Journal of Health and Biology
https://journalhb.org/
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Review Article

Genetically modified CAR T-cell and its application in immunotherapy

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DOI: https://doi.org/10.71428/JHB.2026.0109

Abstract

Chimeric antigen receptor T-cell therapy, or CAR, is a treatment that alters a patient's T cells so they can detect and destroy tumor cells. Immune-modified cells called chimeric antigen receptors, or CAR cells, are used to treat a variety of cancers and immune system illnesses. CAR-T cells, which are genetically engineered combinations of antibodies and T cells, have three types of domains: transmembrane domains, intracellular signaling domains, and antibody-identical surface domains.

Car T cell engineering requires careful management due to its complexity. A group of unstimulated leukocytes is produced by Leukapheresis. Numerous methods, including separating cells by size and density, removing monocytes, isolating lymphocytes, and separating red blood cells and platelet contaminants using a density gradient, will be used to isolate T cells.

CAR-T cell action depends on receptors, called second- or next-generation receptors, in the system of "living drugs." Initially, two CAR T-cell medications were selected for marketing agreements in the USA for treating symptoms of hematological cancer, demonstrating the remarkable advancements made in cancer care through "adoptive cell immunotherapy." Immunotherapy has resurfaced as a novel treatment approach in recent years. A potential strategy to strengthen or replenish the immune system's ability to fight cancer and control autoimmune disorders is the concept of precisely altering the immune response. Today, CAR T-cells have been developed to treat a variety of illnesses, such as multiple myeloma, breast cancer, and CNS tumors. Ideally, CAR T-cell medical care will replace stem cell transplantation and chemotherapy in the long run.

Keywords: CAR -T cells, T-cell therapy, immunotherapy, chimeric antigen receptors.

Introduction

Chimeric antigen receptor (CAR) T-cell therapy modifies a patient's T cells so they can recognize and attack malignant tumor cells. CAR T cells are immune-modified cells used as therapeutic agents for many malignant tumors and immune system diseases. In a process called "apheresis," a patient's white blood cells are collected and sent to a lab. There, T cells are isolated and engineered to express a synthetic receptor on their surface, creating modified T cells that can identify and target cancer cells. These artificial receptors are called "chimeric antigen receptors." The lab or manufacturing facility produces larger quantities of engineered CAR T cells. T cells can be frozen and shipped to the treatment center if needed (1).

There, the CAR T cells can be frozen and infused back into the patient via intravenous administration. The most commonly used antigen for leukemia and lymphoma in CAR T-cell immunotherapy is "cluster

Received: January 20, 2026. Accepted: April 2, 2026. Published: May 11, 2026

of differentiation 19" (CD19). Both malignant and healthy B-cells, including those in lymphoma and leukemia, express the CD19 antigen on their surface. Because only B-cells carry the CD19 antigen and patients can tolerate long-term B-cell suppression, CD19 is considered the ideal target antigen for CAR T-cell therapy. There are also ongoing trials of CAR T cells targeting other antigens found in different types of hematologic malignancies (1).

Family of CAR T Cell

Three types of domains, including antibody- derived surface domains, intracellular signaling domains, and transmembrane domains, are present in CAR- T cells, which are genetically engineered to express various antibodies and T- cell receptors. The single-chain variable fragment, an extracellular element, is derived from an antibody and consists of both light and heavy chains. To enable the receptor to identify tumor antigens, it reroutes its specificity without the use of major histocompatibility complex proteins. Typically, the transmembrane gap that joins the extracellular and intracellular components is constructed using CD8 or IgG4 molecules. The intracellular signaling domain consists of the CD3 ζ chain and a costimulatory domain, which together trigger T- cell activation. For several reasons, CD19 has been chosen as the most prevalent target antigen. It is restricted to the B- cell lineage in healthy tissue, has broader expression than other possible targets like CD 20 or CD 22, and is expressed in B- cell leukemias and lymphomas. This means that monthly intravenous immunoglobulin replacement therapy can reduce B- cell aplasia, a common off-target outcome following CAR T cell infusion. The receptors have undergone considerable design changes over time. Only the CD3 ζ domain was used in the engineering of the first generation of CARs. They were unable to activate resting T cells, control long- lasting T- cell responses, or maintain cytokine release because of their low signaling capacity (2,3). When the modified T cells were coupled with other costimulatory signal domains (such as CD28 and 4-1 BB), their stimulation, persistence, and ability to proliferate were all improved (4). The current authorized CAR T cell action depends on receptors, which are called second or next- generation receptors in the system of "living drugs." CAR T cells of the third generation combine the signaling capabilities of two costimulatory domains (e. g., both CD28 and 4- 1 BB). Fourth- generation CARs, also known as TRUCKs (T- cells redirected for universal cytokine- mediated death), have increased anticancer potency due to further genetic modification. Transgenes for the production of cytokines (such as IL- 12) or additional ligands for stimulation are two examples of these (5).

