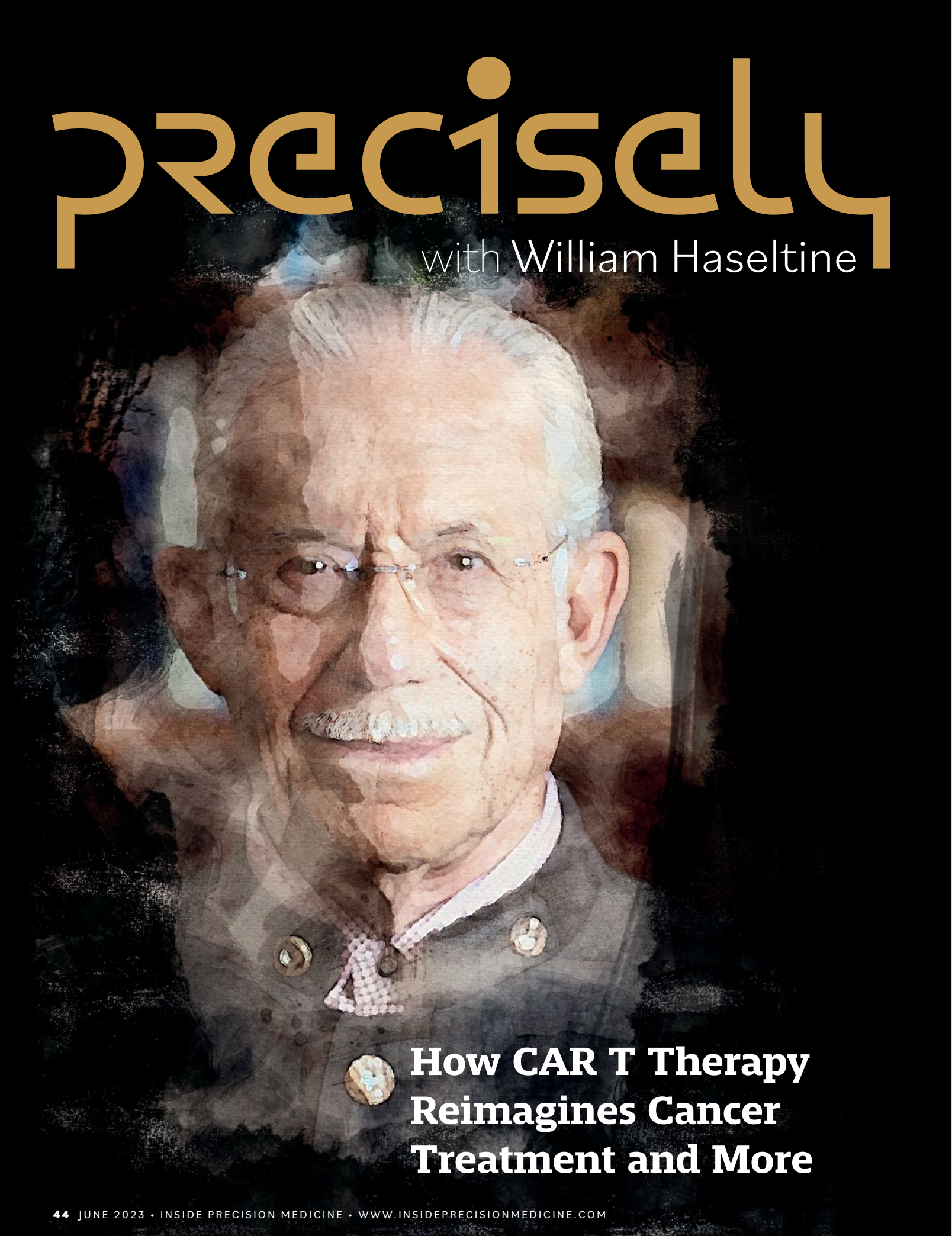
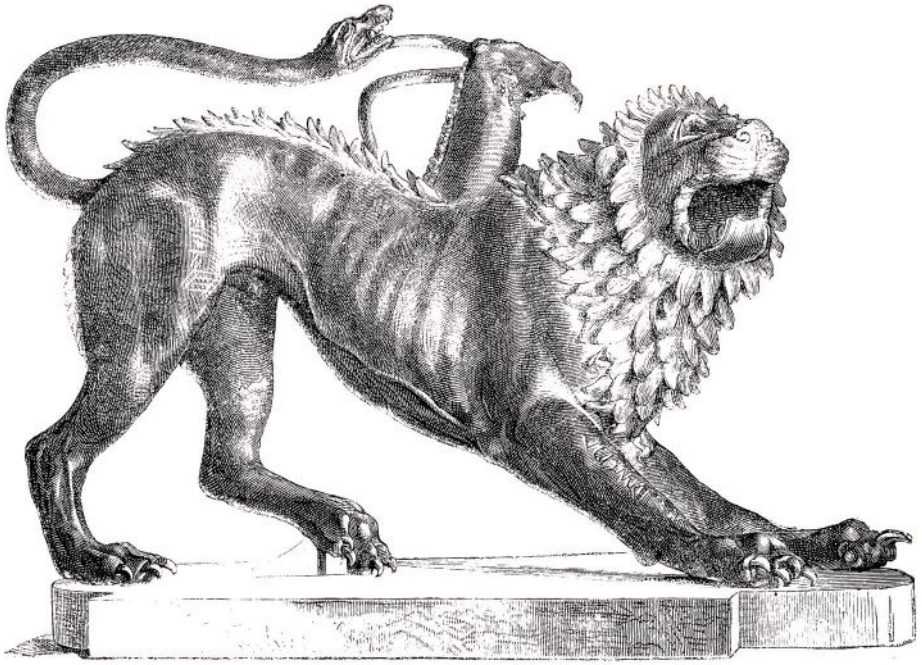
precisely

