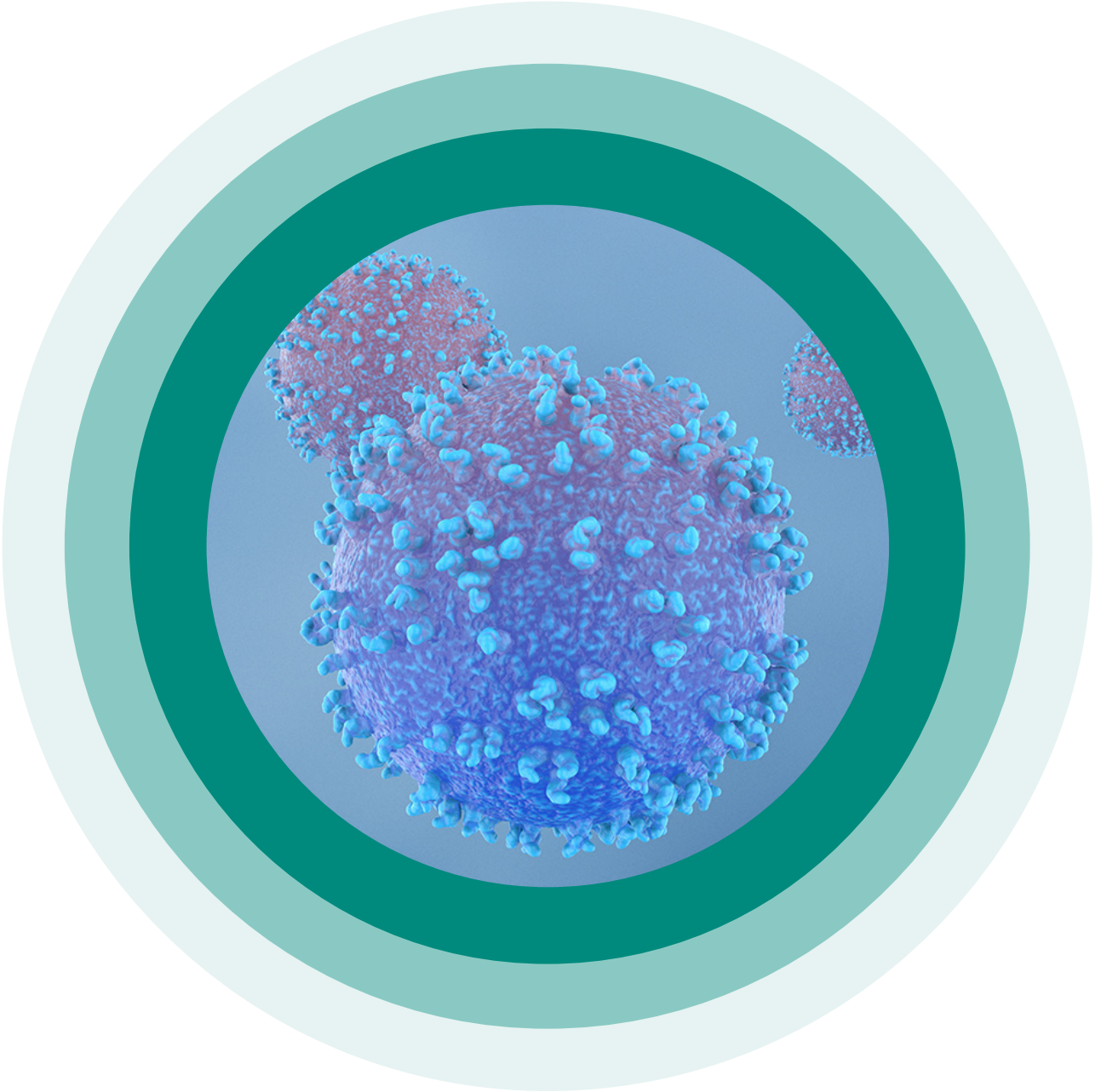
Approaching the CAR T-cell therapy horizon

Clinical advancements expanding the potential of CAR T-cell therapies



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Executive Summary

Approved CAR T-cell therapies have shown remarkable results in patients with certain types of blood cancers, but further innovations are needed before the technology can reach its full potential. This whitepaper explores the most exciting clinical developments in CAR T-cell therapy in the context of existing challenges with efficacy, safety and logistics.

Efficacy: Currently, approved CAR T-cell therapies are limited to a few types of blood cancers, and the therapies have long-term survival benefits for less than 50 percent of treated patients.

Safety: Many patients who receive CAR T-cell therapy experience life-threatening side effects that require close monitoring and management in specialised centres.

Logistics: The logistical complexity of extracting, engineering and readministering personalised CAR T-cell therapies is costly andt limits scalability; treatment for a single patient in an academic hospital inpatient setting can cost more than $450,000.

In this whitepaper, we explore key challenges in the context of questions that inform the safety, efficacy and logistics of future CAR T-cell therapies. Promising developments in the field include combination therapies and modifications to CAR T-cells that could help improve CAR T-cell's ability to:

– target cancer cells with greater precision, including solid tumours

– survive in an immunosuppressive environment

– control the level of CAR T-cell proliferation and activity following administration

Alternatives to autologous CAR T-cell therapies may help to overcome current logistic challenges and constraints of scale.

As CAR T-cell therapies evolve, a synthesis of innovations are poised to bring CAR T-cell therapies targeted against more cancers to a greater number of patients.

Where are we now?

A novel immunotherapy saves lives

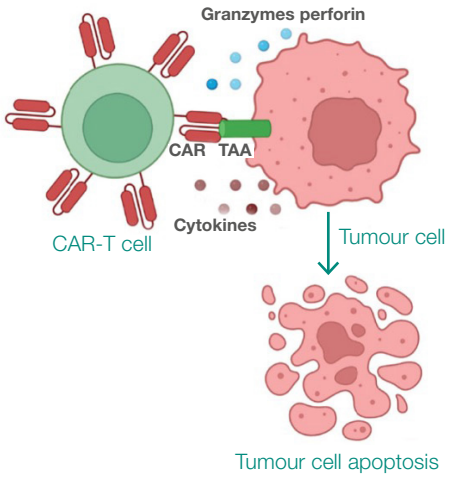
Chimeric antigen receptor (CAR) T-cell therapies have garnered excitement as a new therapeutic option for cancer treatment, with six CAR T-cell therapies approved since 2017.[1] In principle, the therapy works by introducing immune cells (T cells) into a patient that are specifically engineered to identify and combat a patient's cancer. These cells can directly attack the cancer and coordinate with the patient's own immune response to make it stronger and more enduring.

