAR-T cell therapy has proven very effective in patients with chemotherapy-refractory hematologic malignancies. However, for solid tumors, the immunosuppressive microenvironment poses a huge obstacle for the CAR-T cells that exhausts them and lowers their success in fighting the tumor. The addition of CPB agents to the CAR-T cell, however, mitigates some of the weaknesses undermining the efficiency of CAR-T cell by ensuring it's performance isn't hampered by this immune inhibitory environment (16). Overcoming this immunosuppressive microenvironment of the tumor is crucial to the functionality of the CAR-T cells as it ensures the T cells are able to successfully identify and destroy the correct tumor antigens, furthering the body's ability to eliminate the tumor.

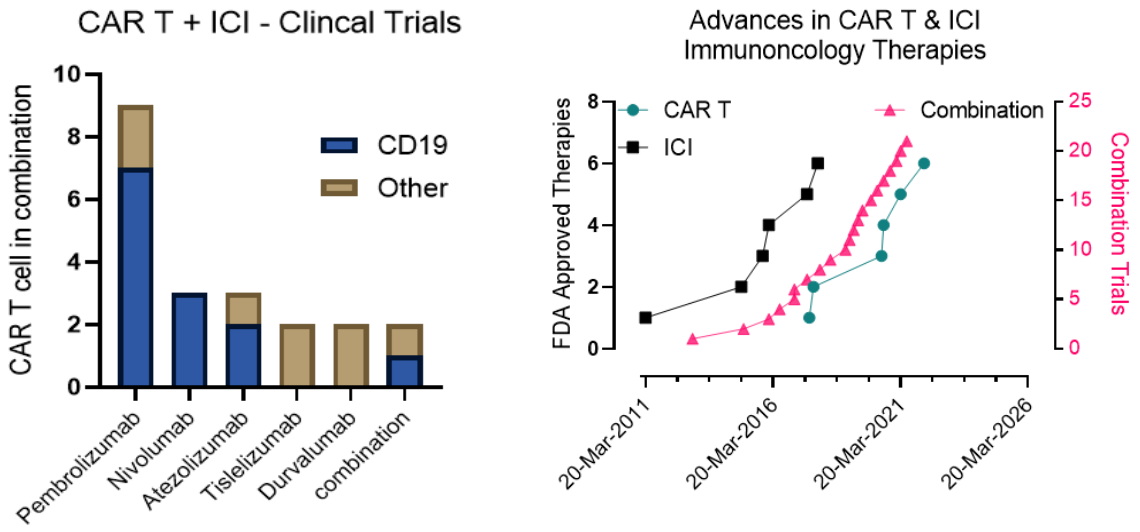


**Figure 1.** Improving CAR T cell therapies with immune checkpoint inhibitors and vice versa for immuno-oncology therapy. (A) Trajectory of FDA approved ICI and CAR T therapies since the first ICI approval in 2011 and the first CAR T cell therapy in 2017. Clinical trials of combination therapies of CAR T and ICI therapies began as early as 2013. (B) Graphical representation of which ICI and CAR T therapies are primarily being tested in the clinic. Data derived from Rossetti et al., 2022 and ClinicalTrials.gov online repository.

More recently, clinical trials were conducted to test this combination therapy, specifically between HER2-targeted CAR T cells and PD-1 blockade. These trials concluded that using pembrolizumab (a type of CAR T cell therapy) led to an improved T-cell function and survival after repeatedly stimulating antigens that are against PD-L1-positive tumor cells. In these trials, these PD-1 checkpoint proteins blockage antibodies were shown to be able to restore the effector activity of worn-out CAR T cells. However, tumor recurrence was seen when therapy stopped, suggesting that the PD-1 antibody's effectiveness depends on frequent delivery of the antibody (17).

Not surprisingly, as shown in figure 1, these clinical trials combining ICI and CAR T therapies began before the first CAR T therapy was FDA approved. It's clear that scientists and clinicians saw the promise in combining the two immunotherapies in order to target and eliminate cancer more effectively. The first combination therapy started only 2 years after the first ICI therapy had been approved, while it took the CAR T cell therapies around 6 years more than the ICIs, three times the length from combination therapies (18). This demonstrates how it was apparent to scientists early on that combination therapy had the potential to be highly successful.

## Discussion

The use of CAR T cell treatment in conjunction with immune checkpoint inhibitors is very beneficial since CAR T cells can infiltrate solid tumors and the immune checkpoint inhibitors can override CAR T cell inhibition and restore functional persistence. Through a plethora of clinical trials, this very advantage has been displayed, but there is room for possible improvements to this combination therapy, specifically for immune checkpoint inhibitors. Current ICI therapies have not always proven to be effective, with only around 12.5% of cancers responding to this treatment (19). In order to address the other unresponsive cancers, a potential solution can be finding other modulatory targets for immune blockades. Recent studies have identified potential new targets such as lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin and mucin-domain containing-3 (TIM-

3), T cell immunoglobulin and ITIM domain (TIGIT), V-domain Ig suppressor of T cell activation (VISTA), and more (20). Hopefully by focusing on these other targets, there can be an increase in the success rate of the ICI therapy, furthering the overall effectiveness of the CAR T cell and ICI combination therapy.

However, the other drawbacks associated with this combination therapy method demonstrates the need for further advances in immuno-oncology therapies in the future that can hopefully bear more positive results than those shown due to the combination of CAR T cells and immune checkpoint inhibitors. One such example can be expanding the availability and success of cancer vaccines. By recognizing the potential of cancer vaccines and focusing efforts on creating new ones that target a more expanded field of cancers, these therapies can be an efficient solution to help destroy numerous types of cancers. A newer approach to immunotherapy appeared as a result of neoantigens, a new protein that develops on cancer cells when certain DNA mutations take place in tumors. Studies have shown that there is a relationship between these neoantigens and the ability of T cells to recognize the cancer cells (21). Another possible new therapy that should be further explored is personalized or genetic based medicine. This involves examining a patient's unique genes and proteins present in their cancer cells to figure out if changes to these aspects can benefit their wellbeing (22). One type of personalized medicine involves combining the two previously mentioned therapies, cancer vaccines and neoantigens to create a treatment plan more specific to a patient's cells, cancer, and overall situation. Surprisingly, clinical trials conducted over this new method have shown promising results (23).

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