with William Haseltine



How CAR T Therapy Reimagines Cancer Treatment and More



ilbusca / Getty Images

Ancient Greek mythology depicts a chimera as a hybrid fire-breathing monster with the head and body of a lion and a snake's head for a tail. A goat's head also protrudes from the back. The patchwork of this mythic animal resonates with the mix of artificial and natural components in a chimeric antigen receptor.

Chimeric antigen receptor T cell therapy, commonly referred to as CAR T therapy, is an intervention that uses a patient's own cells to fight their cancer. The therapy first received US Food and Drug Administration (FDA) approval in 2017. For some patients, this meant long term remission and even cures of their disease.

And yet CAR T has the potential to do much more. The groundwork paved by basic forms of CAR T therapy for cancer may also be applied in the future to combat conditions such as heart disease and rheumatoid arthritis. CAR T cells provide an adaptable vehicle to rewire fundamental immune processes—one that can synergize with other contemporary technologies to become more accurate, potent, or accessible.

Here we detail how CAR T cell therapy works, describe its current uses to treat cancer, and summarize progress toward applying it to treat other illnesses.

How CAR T therapy works

Described simply, CAR T cells improve the body's own defenses, pitting a patient's immune cells against cancer. For an eligible patient, the journey begins in the hospital. The first step involves leukapheresis, a process which separates a portion of white blood cells from circulated blood; the remaining components are returned back to the body. The collected white blood cells are then sent to a lab for further isolation and modification.

From Killer T Cells to CAR T Cells

In the lab, the white blood cells are isolated and purified to extract a specific type of immune cell: cytotoxic CD8+ T cells, also known as "killer" T cells. CAR T therapy borrows this cell's natural ability to destroy infected and cancerous cells.

Normal cytotoxic T cells require a two-step activation process to kill. First, an antigen must be presented to the T cell's receptor; eventually, the T cell will identify any cell with that antigen as an enemy. Second, the T cell must receive a co-stimulatory signal at a distinct receptor. Naïve T cells require at least two signals for activation. Both are provided by an antigen-presenting cell, which is usually a dendritic cell: signal 1 is provided by MHC-peptide complexes binding to T cell receptors, while signal 2 is mainly provided by B7 costimulatory proteins binding to CD28 on the T cell surface.

Activation now complete, the T cell gains the ability to recognize and bind to a target cell. The bound and activated T cell releases a set of killer proteins and other chemicals that destroy the target cell (*see Figure 1*).