CAR -T Cells Mechanisms

Because CAR-T cell technology is so intricate, it needs to be carefully managed. Leukapheresis yields a group of unstimulated leukocytes. T cells can be separated using a variety of techniques, such as removing red blood cells using a density gradient and platelet contaminants, dividing cells based on size and density, removing monocytes, and separating the lymphocytes. Additional steps allow segregation into "CD4, CD8, CD25, or CD62L T-cell" subsections. The allowed yields for T-cell process enhancement differ depending on the therapeutic indication. Therefore, Kymriah and Yescarta have different starting amounts for CAR T production, while the importance in terms of safety and efficacy is still unclear (6-8).

Viral vectors genetically modify the harvested T-cells and their enriched starting population. Compared with the gamma retroviral vector, the lentiviral vector can offer safer genomic incorporation (9). Accordingly, lentiviral vectors have been employed in clinical analyses of CAR T-cell treatments. The transposon-transposase system is a modern approach that employs a plasmid-dependent expression system, which adds anti-CD19 CARs to the T cell via electroporation (10).

Anti-CD3 antibodies, anti-CD3-anti-CD28 magnetic beads, or antigen-presenting cells, such as

stem cells or synthetic antigen-presenting cells, can be used to stimulate CAR T cells (11). To confirm propagation, the latter can be synthesized using several costimulatory ligands. To provide therapeutic doses of CAR-T cells, various stages are available that enable rapid cell proliferation to reduce contamination (12). Commercial harvests are produced under uncertain conditions using CD3/CD28 droplets and specific cytokines; however, due to proprietary holdings, not all information is disclosed by the firms (13). Following the manufacture of patient-specific CAR-T cells and blood assembly, cells are transported to a handling and thawing facility. The final phase is the fermentation of CAR-T cells in the patient, achieved through lymphodepleting chemotherapy to stimulate development (12,13).

Car's Passage: Finish Line of medical care

In the new year, CAR-T cell care has advanced rapidly. Initially, two CAR T cell medications were selected for marketing agreements for the treatment of hematological cancers in the USA, demonstrating the remarkable advancements made in cancer treatment through "adoptive cell immunotherapy." In 2018, the European Commission approved the use of axicabtagene ciloleucel (Yescarta®, Gilead) and tisagenlecleucel (Kymriah®, Novartis) to treat severe non-Hodgkin lymphoma (NHL: diffuse large B cell lymphoma [DLBCL] and primary mediastinal large B cell lymphoma) and r/r B-cell precursor ALL in children and young adults. In the comprehensive worldwide ELIANA trial (NCT02435849), the use of tisagenlecleucel, the CD-19-targeted genetically modified autologous T cell product, in pediatric and young-adult patients with r/r B-cell ALL was examined. Tisagenlecleucel was administered just once to 75 individuals in this phase II study. A review of the most recent long-term outcomes of the ELIANA trial was published earlier this year. Sixty-one percent of patients had undergone allo SCT before enrollment, with a median of three previous treatments. The median follow-up was over a year, and the overall remission rate (CR/CRi) was 81%. At six months, the median duration of remission had not been reached, while overall survival was 90%, with an event-free survival rate of 73%. The long-term durability of tisagenlecleucel was evidenced by patients who received it for up to 20 months (14).

CAR T cell applications

The fact that CAR T can be used for any disease treatment makes it a huge success. New research is being conducted to extend the application of CAR T cell therapy to additional malignancies after its notable success in treating B-cell malignancies and ALL lymphoma. Additionally, the initial endeavor to target solid tumors was successful (16). Treatment using CAR T-cells for solid tumors has produced a range of results thus far (17). As shown in numerous clinical trials, discovering a strong marker antigen that is expressed in tumors but not in healthy tissues is an important challenge to prevent acute toxicities (15-17). Treatment with B-cell maturing antigen-targeting CAR- T cell. Individuals with r-r aggregate myeloma are receiving treatment with (bb2121, a second-generation CARs). Updated data from the ongoing 2-Part state I survey (CRB-401) show that bb2121 significantly and sustainably improved the response of many myeloma patients undergoing harsh primary treatment. CAR-T cell therapy for (bb2121 anti-B cell) matured antigens may be a new therapeutic option for r/r aggregate myeloma since (CRS) and neurotoxicity were controlled (18).