Existing CAR T-cell therapies accomplish this autologously, by extracting a patient's T cells and genetically engineering them in a laboratory to express a synthetic receptor called a chimeric antigen receptor. This receptor is designed to bind to a single, specific protein (antigen) found on the surface of the patient's cancer cells. The modified T cells are infused back into the patient, where they proliferate and coordinate a targeted immune response once they encounter their target antigen.

Approved CAR T-cell therapies have shown remarkable results in patients with certain types of blood cancers, including acute lymphoblastic leukaemia, chronic lymphocytic leukaemia, non-Hodgkin lymphoma and multiple myeloma. Many patients who achieved complete remission after receiving CAR T-cell therapy had exhausted all other treatment options.

For example, the first person to receive CAR T-cell therapy, Emily Whitehead, was diagnosed with acute lymphoblastic leukaemia at age five and relapsed twice after chemotherapy. She received an experimental CAR T-cell therapy that targeted a protein called CD19 on her leukaemia cells. While she experienced life-threatening side effects as a result of the therapy, which required two weeks of intensive care, the treatment was ultimately curative. Within a month after receiving CAR T-cell therapy her cancer was undetectable, and she remains cancer-free eleven years later.[2]

Figure 1: Progressive evolution of CAR-T cells



Source:
https://www.frontiersin.org/articles/10.3389/fimmu.2022.903562/full

Limitations of current CAR T-cell therapies

While therapeutically game-changing, existing CAR T-cell therapies face major problems with efficacy, safety and logistics. Accelerating research in the field aims to address these challenges. Between 2020 and 2022, there were 857 trials and 1,432 pipelines for CAR T-cell therapies globally – more than any other immunotherapy.[3]

Efficacy

Currently, the efficacy of approved CAR T-cell therapies varies between patients, and their response is difficult to predict. Approved CAR T-cells in lymphomas and leukaemias lead to long-term survival benefits in less than 50 percent of treated patients.[4] Increasingly, researchers are recognising how cancer turns the immune system against itself, and that the unique interactions between a patient's CAR T-cells, cancer and native immune system impact the likelihood that CAR T-cell therapy will be successful.[5,6]

For example, some cases of cancer resistance or recurrence following CAR T-cell treatment has been attributed to a patient's cancer cells losing or down regulating the expression of the antigen that their CAR T-cells were engineered to target.[7] A more nuanced understanding of cancer's immune evasion and suppression tactics have proved crucial for research efforts aimed at developing CAR T-cells for solid tumours, where an immunosuppressive tumour microenvironment is especially hostile to CAR T-cells.[8]

In addition to developing CAR T-cells that can counter cancer's immune evasion and suppression tactics, researchers are working to develop more precise ways for CAR T-cells to target cancer cells without targeting healthy cells. Currently, successful applications of CAR T-cell therapy are limited to a few types of blood cancers that express a single antigen (CD19 or BCMA).

Equally suitable antigen targets for solid tumours have proved extremely challenging to identify, because there is considerable overlap in antigen expression between solid tumours and healthy tissue, and because solid tumour antigen expression is more diverse and variable than antigen expression on blood cancers.[9] New methods of targeting and personalising CAR T-cells to a patient's unique cancer need to be developed to extend CAR T-cell therapies to solid tumours, and to reduce the number of patients with blood cancers who relapse or fail to respond to approved therapies.

Safety

Like Emily Whitehead, many patients who receive CAR T-cell therapy experience severe side-effects, including cytokine release syndrome and neurotoxicity, that require close monitoring and management by experienced clinicians in specialised centres. According to one study, nearly 50 percent of patients enrolled in early clinical trials using CAR T-cell therapies required intensive care management.[10]

Side effects are often the result of an overstimulated immune system or CAR T-cell's on-target but off-tumour attack of healthy cells expressing the target antigen. The extent and severity of these side effects are compounded by the inability to control the expansion and activity of approved CAR T-cell therapies following their administration.[11] Research efforts focused on improving the safety of CAR T-cell therapies are working to make patient responses more predictable, improve CAR T-cells' ability to differentiate healthy cells from cancerous cells, and increase control over CAR T-cell activity after the therapy has been administered.

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Logistics

The logistics of extracting, engineering and readministering personalised CAR T-cell therapies is also a major challenge, and the cost is very high: more than $450,000 per patient treated in an academic hospital inpatient setting.[12] Manufacturing and delivering a single autologous CAR T-cell therapy involves precise and adaptive coordination between patients, clinicians, specialised manufacturing facilities and couriers. And, the process is lengthy. For Emily, the vein-to-vein time between extracting her T cells (leukapheresis) and readministering CAR T-cells took more than a month.[2] All of this complexity and personalisation creates challenges for quality control, standardisation, scalability and cost-effectiveness of autologous CAR T-cell therapies. Alternatives to autologous therapies – including allogeneic therapies and generating CAR T-cells within the patient's body through in vivo methods – aim to make CAR T-cell therapies easier to manufacture and deliver.

Research horizons for CAR T-cell therapy

In the evolution of CAR T-cell therapy, the first major hurdle was to engineer a working T-cell receptor that was cancer-specific. But, as the limitations of existing CAR T-cells have come more fully into view, it is clear that the next generation of therapies need more than a well-targeted receptor. Researchers envision an engineered T cell capable of travelling to cancer cells, distinguishing cancerous cells from healthy ones, overcoming any immune-evasion tactics, coordinating an immune response with the rest of the immune system, and surviving long term. In addition, the activity of this ideal CAR T-cell therapy would be controllable, so that patients would not be endangered by their immune response. From a logistic perspective, the engineered CAR T-cell is quick and easy to manufacture in a standard clinical setting.