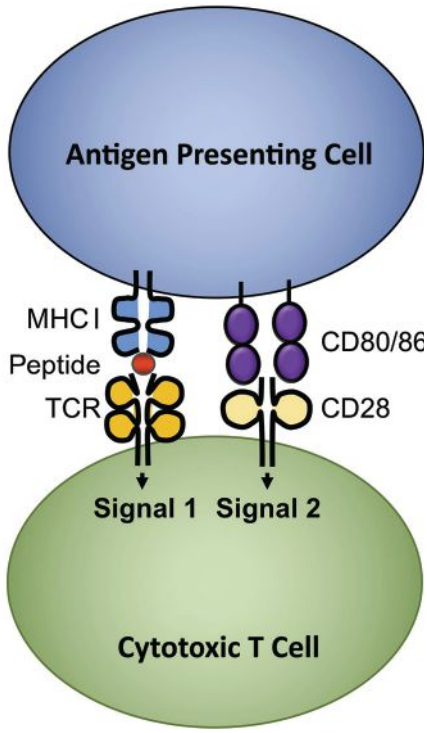


FIGURE 1: Cytotoxic T cells must be activated in two steps. An antigen presenting cell (APC) such as a dendritic cell brings an antigen to a molecule called the class I major histocompatibility complex (MHC I) found on the T cell's surface; this is the first signal. Simultaneously, the T cell's CD28 coreceptor receives a costimulatory signal that helps activate the cell. Both signals received, the T cell can then identify and bind to matching antigens present on the surface of other cells. Once bound to a target, the T cell releases chemicals that trigger the target cell's death.

Weinkove, R., George, P., Dasyam, N. and McLellan, A.D. (2019), Selecting costimulatory domains for chimeric antigen receptors: functional and clinical considerations. Clin Transl Immunol, 8: e1049. doi.org/10.1002/cti2.1049

CAR T technology takes advantage of the killer T cell's anticancer abilities and improves on them by removing the requirement for a secondary signal. Scientists use viral vectors to deliver new genes to change the T cell receptor. The resulting hybrid protein recognizes target antigens on cells and activates the T cell in the same step.

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The first-generation version of this hybrid protein, the CAR, has three parts, as illustrated in *Figure 2*. The protein on the outside of the cell is a modified monoclonal antibody that sticks to the target protein. This exterior, antibody-like receptor is joined to a linker that penetrates the cell membrane. The linker, in turn, is connected to the native intracellular activation domain of the killer T cell receptor. When the exterior protein binds to the target cell, it activates the CAR T cell program. The "C" in CAR T reflects the hybrid, chimera-like nature of this receptor.

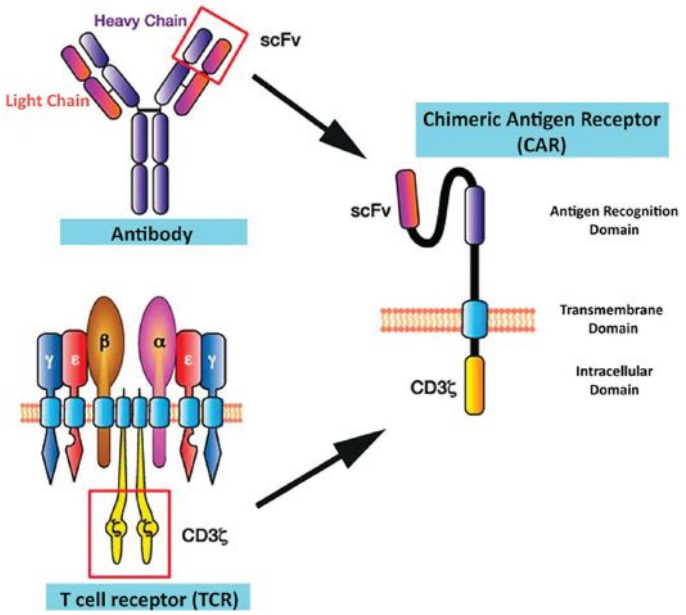

FIGURE 2: First generation CAR T cell design. A chimeric antigen receptor is composed of natural and artificial components. At its most basic form, the native T cell receptor signaling machinery (CD3ζ) is combined with a protein derived from antibodies called a single chain variable fragment (scFv). The single chain variable fragment can precisely recognize and bind to an antigen target such as CD19 or BCMA (B-cell maturation antigen).