It is anticipated that future CAR-T cell handling will target numerous antigens, making the loss of single molecules less critical to therapeutic effectiveness. The next phase of development entails delivering CAR-T cells continuously to the tumor site to reduce systemic exposure. For example, CAR-T cells targeting IL-13, fused into polymorphic glioblastoma, led to tumor shrinkage without adverse side effects (19).

The HER2 protein is another notable target antigen present in a variety of adolescent brain tumors, in addition to breast cancer. Third-generation CAR T

cells, which are administered directly via a CNS catheter and target HER2-expressing tumor cells, were first evaluated in the stage I Brain-Child-01 trial (NCT03500991). Treatment resistance is largely driven by the immunosuppressive environment of solid tumors. Because it remains unclear how much T-cell proliferation and persistence within the tumor are necessary for therapeutic success, penetrating CAR T cells into solid tissues is challenging (20).

By upregulating repressive receptors such as CTLA-4 and PD-1/PD-L1, solid tumors can withstand treatment. To tackle this, checkpoint inhibitors and CAR-T cells were investigated in mice, with encouraging outcomes (21). Furthermore, combining CAR-T cells with an oncolytic adenovirus should help overcome the limitations of monotherapy. Achieving such encouraging outcomes in solid tumor treatments illustrates the tremendous potential of CAR-T cell therapies and emphasizes the importance of their ongoing research (22).

Until recently, only a very small percentage of individuals with late-stage hematological malignancy could receive CAR T-cell therapy. Although this emerging therapy has primarily been tested in small clinical studies, it has attracted significant attention from researchers and physicians alike. Exceptional results have been observed in both adults and children. CAR T cells could one day be used to eliminate residual disease in patients with acute lymphoblastic leukemia (ALL). While much remains unknown, urgent issues require immediate attention, particularly the management of severe adverse events caused by strong immune activation, which is critical for the effective and objective deployment of CAR T cells.

The Immune Task Force MU at Ludwig-Maximilian University Munich (LMU) is one example of this, providing a 24-hour on-call service staffed by hematologists, neurologists, and neuroradiologists skilled in T-cell therapy. Their goal is to manage CAR T-cell patients effectively while ensuring patient safety, as inappropriate care may impede CAR T-cell development (23).

Other significant issues include streamlining the complex manufacturing process and providing low-cost ways to expand the use of this technology across multiple cancers. After the first CD-19-specific CAR-T cell therapy was authorized in the US and Europe, CAR T cells are now being developed to treat various diseases, including multiple myeloma, breast cancer, and CNS tumors. Ideally, CAR T-cell therapy will replace stem cell transplantation and chemotherapy in the long run (24).

However, in solid tumors, outcomes have been inconsistent. Solid tumor treatment is more difficult because it is challenging to identify suitable target antigens and to ensure that T lymphocytes can infiltrate and persist within the tissue long enough to elicit an anticancer response. While CAR T-cell treatment for tumors other than lymphoid malignancies is still in its early stages, success in future trials could greatly increase the use of this groundbreaking immunotherapy. Managing adverse responses and implementing risk-reduction measures, such as specialist treatment centers, staff training, and provisions for cytokine release syndrome (CRS), will become even more important. CAR T-cell technology marks the beginning of a new era in cancer treatment. The potential and challenges that await us remain to be properly explored (23). Chimeric antigen receptors (CARs) are fusion proteins that enable T cells to detect surface molecules on tumor cells instead of relying on the conventional T cell receptor (TCR) and major histocompatibility complex (MHC) interactions. CARs are introduced into T cells by gene transfer procedures (25).

Chimeric antigen receptor (CAR) T cells, a type of genetically modified cell, are a revolutionary form of cancer immunotherapy. The development of adoptive cellular therapy using CAR T cells has spanned more than three decades, with Gross et al. establishing the foundations for first-generation CAR T cells. These synthetic receptors are designed

to target surface antigens in their native state. CARs consist of a tumor-targeting component (usually a single-chain variable fragment of an antibody), a hinge region, a transmembrane domain, and an intracellular signaling motif derived from the TCR complex's CD3-zeta (CD3ζ) unit (4).