Before an optimal CAR T-cell therapy can be conceived, research must first focus on developing solutions to specific problems. The iterative efforts to improve CAR T-cell therapies are often context-specific and can be confusing. A foundational understanding of the problems researchers are wrestling with can help relate these efforts to what researchers hope CAR T-cell therapy will be able to achieve, and create a clearer vision of how ongoing efforts could complement or compete with each other as CAR T-cell therapies evolve.

Here, we discuss the most exciting clinical developments for CAR T-cell therapies in the context of discrete questions guiding research and development that contribute to the safety, efficacy and logistics of CAR T-cell therapies:

– How can CAR T-cell therapies be more targeted?

– How can CAR T-cell therapies overcome immunosuppression or evasion?

– How can CAR T-cell therapy be more controlled?

– How can clinicians better predict a patient's response to CAR T-cells?

– How can CAR T-cell therapies be easier to manufacture and deliver?

How can CAR T-cell therapies be more targeted?

The first hurdle for a viable CAR T-cell therapy is the identification of an antigen that is reliably expressed on cancer cells, and not on vital healthy cells, where toxicities from "on-target, off-tumour" antigen recognition by CAR T-cells can be fatal. So far, suitable antigens have been identified only for blood cancers. However, efficacy of approved CAR T-cell therapies has been inconsistent, with the loss of target antigens on cancer cells thought to be a contributing factor. Meanwhile, antigen selection has proved to be a major roadblock in the development of CAR T-cells for solid tumours.

Identify cancer antigens not expressed on vital tissue

All FDA-approved CAR T-cell therapies target either the proteins CD19 or B-cell maturation antigen (BCMA), which are antigens associated with cell development that are highly expressed on blood cancer cells but have limited expression on healthy cells. CAR T-cell therapies that target CD19 or BCMA lead to the initial depletion of healthy immune cells. But, this does not immediately endanger the patient. In contrast, solid tumours have a wider array of potential antigen targets than blood cancers, and the antigens expressed on solid tumours are often also found at low levels on healthy tissue, making the identification of a single, reliably expressed and cancer-specific antigen very challenging.

While no CAR T-cell therapies targeting solid tumour antigens have yet been approved, a few solid tumour antigens have demonstrated their potential as CAR T-cell therapy targets in early clinical trials. One of these antigen targets, CLDN6, is an embryonic gene that enables cancers to metastasize.[13] The antigen is present in most testicular cancers, as well as in some ovarian, non-small cell lung, gastric, breast and endometrial cancers. Despite normally being silenced at birth, the CLDN6 antigen does carry some danger of off-tumour effects: It is expressed at low levels in the pancreas and liver, and a protein with a similar structure, CLDN9, is expressed throughout the body.

In a 2022 phase 1/2a trial, a CLDN6-directed CAR T-cell therapy, called BNT211-01, displayed one of the first examples of CAR T-cell efficacy in solid tumours.[14] Initial trial results reported tumour shrinkage in 33 percent of 21 patients with seven different tumour types, including one testicular cancer patient who went into complete remission.

The therapy also proved tolerable, with approximately 40 percent of treated patients developing manageable cytokine release syndrome. Two additional solid tumour targets have also shown preliminary promise in clinical trials: the protein IL13Ra2 is being investigated as a CAR T-cell target for glioblastoma and prostate stem cell antigen (PSCA) is under investigation for prostate cancer.[15,16]

The search for suitable solid tumour antigen targets has been slowed by the poor translation of preclinical research to first-in-human trials, as antigen expression in current animal models often do not reflect antigen expression in humans. In practice, this has led to patients experiencing severe toxicities in many early solid tumour CAR T-cell therapy clinical trials, because there was overlap in antigen expression between tumour and healthy cells that was not predicted by earlier animal models.[17] Identification of new antigen targets for solid tumours could be accelerated by the development of preclinical models that better represented human immune system and the antigen expression of healthy cells.

Upregulate tumour antigen expression

The challenge of selecting a tumour antigen for CAR T-cells that is not expressed on healthy cells is compounded by the challenge of identifying a tumour antigen that is also reliably expressed on cancer cells. Most solid tumours have heterogeneous antigen expression, meaning that some tumour cells may express an antigen more strongly than others, and some may not express that antigen at all. This heterogeneity is likely one important reason for the diminished initial and durable CAR T-cell response.[9] A CAR T-cell therapy targeting a cancer-specific antigen that was heterogeneously expressed would miss tumour cells not expressing that antigen, and would be less effective on cells weakly expressing that target.

Even for blood cancers, where some antigens are more reliably expressed, antigen loss can limit a CAR T-cell therapy's effectiveness. Antigen loss (or escape) refers to the phenomenon where cancer cells either lose or downregulate the expression of the target antigen, leading to cancer resistance or recurrence. This affects the durability of approved CAR T-cell therapies. In fact, the most common cause of relapse following CAR T-cell therapy for B-cell acute lymphoblastic leukaemia has been attributed to the loss or downregulation of target antigens on cancer cells.[7]

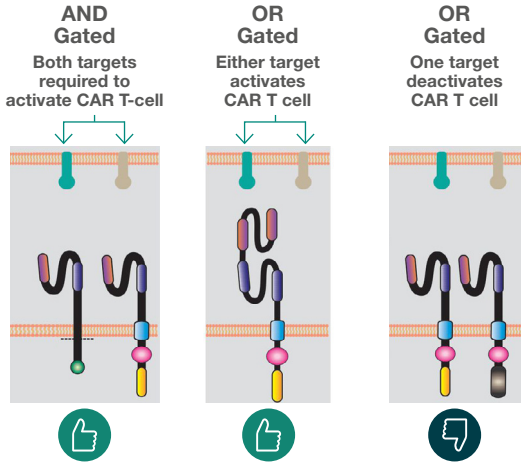
In cases where antigen loss is a problem, drugs that upregulate tumour antigen expression may increase the durability of CAR T-cell therapies. For example, inhibition of the enzyme gamma-secretase can result in the increased expression of BCMA on the tumour cell surface and reduce soluble and shed BCMA – which act as a decoy for BCMA-specific CAR T-cells.[18] Early phase clinical studies have demonstrated that injections of a gamma-secretase inhibitor can increase the durability of response to a BCMA-specific CAR T-cell therapy in patients with relapsed or refractory multiple myeloma.[19] Other drugs in development for the upregulation of tumour antigen expression include an inhibitor of the enhancer of zeste homolog 2 (EZH2) for the increased expression of a bone cancer antigen (GD2), inhibitors of histone deacetylase; and inhibitors of DNA methyltransferase.[20–22]