Hughes-Parry HE, Cross RS, Jenkins MR. The Evolving Protein Engineering in the Design of Chimeric Antigen Receptor T Cells. *International Journal of Molecular Sciences*. 2020; 21(1):204. doi.org/10.3390/ijms21010204

The killer T cells, now equipped with new receptors, need to be multiplied. Exposing the cells to a culture medium and to certain cytokines—immune-signaling chemicals found in humans—stimulates the cells to expand to large numbers. The final product is then cryopreserved and returned to the patient via infusion.

Preparing for Infusion

A few days before infusion, a patient will undergo a short course of preparatory chemotherapy using drugs such as fludarabine and cyclophosphamide. This step does not treat the cancer, but rather decreases the number of other white blood cells in the body. The resulting slightly suppressed immune system is less likely to reject the influx of CAR T cells; similarly, the empty space formed leaves room for the thousands to millions of engineered T cells to grow and multiply. The final step, the CAR T cell infusion, often takes less than an hour to complete.

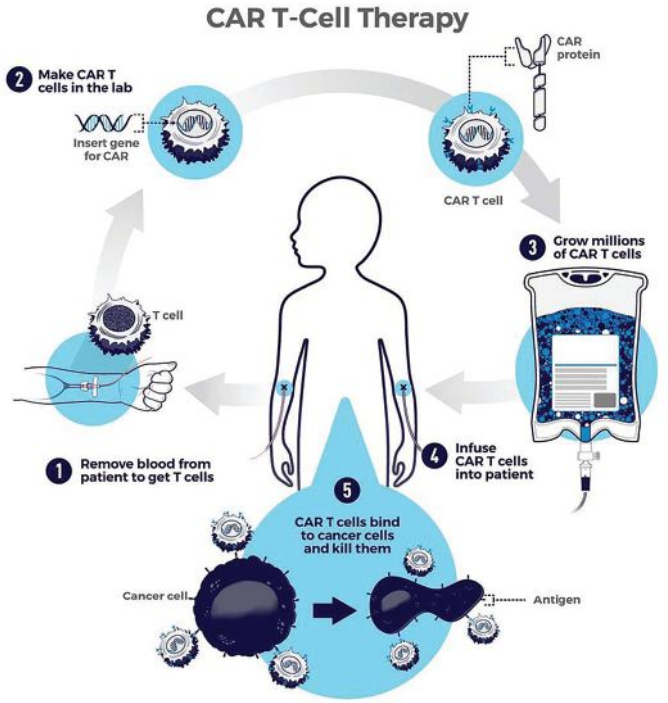

FIGURE 3: Summary of the CAR T therapy process. T cells are isolated from the blood and genetically modified to express a new chimeric antigen receptor. The modified and expanded cells are returned to the body via infusion.

(2022). CAR T Cells: Engineering immune cells to treat cancer. *National Cancer Institute.* www.cancer.gov/about-cancer/treatment/research/car-t-cells

Proof of principle

CAR T Therapy first demonstrated its feasibility with B cell-derived blood cancers, including certain leukemias and lymphomas, and multiple myeloma. These cancers involve the uncontrollable growth of B cells, white blood cells that create antibodies when mature. Early forms of B cells have no specific function and must gain specific purpose through a process called differentiation (*see Figure 4*).

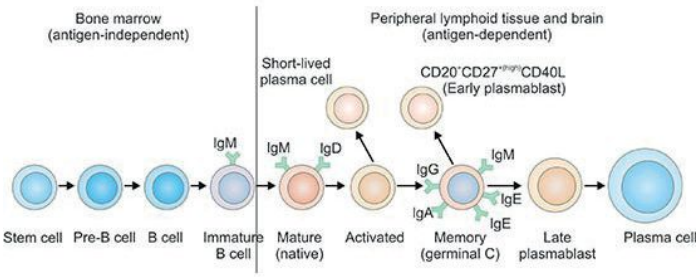

FIGURE 4: B cells can be found in various stages of development. Similarly to T cells, B cells start as stem cells and gain a specific function through a process of differentiation. Plasma B cells are a mature type of B cell which produce essential antibodies needed to tag threats to the immune system. Cancer can occur if B cells, matured or not, grow erratically.

What is The Cell Surface Marker: B Cell. CUSABIO. www.cusabio.com/Cell-Marker/B-Cell.html

Most B cells, cancerous or not, share similar antigens on their cell surface. CAR T therapies are designed to detect common B cell antigens such as CD19 or B cell maturation antigen (BCMA).