CAR-T cells are not limited by the major histocompatibility complex (MHC) because of their molecular characteristics. Second-generation CARs, which feature a costimulatory signaling domain (typically CD28 or 4-1BB), have been demonstrated to enhance T-cell proliferation and cytokine production in more recent clinical trials (4). CARs with 4-1BB may lead to longer T-cell persistence, per a number of studies (5-7). Additional clinical research is necessary to determine whether third-generation CARs with at least two costimulatory domains are more effective than those with just one costimulatory domain, but they have also shown activity (26).

White blood cells (WBCs) are typically collected from the patient via leukapheresis, the first step in autologous CAR T-cell therapy. After activation (often with anti-CD3/anti-CD28 beads), T cells undergo lentiviral or retroviral transduction to modify their genetic makeup. CAR T cells are produced, expanded to clinically meaningful numbers, evaluated for quality, and cryopreserved. Central facilities produce commercial CAR T-cell products.

requiring the transfer of the apheresis sample to the manufacturing site and the return of the cryopreserved CAR T-cell product to the therapy location.

Before receiving CAR T cells, the patient usually receives lymphodepleting chemotherapy to create a favorable immunological environment for the therapy to work well. The patient is then given a single intravenous infusion of the CAR T-cell product (10). Several factors can influence the efficacy and toxicity of infused CAR T cells, including the extent of prior treatments, the intensity of lymphodepleting chemotherapy, and the disease burden at the time of infusion. Immunogenicity, immunosuppressive components in the tumor microenvironment, and on-target/off-tumor effects may also affect CAR T-cell effectiveness. Various techniques are now being studied to develop CAR T-cell designs that aim to maximize therapeutic usefulness while decreasing toxicity, and these will be covered in subsequent sections.
(27-29).

The immune system, composed of two major components, plays an essential role in maintaining the body's homeostasis. T cells, part of the adaptive immune system, are classified into subgroups based on markers and functional properties. Double-negative (DN) T cells are distinct in that they express CD3 while lacking both CD4 and CD8 coreceptors. This sets them apart from normal T cells, which express either CD4 or CD8. DN T cells express either TCRαβ or TCRγδ and lack NK T cell markers, distinguishing them from other T lymphocytes.

Although modest, the DN T-cell population accounts for about 3-5% of total T cells in peripheral blood. DN T-cells, unlike conventional T cells that occur only within the adaptive immune system, have been found to possess both innate and adaptive immunological capacities. Although rare, they are an interesting category of immune response pathways due to their dual capacity. This review aims to present the less frequent subgroup of TCRαβ+ DN T-cells, which have gained interest for their particular immune role (30).

The lack of peripheral or central tolerance can lead to autoimmune diseases. It has been established that even healthy individuals have an autoimmune repertoire, and thymic ablation does not entirely remove autoreactive T cells. However, the majority of humans and animals do not develop autoimmune diseases. A key factor in the induction of autoimmune diseases is the failure of regulatory T

cells (Tregs), which are important for maintaining peripheral tolerance.

To understand the mechanism of action of Tregs, start by examining how a small number of autoreactive T cells can be transformed into powerful effector cells. This usually results from a breakdown in the regulation that typically keeps these potentially toxic T cells in check. Tregs play a vital role in preventing such conversions by regulating autoreactive T cell activation and activity, thereby maintaining immune homeostasis and preventing autoimmunity. Understanding the processes behind Treg dysfunction is critical to understanding how autoimmune disorders emerge and to developing effective therapeutics targeting these pathways.

To defend the host against infection and cancer, immune cells must respond quickly to antigens and danger signals. The response includes T and B cell hyperexpansion, migration to sites of tissue injury, and the production of cytokines and effector molecules. Immunological activation thus entails high energy and biosynthetic resource demands. To meet these demands, lymphocytes immediately alter their metabolism, increasing energy production and the synthesis of required building blocks (31).

In the context of Treg (regulatory T cell) activity in human autoimmune disease, preliminary studies of patients have indicated that the majority of autoimmune diseases are associated with a decrease in the number or function of Treg cells isolated from peripheral blood. These findings have been repeatedly confirmed by in vivo models of disease that further define the pivotal role played by Tregs in immune system balance. Impairment of Treg cells will lead to a failure to modulate the immune response properly and hence promote the onset of autoimmune diseases (32).