Target cancer cells with varied antigen expression

Another way to circumvent the lack of cancer-specific, consistently expressed antigens is engineering CAR T-cell therapies to have multiple antigen targets.[23] This has been approached through genetically engineering multiple antigen-specific receptors into the same T-cell, known as a dual CAR T-cell. In this case – commonly referred to as "OR gating" – recognition of an antigen by either receptor activates the CAR T-cell.[24] Alternatively, sequentially administering CAR T-cell therapies that target different antigens, also known as a "cocktail treatment," has been tested in clinical trials, although patients experienced severe toxicities.[25] CAR T-cells that destroy surrounding cells, regardless of their expression of the target antigen, through a "bystander killing effect," may also help to overcome challenges with tumour antigen heterogeneity or antigen loss.[26]

CAR T-cell therapies that can target more than one antigen may be less likely to miss cancerous cells with heterogenous antigen expression. However, they carry an elevated risk of on-target, off-tumour toxicities.[27] An alternative application for CAR T-cells engineered with multiple receptors are therapies in which one receptor is targeted to a cancer antigen, and another receptor is targeted to a healthy cell antigen. In this case, the receptor that identifies healthy cell antigens prevents CAR T-cell activity, and serves as a safety mechanism against off-tumour effects, often referred to as "NOT gating". CAR T-cells can also be engineered with two different cancer-antigen-specific receptors, both of which need to recognise an antigen before the CAR T-cell is activated. This safety mechanism, referred to as "AND gating" can also help to prevent targeting of healthy cells if those cells express one but not both antigens. However, this method of gating increases the risk of missing cancer cells.[28]

Figure 2: Biological logic gating



Source: https://jitc.bmj.com/content/10/4/e004185

How can CAR T-cell therapies overcome immunosuppression or evasion?

While the immune system is often thought of as a defence force that protects the self by identifying and destroying foreign invaders, the reality is more nuanced. The primary job of a person's immune system is to keep that person healthy and intact. The immune system identifies and destroys pathogens and compromised or damaged cells – including cancerous cells. However, it also actively self-regulates to avoid mis-identifying healthy cells as targets, and to control the level of collateral damage during an immune-mediated attack. Cancers that have survived long enough to endanger one's health have generally accomplished this by taking advantage of the immune system's mechanisms of self-regulation in order to resist or evade immune attack.[5]

For example, the immune system employs T cells to target and destroy host cells that have lost the ability to self-regulate, so that they do not grow out of control. Some cancers activate a self-destruct mechanism on T cells called an immune checkpoint. Normally this prevents T cells, whose receptors bind to healthy cells, from causing an autoimmune disease. But, some cancers co-opt this mechanism to evade both native T cells and CAR T-cells. This immune-evasion strategy informed the development of the first immunotherapies against cancer – immune checkpoint inhibitors – which prevent cancer cells from using the immune checkpoint to their advantage.[6]

Cancer manipulates the immune system and its surrounding environment in other ways as well. For example, immune cells often have difficulty penetrating and surviving in the environment created by solid tumours, because they are surrounded by physical barriers, including the extracellular matrix and the vasculature. In addition, the tumour environment is anoxic and can contain cells and compounds that actively suppress or confuse the immune system.[8]

Ongoing research efforts are now attempting to develop CAR T-cell therapies with the contextual efficacy to overcome specific immune evasion and suppression tactics. Approaches to improving CAR T-cell efficacy include methods of engineering the CAR T-cell itself and combination CAR T-cell therapies, in which CAR T-cell therapy is combined with other immunotherapies or drugs that augment its performance.

Combination therapies

Combining CAR T-cell therapy with immunotherapies, such as immune checkpoint inhibitors or therapeutic vaccination, may augment their function by helping to counter cancer's immunosuppressive effects. For example, mRNA vaccines that encode a cancer antigen can boost the efficacy of CAR T-cells that target the same antigen. When the mRNA vaccine is administered, it instructs immune cells called dendritic cells to produce an antigen encoded in the vaccine's mRNA. The dendritic cells display this antigen on their cell surface, and activate CAR T-cells that recognise the antigen. The resulting CAR T-cell proliferation helps the therapy persist in the immunosuppressive tumour microenvironment and allows it to be administered at lower initial doses.[29]

This approach has already demonstrated early clinical promise. A September 2022 phase 1/2a trial of a CLDN6-directed CAR T-cell therapy, in combination with an mRNA vaccine for the CLDN6 antigen, displayed one of the first examples of CAR T-cell efficacy in solid tumours, with 43 percent of patients treated with the combination therapy exhibiting tumour shrinkage.[14]

Armoring CAR T-cells

Other research efforts are dedicated to optimising CAR T-cell function by engineering the cells themselves. For example, CAR T-cells can be engineered to alter their expression of cytokines – small proteins that immune cells use to signal each other and orchestrate the immune response – or cytokine receptors. Expression of the cytokine IL-2 has been used to increase CAR T-cell proliferation following administration.[30] CAR T-cells engineered to express other immunostimulatory cytokines may be able to counter local immunosuppression.[31] Increased expression of cytokine receptors could improve CAR T-cell penetration of solid tumours, if the CAR T-cells were engineered with receptors for cytokines highly present in the tumour microenvironment.[32,33]

How can CAR T-cell therapy be more controlled?