Once a patient receives a CAR T cell infusion, the treatment begins eliminating any B cells it encounters—regardless of whether they are malignant or not. This can decimate healthy B cells. Interestingly, medical professionals see B cell deficiency as proof of the therapy's success. Post procedure, a patient may need to replenish the lost antibodies with immunoglobulin replacement therapy.

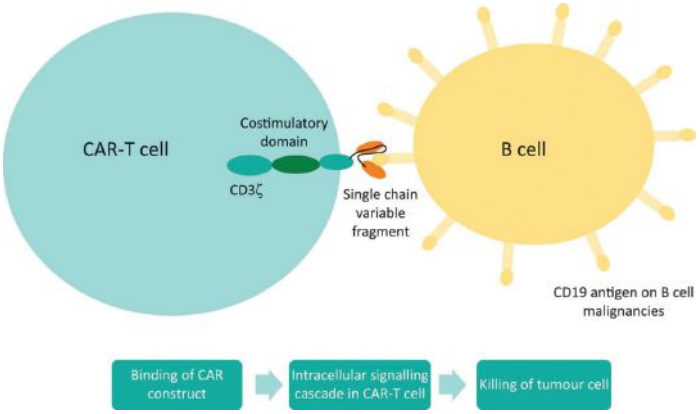

FIGURE 5: Anti-CD19 CAR T cell for certain B cell cancers. Most federally approved CAR T therapies use single chain variable fragments (scFv) that target antigen CD19 or B cell maturation antigen (BCMA); these antigens commonly reside on the surface of B cells, whether cancerous or noncancerous. The CAR T cell binds to any encountered cell with CD19 or BCMA and releases signaling molecules to eliminate it. This process can often leave a patient with B cell aplasia, otherwise known as a deficiency of healthy B cells.

Charlotte Graham, Rebecca Hewitson, Antonio Pagliuca, Reuben Benjamin (2018). Cancer immunotherapy with CAR-T cells - behold the future. Clinical Medicine (4) 324-328. DOI: 10.7861/clinmedicine.18-4-324

Clinical success for B Cell cancers

CAR T therapy shines in some cases where other cancer treatments have failed. It can reduce signs of cancer and extend life expectancy, giving patients another chance at life. While the recovery process, as for any cancer treatment, is physically and mentally demanding, a recent study published in *Blood Advances* noted that patient quality of life typically improves around six months after the procedure.

For the therapy to be a success, it must cause some form of remission, meaning a reduction in cancer signs or symptoms. A complete remission occurs if the cancer can no longer be detected. In a best case scenario, a patient may be considered 'cured' if their remission lasts more than five years.

Initial remission rates for CAR T therapy can range as high as 98%. For a minority of patients, the treatment can send cancer into complete remission. For example, one clinical study found that CAR T therapy achieved remission for more than 82% of young patients with aggressive acute lymphoblastic leukemia. Of that group, however, less than half of the patients achieved complete remission and lived without relapse for more than five years.

Overcoming limitations

CAR T therapy has morphed over the years. Design variations replace or add components to better engineer the T cells' antigen targeting, persistence, and proliferation.

First, Second, and Third Generation

First generation CAR T cells, the simplest form of CAR T, struggled to survive and multiply once infused. These cells also demonstrated poor efficacy due to T cell exhaustion. The basic design lacked costimulatory molecules, proteins needed to bolster the T cells' activation signal.

This activation signal widely influences the T cells' fate. According to elegant research, while the antigen binding domain is crucial for target finding, it is the resulting signaling cascade that defines the nature of the cell's response. How long the T cell survives and grows, and how well it destroys cancer, depends on the cell's signal transduction.

Second and third generation CAR T cells include additional costimulatory molecules, as seen in *Figure 6*. The number, combination, and position of the molecules yield different results. Two common options include molecules 4-1BB, which can increase T cell memory and persistence, and CD28, which is associated with effective killing but reduced persistence. Overall, second generation design produces longer half-lives, more durable immune responses, and more potent cytotoxicity compared to its predecessor. The third displays even better persistence and proliferation, but the efficacy remained similar to second generation design.