Regulatory T cells (Tregs) have high therapeutic value for treating autoimmune disorders and preventing transplant rejection because of their ability to actively suppress immune responses. Although the transcription factor forkhead box protein 3 (Foxp3) has been conclusively established as a marker for Tregs in mice, evidence for other unique markers in humans is scarce. Tregs do have a defined set of surface molecules, however. These include CD58, folate receptor-4, neuropilin 1, human leukocyte antigen HLA-DR, and the classic markers CTLA-4 and CD25. Co-expression of these markers helps identify and characterize Tregs, which play an important role in maintaining immune tolerance and preventing excessive immune activation that causes autoimmune disease (33).

Immunotherapy emerged a few years ago as a novel therapeutic approach. The concept of precisely altering the immune response offers a viable way to improve or restore the immune system's capacity to combat cancer and control autoimmune disorders. One of the more hopeful approaches of this type is immune cell-based therapy, or the use of the patient's cells after they are expanded and/or modified in vitro. This approach has the potential to be used to cure not only autoimmune disease but also cancer by leveraging and enhancing the patient's immune system (34).

The natural history of autoimmune diabetes, like that of other autoimmune diseases, is complex and typically involves Treg (regulatory T cell) dysfunction, with Tregs playing a central role in peripheral tolerance. Treg failure in autoimmune disease allows autoreactive T cells to evade regulation and mature into pathogenic effector cells. To understand how this happens, it is necessary to know how autoreactive T cells arise and become pathogenic.

Autoreactive T cells, similar to naïve T cells, migrate throughout the body in response to chemokines such as CCL19 and CCL21. These T cells enter the lymph nodes via high endothelial venules (HEVs) and subsequently reside in the T-cell zones within the lymph nodes, where they may become activated. In autoimmune diabetes, this dysregulated activation of

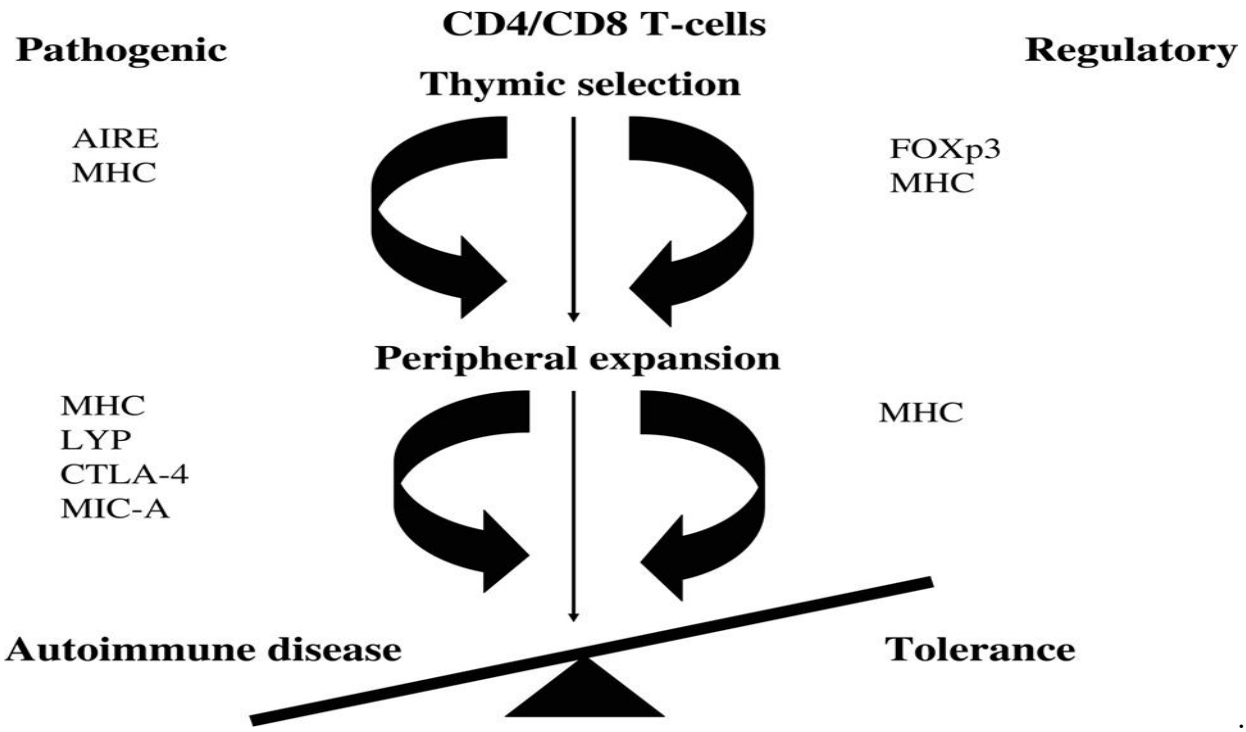
autoreactive T cells contributes to the autoimmune destruction of pancreatic β-cells, leading to the development of the disease (35)

In autoimmune diabetes, particularly in the non-obese diabetic (NOD) mouse model, activation of autoreactive T cells within lymph nodes (LNs) is a crucial step in disease induction.