Immunotherapies, including CAR T-cell therapies, which intend to enhance the immune response to cancer, often accomplish this by reducing or altering the immune system's ability to regulate itself so it does not damage the body. As a result, severe complications from approved CAR T-cell therapies are quite common. Cytokine release syndrome usually occurs within a week of infusion with a CAR T-cell therapy, and the rate of cytokine release syndrome can be as high as 93 percent in patients with lymphoma or leukaemia.[10]

The severity of the side effects from CAR T-cell therapy is exacerbated by a lack of control over the level and duration of CAR T-cell activity in the body. With current technology, there is no way to regulate how much the CAR T-cells expand, express their genes or stimulate the immune system once they are infused into the patient. To overcome these challenges, researchers are working on developing new strategies to control the level of CAR T-cell proliferation and activity.

Modular CAR T-cell therapies

One promising approach to improving the safety of CAR T-cell therapy involves separating the CAR T-cells into multiple modular pieces, which makes it possible to control their activity.[11] Instead of directly recognising cancer antigens, a CAR T-cell is engineered to recognise a middle-man – the antibody "switch," which is engineered to bind the cancer antigen. The CAR T-cell becomes functional when it binds to the antibody switch that is bound to the cancer antigen. In practice, this allows the clinician to control the degree of CAR T-cell activation based on how much of the antibody switch is administered. When that antibody switch is naturally eliminated and no longer present in the body, then the CAR T-cells become inactive, making the pharmacodynamics analogous to a normal drug. In September of 2022, a first-in-human trial using a modular CAR T-cell therapy in blood-cancer patients demonstrated that adjusting the antibody switch dosage shortened the duration and severity of cytokine release syndrome and neurotoxicity events compared to approved CAR T-cell therapies.[34]

Expression switches

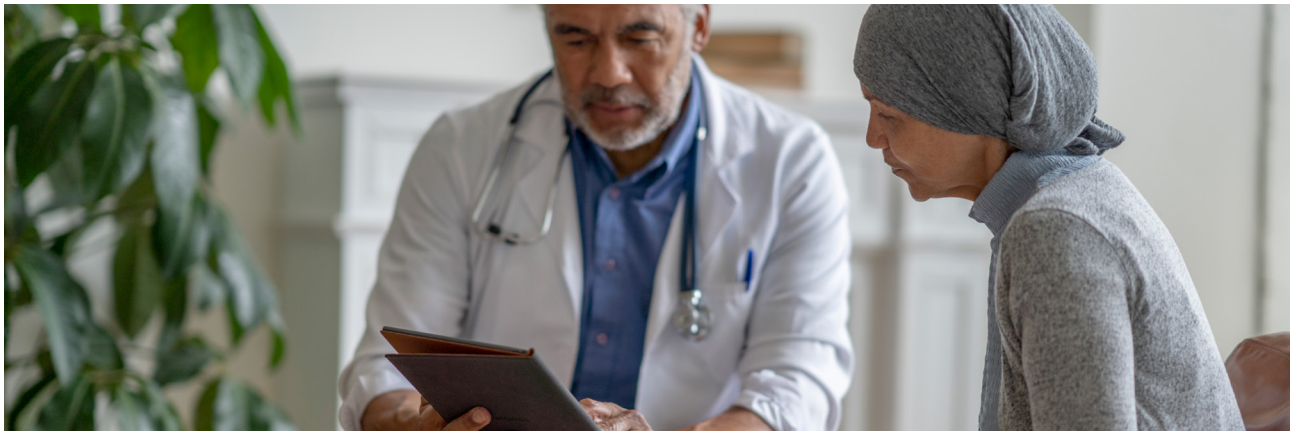
A similar approach to modular CAR T-cell therapy involves engineering CAR T-cells with expression switches that can regulate the level of receptors expressed on the CAR T-cell in response to specific conditions or small molecules.[11] The modulation of CAR expression can be regulated by small molecules, such as rapamycin, or by specific conditions – such as anoxia – as a lack of oxygen is characteristic of tumour microenvironments.

Most expression switches for CAR T-cell therapies are in preclinical development. Yet, the possible applications are noteworthy. One research team led by Ahmad Khalil, Ph.D., of Boston University has developed synthetic zinc finger transcription regulators (synZiFTRs), that use two different small-molecule inducers to first control T-cell proliferation, and then induce antitumour activity.[30] Control of T-cell proliferation was achieved through the induced expression of a T-cell stimulating cytokine, IL-2, that helps T-cells grow and survive, followed by induced expression of CARs by a second small molecule. In principle, the combination of multiple drug-induced switches within a single CAR T-therapy could allow for the fine-tuning of multiple elements of CAR T-cell proliferation and activity, and enable the termination of CAR T-cell therapy by withdrawing a small molecule inducer.

Suicide genes

Another, more permanent, method of terminating a CAR T-cell therapy is through the activation of a suicide gene. In contrast to expression switches, suicide genes are not fine-tunable – they work by rapidly inducing CAR T-cell death. One commonly used suicide gene system relies on the modified protein Caspase-9, which can trigger the death of a T-cell when it is cross-linked by a small molecule with another protein.[11] Rimiducid, one of the small molecules that can trigger the Caspase-9 suicide-gene, has recently been demonstrated as an effective "safety switch" in a phase 1/2a trial of a novel CAR T-cell therapy for advanced neuroblastoma. In the trial, one patient who experienced especially severe side effects received two infusions of rimiducid, which rapidly eliminated the CAR T-cell therapy and resolved life-threatening toxicities.[35] CAR T-cell control through suicide genes and expression switches may prove especially useful in early trials of CAR T-cells, where the safety of the therapy is difficult to predict.

How can a patient's response to CAR T-cell therapy be more predictable?



As previously mentioned, there is a lot of variability in a patient's response to approved CAR T-cell therapies. For many patients with advanced blood cancers, CAR T-cell therapy results in long-term remission, but others do not respond to treatment at all, and some respond initially, but relapse.

Then, there is the variability in safety – two patients receiving the same CAR T-cell therapy can experience great differences in the severity of side effects. For some, complications, such as neurotoxicity and cytokine release syndrome, will be life-threatening. Others may only experience nausea, fatigue and fever.