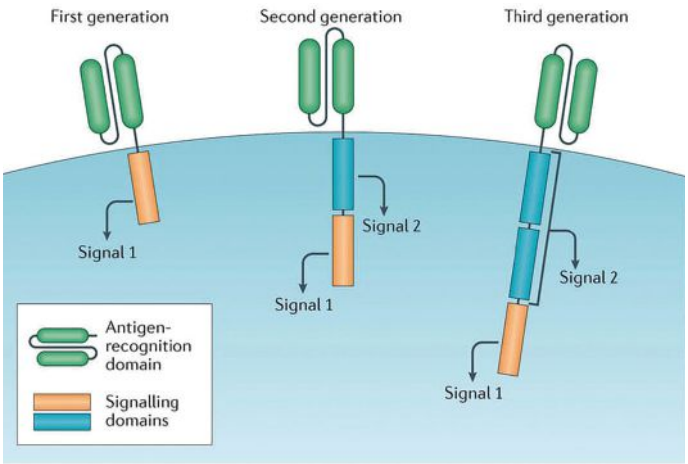

FIGURE 6: Later generations of CAR T therapy include costimulatory signaling domains to improve T cell expansion after infusion and T cell survival once in circulation. Signal 1 refers to the CD3ζ molecule; Signal 2 represents any additional costimulatory molecules (ex: 4-1BB or CD28).

Brentjens R, et al. "Driving CAR T cells forward." Nat Rev Clin Oncol. 2016 13, 370-383.

All CAR T cells approved by the FDA use second generation design. Kymriah was the first to be approved for clinical use, in 2017. Since then, five more CAR T therapies have joined the ranks. Table 1 lists all available therapies and highlights the differences in their construction.

CAR T Therapy for Solid Tumors

Blood cancers represent a mere 10% of all diagnosed cancer cases in the United States. The hope is to translate CAR T therapy to solid tumors such as prostate cancer, lung cancer, pancreatic cancer, and others.

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Three obstacles block the way: CAR T cell antigen targeting, tumor infiltration, and persistence. Antigens found on solid tumors vary greatly, and often overlap with antigens found on healthy tissues and organs; if the CAR T cells destroy healthy tissue, it cannot be repaired and supplemented as B cell deficiency can. It is also arduous for a CAR T cell to reach the tumor's center due to the issue barrier. Lastly, the solid tumor environment actively suppresses immune responses, thus blunting T cells' efficacy.

One potential solution involves using CRISPR technology to create a chimeric receptor with multiple antigen targets. Another possibility involves editing the T cell receptor genes to activate more efficiently. A study of mice with ovarian cancer found that mutating the CAR T cells' signaling domain led to reduced tumor burden. This suggests that streamlined T cell activation could significantly impact the cells' persistence and antitumor response—two factors needed to fight solid tumors.

List of FDA-approved CAR T cell therapies

Brand Name	Date Approved	Antigen Target	Antigen Recognition Domain	Intracellular Signaling Domain	Diseases Targeted/ Line of Therapy
Kymriah	8/2017	CD19	scFV	4-1BB—CD3ζ	B cell precursor adult lymphoblastic leukemia (3rd line) Diffuse large B cell lymphoma (3rd line) Follicular lymphoma (3rd line)
Yescarta	10/2017	CD19	scFV	CD28—CD3ζ	Diffuse large B cell lymphoma (2nd line) Follicular lymphoma (3rd line)
Tecartus	7/2020	CD19	scFV	CD28—CD3ζ	Mantle cell lymphoma (3rd line) B cell precursor adult lymphoblastic leukemia (3rd line)
Breyanzi	2/2021	CD19	scFV	4-1BB—CD3ζ	Large B cell lymphoma (2nd line)
Abecma	3/2021	BCMA	scFV	4-1BB—CD3ζ	Multiple myeloma (5th line)
Carvykti	2/2022	BCMA	VHH	4-1BB—CD3ζ	Multiple myeloma (5th line)

TABLE 1: Chart of all FDA-approved CAR T cell therapies. Abbreviations: BCMA, B cell maturation antigen | scFV, single chain variable fragment | VHH, single variable domain on a camelid heavy chain antibody (i.e., nanobody).

(2023). Access Health International.

Potential to Treat Autoimmunity

Another burgeoning area for CAR T cells is treatment of autoimmune diseases such as lupus, multiple sclerosis, and rheumatoid arthritis. Current treatments for these conditions only manage symptoms, but CAR T therapy could potentially treat these autoimmune diseases at their source.