T cells aggregate around paracortical T-cell regions and B-cell follicles of LNs, where they get activated by dendritic cells (DCs) presenting the cognate antigen. This activation process, which is referred to as LN priming, is a critical initial step in the development of autoimmune diseases.

In autoimmune diabetes, the pancreatic lymph nodes are especially critical. The earliest signs of diabetogenic T cell priming appear in these nodes at disease onset. Remarkably, in NOD mice lacking pancreatic LNs, there are too few diabetogenic T cells in the spleen to sustain the disease, indicating the initiating role of the pancreatic LNs in the autoimmune attack.

Furthermore, the B7-2 costimulatory molecule has been found to play a critical role in T-cell priming within LNs. In NOD mice lacking B7-2, LN priming of islet-specific T cells decreases significantly, thereby protecting against diabetes. This highlights the central role of B7-2 and pancreatic LNs in the induction of autoimmune diabetes by facilitating the proliferation of autoreactive T cells at low frequency. Generally, the preparation of autoreactive T cells in pancreatic LNs is necessary for the disease pathophysiology (36).



The diagram provides a clear and informative overview of the generation of regulatory and pathogenic T cells, with an emphasis on the roles of thymic selection and peripheral expansion.

This diagram provides a clear and informative overview of the generation of regulatory and pathogenic T cells, with an emphasis on the roles of thymic selection and peripheral expansion. The diagram is divided into two main processes: thymic selection and peripheral expansion, with corresponding regions for regulatory T cells (Tregs) and pathogenic T cells on the left and right sides, respectively (37).

- Thymic Selection: It is the process of T cell maturation in the thymus. During this stage, pathogenic T cells and regulatory T cells both undergo selection. For example:
- Regulatory T cells (Tregs) are selected based on their responsiveness to self-antigens without inducing autoimmunity. The FOXP3 gene, which is crucial for the development and function of Tregs, is most active in this regard.
- Pathogenic T cells, which can lead to autoimmune diseases, are typically selected because they respond to self-antigens. A gene such as AIRE (Autoimmune Regulator) plays an essential role in the preferential expression of tissue-specific antigens within the thymus, thereby eliminating potentially autoreactive T cells.
- Peripheral Expansion: After thymic selection, both Tregs and pathogenic T cells enter the peripheral circulation. When they encounter specific antigens in peripheral tissues, these cells can expand.
- Regulatory T cells (Tregs) expand in the peripheral immune system to maintain tolerance and prevent autoimmunity. Some genes involved in this process include CTLA-4, which contributes to Treg-mediated suppression.
- Pathogenic T cells expand in response to environmental or immune stimuli and can contribute to autoimmune diseases. The MHC (Major Histocompatibility Complex) genes are involved in antigen presentation and play a role in both Treg and pathogenic T cell responses.

Each of these genes is placed in specific regions of action within the schematic. While genes like MHC have dual roles in regulatory and pathogenic T cell processes, others like FOXP3 and AIRE are more focused on specific activities during thymic selection or peripheral expansion. The diagram is a valuable tool for visually linking the processes of T cell differentiation, selection, and expansion to the relevant genes that guide their development and function (37).

Conclusion

Over the past few years, immunotherapy has re-emerged as a new therapeutic approach. The idea of specifically modifying the immune response offers a promising way to enhance or restore the immune system's ability to fight cancer and regulate autoimmune diseases. In vitro expansion and/or modification of the patient's cells, known as immune cell-based therapy, is one of the more promising methods of this kind. Chimeric antigen receptor (CAR) T cells are a new medical treatment that has recently led to improvements in the way cancer patients are treated. CAR T cell immunotherapy is a promising new avenue for treating cancer. It uses the immune system's underlying capacity to improve T-cell antigen recognition and reduce cytotoxicity through engineering. Compared with other existing techniques for treating hematological and solid cancers, CAR T cell immunotherapy has fewer adverse effects.

Conflict of interest: NIL

Funding: NIL

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