For example, in clinical trials, rates of immune effector cell–associated neurotoxicity syndrome (ICANS) ranged from 23 percent to 67 percent in adults with advanced B-cell lymphoma and from 40 percent to 62 percent for patients with advanced B-cell acute lymphoblastic leukaemia.[36]

Ongoing research efforts that aim to better anticipate a patient's response to CAR T-cell therapy could go a long way towards personalising patient care so that patients have the greatest chance of a positive outcome, and avoid severe complications.

Improve quality control for CAR T-cells

One of the reasons for a variable response to CAR T-cell therapy is related to the quality of the T cells extracted from the patient, and how well the manufacturing process is able to maintain the fitness of CAR T-cells. Taking measures to improve the quality control of CAR T-cell therapies may be one way to improve the reliability of a patient's response. There are a number of strategies that can be adopted during the manufacturing process that can improve the quality of a patient's T cells.

First, quantification of CAR T-cells per unit of blood, rather than the number of CAR gene copies per host DNA may be a more accurate way to measure the functional activity of CAR T-cells and to evaluate their expansion and persistence.[37] Secondly, extracting T-cells from patients earlier in their disease course, or shifting CAR T-cell therapy to earlier in the disease course, when a patient has had less exposure to cytotoxins, could help to improve the quality and quantity of T cells used for autologous CAR T-cell therapies.[38] In addition, implementing manufacturing methods that limit in vitro expansion in the manufacturing process could help T-cells retain more robust proliferative capacity following administration.[39]

Assess patient's T-cells, cancer and immune environment before treatment

Considering T-cell, tumour and immune-system specific factors before administering CAR T-cell therapy could also help to predict a patient's response and inform optimal patient care.[40]

Assessing the T-cells

In the case of a patient's T cells, intrinsic T cell "fitness" prior to T cell extraction (leukapheresis) is a significant determining factor of initial and durable clinical response. Research has demonstrated that patients who responded poorly to CAR T-cell therapy were more likely to have T cells with reduced intracellular glucose transporter 1 (GLUT1) and had an altered mitochondrial metabolic profile. Meanwhile, patients who experienced an initial complete response had CAR T-cells that had increased mitochondrial mass resulting in a more robust expansion and persistence in the circulation.[41] Researchers have also suggested that engineering CAR T-cells to produce more mitochondria may improve the efficacy of CAR T-cell therapy.[42]

Assessing the cancer

In regards to the patient's tumour, quantifying the density of antigen targets on a patient's tumour cells may help identify whether an antigen-targeted CAR T-cell therapy is likely to be effective.[40] This is in contrast to electing to use a CAR T-cell therapy based only on the qualitative presence of the target antigen on a patient's tumour. A patient whose cancer cells have low levels of the target antigen may be more likely to experience antigen loss or off-tumour toxicity, and might benefit from using a CAR T-cell therapy that targeted a different antigen, or targeted multiple antigens.

Another tumour quality that can impact the efficacy and safety of CAR T-cell therapy is initial tumour burden, which is also a measure of systemic inflammation. Research has associated lower tumour burden with lower levels of inflammation, better initial response to CAR T-cell therapy and a decreased likelihood of severe side effects such as cytokine release syndrome. Early and rapid expansion of CAR T cells related to initial tumour burden is a prognostic marker for initial and durable clinical response.

Assessing the Immune system

The presence of certain inflammatory markers, including inflammatory cytokines, local and systemic inflammation, and presence of suppressor cells, may also serve as indicators that a patient will have a dampened response to CAR T-cell therapy. This is because systemic inflammation negatively affects CAR T-cell expansion post-infusion, which subsequently decreases initial clinical response.

Clinical evidence has related failure of CD-19-directed CAR T-cell therapy in large B cell lymphoma patients to pre-treatment levels of tumour-expressed interferon and elevated levels of systemic inflammatory markers, which include ferritin, C-reactive protein, lactate dehydrogenase, and the inflammatory cytokine IL-6. In addition, patients who did not respond to CAR T-cell therapy had higher levels of circulating monocytic myeloid-derived suppressor cells.[4] These inflammatory markers may also indicate the risk of severe side effects to treatment with CAR T-cell therapy like cytokine release syndrome, and should be assessed ahead of treatment.

How can CAR T-cell therapies be easier to manufacture and deliver?

Even as researchers develop innovative strategies to improve the safety and efficacy of CAR T-cell therapies, additional considerations need to be made to improve the ease and cost of manufacturing and delivering them. Currently, the logistics of autologous therapy are very complex and seriously limit the scalability of CAR T-cell therapies.

For one thing, only a subset of medical facilities have the clinical expertise and certification required to deal with the complexity of handling, administering and managing the side effects of autologous CAR T-cell therapies. Of the approximately 150 medical centres in the United States that are certified to perform CAR T-cell therapies, the vast majority are academic medical centres in urban areas, creating a barrier for rural patient access.[43]