For example, lupus is an autoimmune disease that stems from the overproduction of malignant antibodies. A small study revealed that anti-CD19 CAR T cells could achieve complete remission for patients with severe and treatment-resistant forms of the disease.

One study in a mouse model of rheumatoid arthritis tested cells wielding a chimeric antigen receptor that recognizes and eliminates errant helper T cells, the immune cells responsible for that disease's development (*see Figure 7*). The intervention successfully delayed the onset and severity of rheumatoid arthritis in mice; with more adjustments, the intervention could potentially cure the condition altogether.

An alternative design for multiple sclerosis (MS) also attacks self-reactive helper T cells. The researchers found that these CAR T cells could be implemented at different stages to either prevent disease flare-ups or mitigate symptoms in mice—a mechanism that would benefit patients once translated clinically.

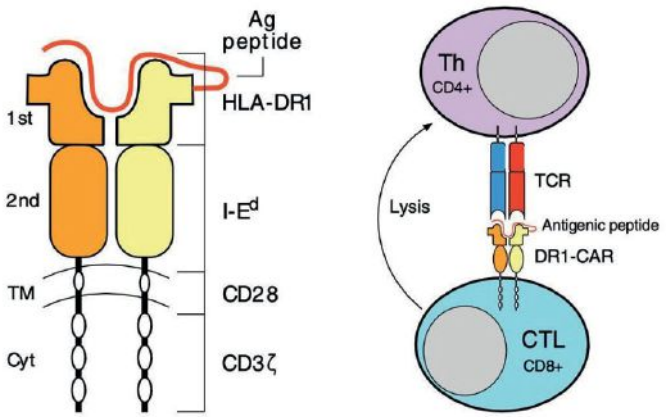

FIGURE 7: CAR T cell design targeting malignant helper T cells. Most notably, the design incorporates a major histocompatibility class II/antigen domain, which enables the cell to interact with pathogenic CD4+ T cells in mice. Abbreviations: CTL, cytotoxic T cell; Th, helper T cell; TCR, T cell receptor.

Karen B. Whittington, Amanda Prislovsky, Jacob Beaty, Lorraine Albritton, Marko Radic, Edward F. Rosloniec; CD8+ T Cells Expressing an HLA-DR1 Chimeric Antigen Receptor Target Autoimmune CD4+ T Cells in an Antigen-Specific Manner and Inhibit the Development of Autoimmune Arthritis. *J Immunol* 1 January 2022; 208 (1): 16–26. doi.org/10.4049/jimmunol.2100643

Treating T Cell Derived Cancers?

It is surprisingly difficult to use CAR T cells to treat T cell leukemia, a form of leukemia originating from the uncontrolled growth of T cells. The main challenge lies with finding an antigen target. Using CAR T cells to target antigens characteristic of the T cell lineage would lead to the destruction of healthy T cells, cancerous T cells, and even CAR T cells themselves. An additional risk is T cell deficiency, a potentially life threatening condition.

One study developed an immunotherapy that, rather than replacing a receptor on T cells, modifies them to secrete a special antibody. The antibody is designed to bind to cancerous T cells on one side and healthy T cells on the other, thus encouraging nearby T cells to target the liquid tumor. The authors described this interaction as "the bystander effect," and the results of their in vitro and in vivo assays suggest that the bystander effect can augment a course of CAR T therapy or even be used effectively alone.

CAR T's drawbacks

CAR T therapy, effective as it may be, does come with several caveats. Clinicians today must tolerate or maneuver carefully around these shortcomings. In the future, these problems may be resolved through innovative solutions in progress today.

Adverse Effects

CAR T cells need to produce strong and durable immune responses to eliminate cancer cells. While these responses are beneficial to an extent, adverse events occur if the immune system becomes overly stimulated from the T cell infusion. Doctors monitor patients for around a month after infusion, watching for common complications such as cytokine release syndrome (CRS) and a neurotoxic condition known as immune effector cell-associated neurotoxicity syndrome (ICANS). Both conditions, while generally reversible, can manifest symptoms that range from mild to fatal.

