BioSpectrum
the business of Bio & Health Sciences
ASIA EDITION

Engineering CAR T-cell sharpshooters

01 April 2024 | Opinion | By Brian Huber, Vice President, Therapeutic Areas, Drug Development and Consulting, ICON

Approved CAR T-cell therapies have shown remarkable results in patients with certain types of blood cancers. However, further innovations are needed before the technology can reach its full potential. In principle, CAR T-cell therapies work because they are engineered with a chimeric antigen receptor (CAR) that is designed to recognise a cancer antigen expressed on the surface of that patient's cancer cells. When the CAR T-cell therapy is administered to the patient, these CAR T-cells coordinate a targeted immune response against the patient's cancer.

Ideally, the antigen for a traditional CAR T-cell (or a T-cell) would be universally and homogeneously expressed on cancer cells, but never expressed on healthy cells. In practice, this "perfect" cancer antigen likely does not exist for solid tumors where antigen expression is heterogeneous, and where overlap between antigen expression on cancer and healthy cells is common.

Two acceptable antigen candidates have been identified for blood cancers (CD19 or B-cell maturation antigen). These antigens are highly expressed in some blood cancers, but they are not cancer-specific. Additionally, in an immune evasion strategy, known as antigen loss, some patients who have received approved CAR T-cell therapies relapse because some of their cancer cells either lack or stop expressing the antigen targeted by their CAR T-cell therapy.

Ongoing challenges with CAR T-cell safety and efficacy reflect fundamental limitations of the initial aim of CAR T-cell development – to engineer cancer-targeting T cells that mirror the function of T cells. Now, efforts to develop more targeted and controlled CAR T-cells reflect a new aim: to engineer CAR T-cells with capabilities that give them distinct advantages over T-cells and first-generation CAR T-cell therapies.

CAR T-cells need to be targeted

To survive, cancers must continuously evade the immune system. Often, cancer cells achieve this by co-opting the immune system's mechanisms of self-regulation that are intended to prevent an overly strong immune response or an immune response against healthy cells. This means that if a cancer has survived long enough to do harm to the body, it has already successfully evaded the efforts of T cells, which normally recognise and then coordinate a targeted immune response against the cancer.

Ongoing efforts are now focused on engineering CAR T-cells with novel functionalities, such as logic gating and expression switches, that anticipate and help to counter mechanisms of immuno-suppression and evasion that have limited the efficacy of T cells and contemporary CAR T-cell therapies.

CAR T-cells need to be controlled

While many limitations of efficacy are shared with T-cells, some critical differences between the first generation of CAR T-cells and T cells contribute to the toxicity of approved CAR T-cell therapies. Because CAR T-cells are engineered outside the body, they are not subject to the mechanisms that the immune system has in place to eliminate T cells that are able to identify antigens expressed on healthy cells. This means that unlike natural T cells, CAR T-cells can be designed to target antigens that are present on cancerous and healthy cells. The resulting auto-immune response from "on target, off tumor" T-cell activity can be fatal.

In addition, CAR T-cell expansion and proliferation are not subject to the same careful control that the immune system normally has over T cells to prevent them from provoking an immune response that is dangerously strong. While the dose of CAR T-cell therapies can be specified when the therapy is administered, the expansion and proliferation of CAR T-cells following administration are poorly controlled. As a result, severe complications from CAR T-cell therapy are common, including cytokine release syndrome and neurotoxicity. Many new CAR T-cell therapies in development are now being engineered so that clinicians retain better control over CAR T-cell activity and proliferation after the therapy has been administered.

Engineering future CAR T-cell sharp-shooters

The most promising elements that can be added to CAR T-cells effectively increase precision and control. Future CAR T-cell therapies may need to be engineered with multiple functionalities at once to improve precision and control. The following methods of CAR T-cell engineering are especially promising:

Multiple receptors

CAR T-cells can be engineered with multiple different antigen-specific receptors to create "logic gates" which can either activate or deactivate the CAR T- cell based on their combined activity. Engineering CAR T-cells with "OR," "AND" or "NOT" logic gates could provide a solution to a number of the challenges presented by CAR T-cells designed to be activated upon recognition of a single antigen.

By making CAR T-cells less specific, "OR" logic gating has the potential to protect against antigen heterogeneity between cancer cells and the likelihood of antigen loss. The "OR" logic gate functions by increasing the number of antigens that can activate a CAR T cell. Meanwhile, by increasing the specificity of CAR T-cells, "NOT" or "AND" logic gates make it possible to safely target a CAR T-cell against a cancer antigen that is expressed on healthy cells, as long as the logic gate prevents the T-cell from activating when it interacts with healthy cells. (In AND gating, a CAR T-cell needs to be activated by more than one antigen at once. While in NOT gating, the CAR T-cell can be deactivated by a receptor bound to a healthy-cell antigen).

Modularity

Modular CAR T-cells aim to improve the control over CAR T-cell activity following administration. This control is accomplished by engineering a CAR T-cell that can be separated into multiple modular pieces. Instead of directly recognising cancer antigens, a CAR T-cell is engineered to recognise a “middleman” – an antibody which is engineered to bind the cancer antigen. The CAR T-cell becomes functional when it binds to the engineered antibody that is bound to the cancer antigen.

In practice, modular CAR T-cells allow a clinician to control the degree of CAR T-cell activation based on how much of the engineered antibody is administered. When that engineered antibody 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 engineered antibody dosage shortened the duration and severity of cytokine release syndrome and neurotoxicity events compared to approved CAR T-cell therapies.

Suicide genes

Another way to control CAR T-cells following administration is engineering them with a suicide gene, which, when triggered by a small molecule, induces the death the of CAR T-cells.This is a more severe method of control than modularity, because it is not fine-tunable. However, the effect of suicide genes are rapid, allowing clinicians to terminate a CAR T-cell therapy in case of severe complications. Suicide genes may prove especially useful in early trials of CAR T-cells, where the safety of the therapy is difficult to predict.

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. 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.

Cytokine expression

CAR T-cells can be engineered to have altered 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. CAR T-cells engineered to express other immunostimulatory cytokines may be able to counter local immunosuppression. Increased expression of cytokine receptors could improve CAR T-cell penetration of solid tumors, if the CAR T-cells were engineered with receptors for cytokines that are highly present in the tumor microenvironment.

Expression switches

Expression switches also offer a mechanism for regulating the activity of CAR T-cells by engineering a mechanism that makes CAR T-cells respond to specific conditions – including anoxia, which is characteristic of the tumor microenvironment – or small molecule drugs such as rapamycin.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 activation. For example, one research team led by Dr Ahmad Khalil of Boston University, has engineered CAR T-cells with two expression switches that use different small-molecule inducers to first control T-cell proliferation, and then induce antitumor activity. Control of T-cell proliferation was achieved through the induced expression of the cytokine IL-2, that helps T-cells grow and survive, followed by induced expression of the chimeric antigen receptor by a second small molecule.

The limitations of a CAR T-cell engineered with a single cancer-specific antigen have become increasingly clear. Although it is not yet evident what the future of CAR T-cells will be, ongoing research efforts suggest that the next wave of CAR T-cells are likely to include additional elements that make them more precisely controlled and adaptable than T-cells and CAR T-cell predecessors.

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