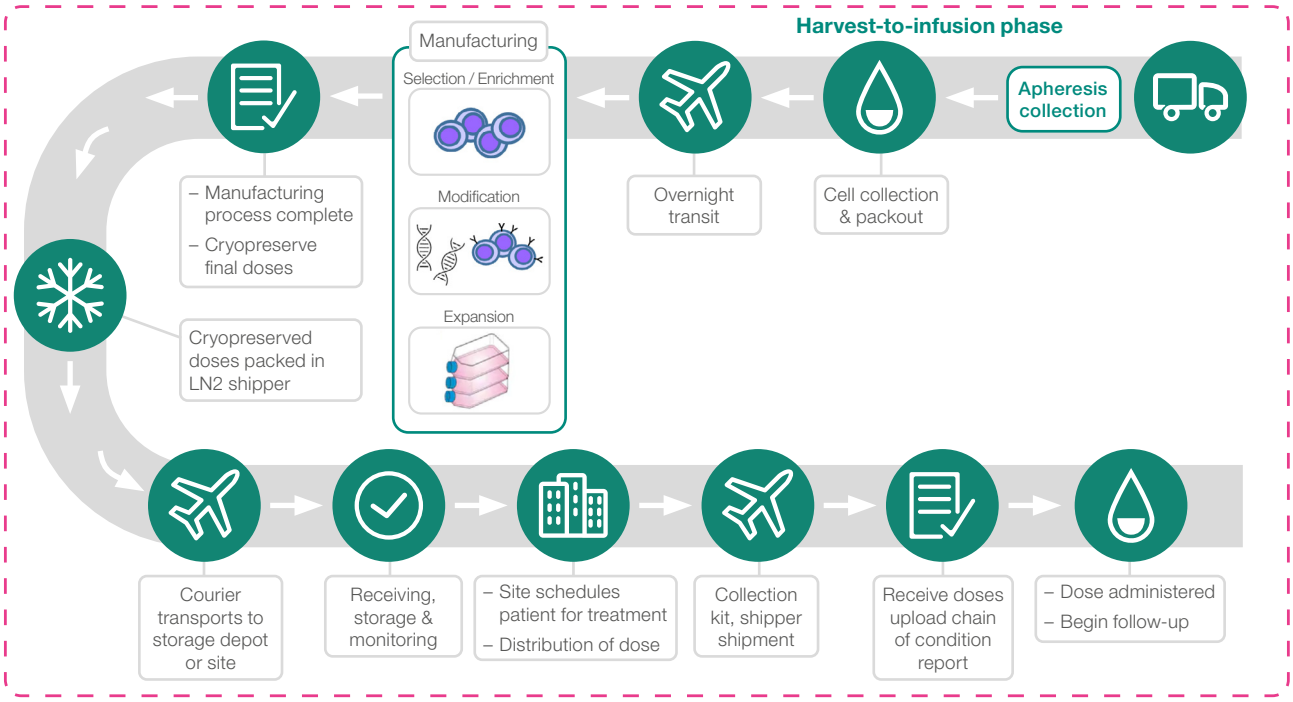
Logistical complexity also contributes to the current expense of CAR T-cell therapies. In a study published in JAMA Network Open, investigators used a decision-tree model to estimate the cost of CAR T-cell therapy from a healthcare practitioner's perspective. Findings showed that the estimated total cost of care for treating a single adult patient with relapsed or refractory large B-cell lymphoma with a CAR T-cell therapy was $454,611 in the academic hospital inpatient setting.[12]

Restrictions on a patient's lifestyle and proximity to a clinic further compound the burden for CAR T-cell recipients and their caregivers. For example, most institutions require CAR T-cell recipients to be less than two hours away from the clinic, and patients may require a skilled caregiver for multiple months. In turn, patient burdens contribute to existing inequities in who is able to access CAR T-cell therapies.

Finding an alternative to autologous therapies, which depend on the patient's own T cells, could help to overcome many of the logistical and economic limitations imposed by existing CAR T-cell therapies. Two such options are allogeneic therapy and in situ CAR T-cell generation.

Figure 3: Manufacturing and delivering CAR T-cell therapies

Tracking chain of identity, chain of condition, chain of custody



Source: https://www.iconplc.com/insights/therapeutics/cell-and-gene-therapies/mainstreaming-cell-gene-therapies

Allogeneic CAR T-cell therapy

Allogeneic therapy uses T cells from healthy donors, which are genetically modified to express CARs and avoid immune rejection by the recipient. This approach has the potential to create off-the-shelf CAR T-cell products that can be readily available for multiple patients, which could streamline manufacturing and eliminate weeks-long delays in treatment. In addition, the use of allogeneic CAR T-cells could expand the number of medical institutions able to perform CAR T-cell therapy, in turn increasing the accessibility of these therapies to patients.

However, development of allogeneic CAR T-cell therapy experienced a significant, but temporary, setback on October 7, 2021, when the FDA placed a hold on five allogeneic CAR T-cell clinical trials based on a report of a chromosomal abnormality detected in a patient treated with anti-CD19 allogeneic CAR T-cells.[44] This patient was being treated with allogeneic CAR T-cells because there had been a manufacturing failure associated with inadequate expansion of their autologous CAR T-cells. Following treatment, a bone marrow biopsy detected a chromosomal abnormality in the allogeneic CAR T-cells that were expanding in the patient.

Ultimately, the FDA removed the clinical hold after concluding that the chromosomal abnormality, which occurred in genes known to undergo rearrangements, was unrelated to the manufacturing process and had no clinical significance. However, the incident did raise concerns that the clinical development of allogeneic CAR T-cells had outpaced the required quality control necessary to implement its safe application. To meet the promise of “off-the-shelf” immunotherapy, allogeneic CAR T-cells require significant ex vivo expansion, likely more so than autologous CAR T-cells manufactured for a single patient. As a consequence, allogeneic CAR T-cells have a higher risk of T-cell exhaustion and loss of function. Yet, there are few meaningful quality control assays to measure allogeneic CAR T-cell potency, batch to batch variability, and batch release criteria.