Cytokine release syndrome, also referred to as a cytokine storm, results when stimulated white blood cells release inflammatory chemicals called cytokines. These chemicals can activate other white blood cells and perpetuate a cycle of inflammation. Fever usually manifests first, often accompanied by headaches, muscle/joint pain, and more. Severe cytokine storms can lower blood pressure and oxygen levels, leading to eventual organ failure and death. The condition can usually be reversed within 5 to 17 days with treatments such as antihistamines, oxygen therapy, or immunosuppressive medications.

Neurotoxicity can also occur, although to a lesser degree. Patients may experience confusion, headaches, tremors, and hallucinations. Symptoms can even, on rare occasions, cause serious delirium, seizure, or coma. Immunosuppressive medicines such as tocilizumab and corticosteroids can alleviate symptoms just as with cytokine storms. The condition, when addressed, typically resolves within 21 days of infusion.

One potential solution to runaway immune responses is to equip CAR T cells with an antibody switch. As shown in *Figure 8*, CAR T cells with such a switch only respond if the switch is bound to both the chimeric receptor and the target antigen on the cancer cell. Using an antibody switch could provide a clinically feasible means to activate or deactivate CAR T cells already inside the body, with the immune response stopping if the switch is no longer present. This research is currently in progress and highly anticipated to improve cytokine release syndrome, in particular.

Barriers to access: Time and money

The greatest hurdle to use of CAR T therapy is its inaccessibility. CAR T cell production is labor intensive because it is customized for each patient; it requires high tech facilities and well-trained personnel. In addition, it can take several weeks to cultivate the retroviruses necessary to deliver new genes to the T cells. Overall demand for viral vectors has increased dramatically due to vaccine production and cell therapy research, causing vector supply shortages and bottlenecks in CAR T cell production during the height of the pandemic.

As a consequence of these factors, the price of the therapy is steep. A single infusion costs between $373,000 and $475,000 in the U.S. depending on the treatment. Including other services such as hospital stay, imaging, and medicine, the total cost of care can easily exceed $500,000, $1 million, or even $1.5 million without health insurance. Medicare approved the intervention for coverage in 2019, but it's clear that CAR T products remain exorbitantly expensive.

Minimizing manufacturing costs and streamlining production will be necessary to solve issues of time and price. One possibility is to use CRISPR technology to edit the genes, a process that is safer and more streamlined than the retroviral alternative. Several studies have successfully manufactured CAR T cells using this method, but the procedure has yet to become an industry standard.

Some place their hopes in an off-the-shelf version of CAR T therapy. These T cells would be derived from donors instead of directly from the patient, and therefore would be cheaper to manufacture in large quantities. It would also be faster to administer the treatment with a stock of ready-made cells at hand. Despite the heightened risk of tissue rejection, clinical trial results published in Nature Medicine demonstrated that the concept can be implemented safely for patients with multiple myeloma.

A Multi-faceted Solution: mRNA Technology

An even more cost-effective solution would be to temporarily create CAR T cells inside the body instead of in the lab. Lipid nanoparticles would deliver the mRNA code for the desired chimeric receptor; as mRNA does not integrate into the genome, the T cells would take up the nanoparticles and transiently express a new receptor. This process mirrors the mRNA technology used in current COVID-19 vaccines,, which can be manufactured in the US for less than $3.00 a dose.

This method could potentially slash prices, but only if proven just as or more effective than standard CAR T therapy. Luckily, preliminary research in mice with cardiac fibrosis successfully used this approach to reduce heart damage. As an additional benefit, the transient nature of mRNA technology means it can be used as an on-and-off switch, thus exerting a more precise level of control than standard CAR T therapies. The study raises hope for CAR T to become more accessible and widely applicable, although further investigation is still needed to test the concept's clinical feasibility.

The future of CAR T therapy

The world of CAR T therapy is continually expanding. Nevertheless, there is room to improve the therapy's safety profile and efficacy, and lower barriers to access. These synthetic cells can currently only be used in certain clinical conditions, and can cause potentially life threatening complications. Most importantly, CAR T cells' benefits will only reach a minority of patients due to the therapy's inaccessible price.

Despite its challenges, the field continues to attract researchers who see the potential of the platform. There are hundreds of ongoing trials investigating strategies to fine-tune CAR T cell design or reimagine it all together. The integration of mRNA technology, in particular, may pave the way for versions of CAR T that exceed current standards on all counts. This forward momentum suggests a bright future for CAR T therapy. ■

JUNE 2023

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