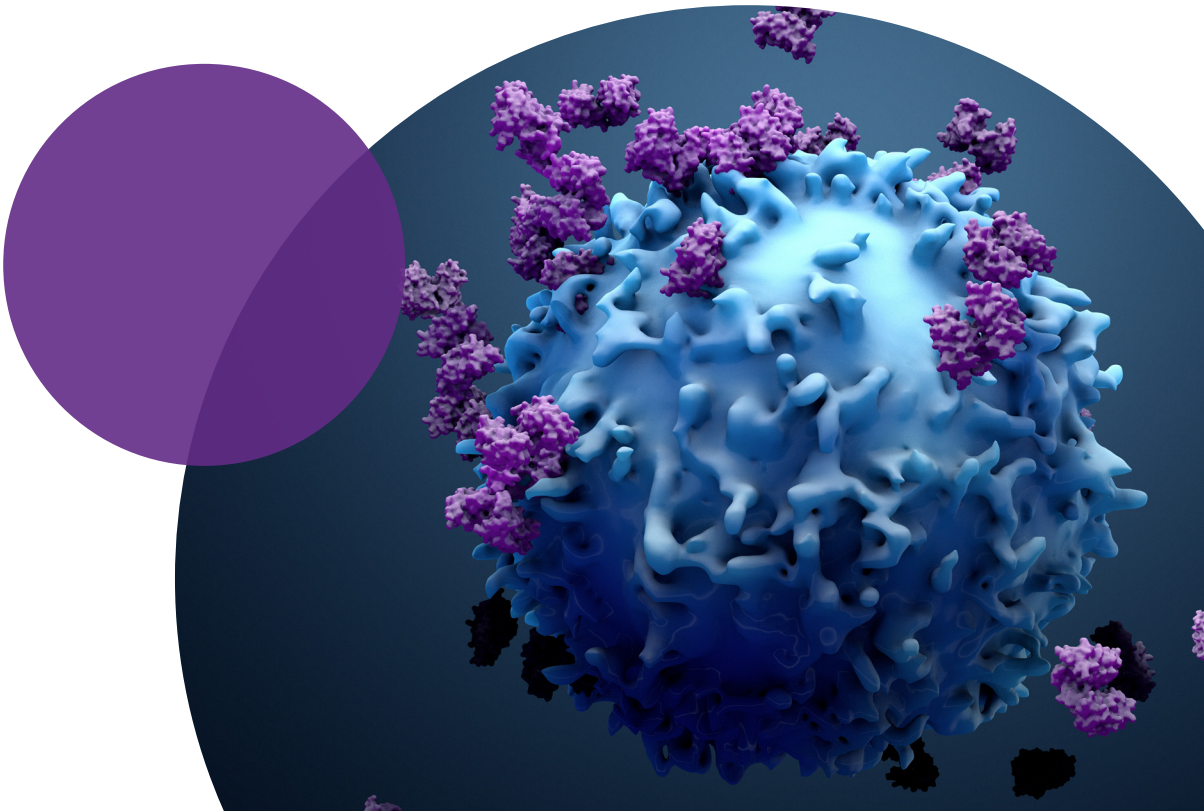
While concerns remain about maintaining the quality of allogeneic CAR T-cells, a few therapies are currently under development. One therapy, CB-011, received fast-track designation in April of 2023, and is in development by Caribou Biosciences for the treatment of advanced multiple myeloma.[45] The therapy uses CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology to address the primary concern with allogeneic technology – the potential for immune rejection. Because allogeneic stem cells do not come from the recipient, there is potential that the patient’s immune system may attack the T cells (host versus graft reaction) or that the allogeneic T cells may attack the patient (graft versus host disease). To avoid this complication, the CRISPR method used by Caribou Biosciences genetically modifies the allogeneic T-cells to “mask” CAR T-cells from the immune system. Specifically, the method creates four edits in allogeneic T cells.[46] Two edits insert a CAR and knock out endogenous T-cell receptor expression, reducing the risk of graft vs host disease. Two edits disable expression of a protein (B2M) involved in communication with other immune cells, and insert an alternative B2M-HLA-E peptide protein fusion gene into the B2M gene, which helps to reduce the risk of immune rejection (host versus graft).[45]

CAR T-cell generation in situ

Another alternative to autologous CAR T-cell therapy – in situ CAR T-cell therapy – is especially promising, because it may address challenges posed by autologous and allogeneic therapies. Instead of extracting the patient's T cells for manufacturing, this method involves genetic manipulation in situ (directly in the patient), with RNA or DNA encoding chimeric antigen receptors – essentially genetically reprogramming a patient's immune system to manufacture their own cancer-targeting T cells.[47]

If successful, this could eliminate the complexity and expense required for individualised external manufacturing of cellular therapies, and possibly the lymphodepletion (elimination of native T cells) required to precondition the patient. In addition, it would eliminate the time delay prior to treatment and most of the logistical challenges. The most common technologies being evaluated for in vivo transduction directly in the patient are lentiviral vectors and lipid nanoparticles.[48]

Lentiviral vectors may provide a more sustained and effective response than current CAR T-cell treatments because they can transduce dendritic cells that process and "present" antigens to T cells to initiate a T cell response. It is hypothesised that by genetically engineering lentiviral vectors to express cancer cell antigens and by using these vectors to transduce dendritic cells in situ, T cells can then be activated to recognise and destroy tumours. Since the dendritic cells would continue to display tumour antigens, this may provide a sustained T cell response. Additionally, lentiviral vectors may be able to encode multiple tumour antigens, potentially making the T cell response more robust and effective. Preclinical results, to date, suggest that lentiviral vectors are safe and effective. However, the method has not been tested in a clinical setting.[49] Meanwhile, lipid nanoparticles are under investigation, because they are an especially effective nonviral gene delivery system, which may help reduce immunogenicity. Chemical modifications of lipid nanoparticles may also enhance their stability and allow for tissue-specific targeting.[50]



Conclusion

The field of CAR T-cell therapy is rapidly evolving and expanding, with many promising avenues of research and development. However, it is important to keep in mind the challenges and complexities of this innovative approach to cancer treatment. CAR T-cell therapy is not a one-size-fits-all solution. It requires careful design, optimisation, and personalisation for each patient and tumour type.

Scientific discovery is a long and nonlinear process, and it takes patience and perseverance to overcome the hurdles and uncertainties. Nevertheless, the progress made in the last decade has been remarkable, and the future of CAR T-cell therapy is bright. In the last two years, early phase clinical trials have demonstrated the efficacy of CAR T-cells against solid tumours when administered in combination with other immunotherapies.[14] Many other innovative strategies for engineering and delivering CAR T-cells are also likely to make the CAR T-cells of the future more targeted, safe and controlled.

Perhaps more important than any one research effort, the recognition that cancer is not a homogeneous disease, but rather a heterogeneous and dynamic one, has led to a paradigm shift in how investigators approach cancer immunotherapy.

By tailoring CAR T-cell therapy to the specific characteristics of each patient's tumour and immune system, we can hope to achieve more personalised, effective and durable responses for a wider range of cancers.

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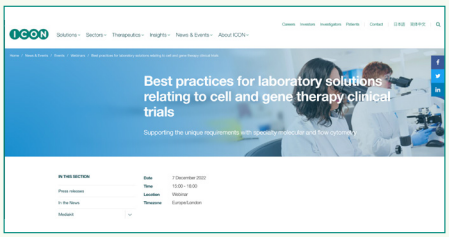

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