MD ANDERSON CANCER CENTER

Oncologist Katy Rezvani has engineered the immune system's natural killer cells to target certain tumour cells.

The less-personal touch

CAR-T immunotherapy is a specialist and complex treatment for cancer. Now, researchers are looking to provide an off-the-shelf version to make the therapy available to more people. **By Anthony King**

The success of chimeric antigen receptor T-cell (CAR-T) therapy has electrified the oncology field. Many specialists gush about its promise. But most people with cancers that the therapy could treat do not currently benefit from it.

The technique used in the clinic involves engineering a person's own immune cells known as T cells to carry a receptor that directs them to attack tumours. Two CAR-T products were approved by the US Food and Drug Administration (FDA) in 2017 for cancers of the blood or blood tissues. "It's the most important therapeutic innovation in a generation in haematology," says Martin Pule, a haematologist at University College London. What was once a "slightly eccentric approach", he says, is now the standard of care for some cancers.

The first of these treatments to be approved was tisagenlecleucel (Kymriah), developed by pharmaceutical company Novartis in Basel, Switzerland. Its use resulted in cancer remission in more than 80% of people with difficult-to-treat leukaemia[1]. Axicabtagene ciloleucel (Yescarta), developed by Kite Pharma in Santa Monica, California, is approved for use in relapsed or treatment-resistant large B-cell lymphoma. In a clinical trial, 65% of people had not relapsed 12 months after their initial response[2].

However, only a fraction of the people in the United States who could benefit from CAR-T therapy are currently receiving it. For people with lymphoma, the figure is around 1 in 5, says Sattva Neelapu, a cancer scientist at the University of Texas MD Anderson Cancer Center in Houston, who led the trial that resulted in Yescarta's approval.

The main reason for this is that both Kymriah and Yescarta are challenging to produce. Both are autologous therapies, which means they use a person's own cells. Manufacture begins with the collection of a person's blood. T cells are then isolated from the sample and shipped to a centralized manufacturing facility, where they are genetically modified to target a protein on cancer cells. The engineered T cells are grown for 5 to 10 days and, subject to passing rigorous safety and efficacy criteria, shipped back to the hospital and administered to the original donor. "It is a lot of work," says Stephan Grupp, a cancer immunotherapist at the University of

Pennsylvania in Philadelphia, who has treated more than 370 people using autologous CAR-T.

This complex manufacturing process sometimes fails. Neelapu says that about 30% of Kymriah products for lymphoma fall short of criteria set by the FDA. Even when production does go smoothly, it is a lengthy process. Earlier this year, Neelapu and his colleagues looked at data from around 300 people with lymphoma at 17 centres across the United States to see how long it took to produce Yescarta[3]. The average time from donation to receipt of therapy, he says, was more than three weeks. For people with quickly proliferating diseases, such as acute leukaemia, that can be too long to wait. Neelapu estimates that 10–15% of people who are referred for CAR-T therapy either die or are too unwell to risk the treatment by the time it is ready.

The complexity of CAR-T therapy means the treatment can cost upwards of US$350,000, and relatively few US centres are capable of delivering it – a situation that Michel Sadelain, an immunologist at Memorial Sloan Kettering Cancer Center in New York City, who pioneered some of the first CAR-T studies, finds disappointing. "It is really regrettable," he says.

Researchers are searching for ways to make CAR-T therapy accessible to more people. One possibility is to move away from crafting treatment from a person's own cells, and instead to engineer T cells from healthy donors. This allogeneic approach could also be applied to elements of the immune system other than T cells, such as natural killer (NK) cells. However, the use of donor cells is fraught with issues of rejection, leading some to say that the answer instead lies in streamlining and automating existing autologous CAR-T manufacture.

Off-the-shelf promise

Proponents of allogeneic CAR-T therapy see many upsides. Processing cells for not one but dozens of people at a time could lower manufacturing costs and allow hospitals to keep engineered cells on ice, ready to be quickly administered to people in need. "It is more like a drug than the autologous cell process," says Grupp. This off-the-shelf approach to CAR-T therapy could allow it to be offered by hospitals that do not have the ability to extract T cells from people's blood. This process, known as leukapheresis, is usually the preserve of bone-marrow transplant centres.

Using T cells from healthy donors could benefit people whose own T cells are defective, owing to suppression by their cancer or chemotherapy. "One of the main reasons for relapse after CAR-T is because patient T cells were dysfunctional at the time of leukapheresis," says Neelapu. An allogeneic approach could even lead to more-ambitious treatments, involving CAR T cells engineered with multiple targets in mind (see 'A new path to cell therapy'). This is more difficult to achieve, but the risk of needing to start again if a batch of T cells fails might be more acceptable when a person's survival isn't dependent on getting their own cells back quickly. "Tolerance for unsuccessful manufacturing in the allogeneic world is higher," says Grupp.

However, off-the-shelf CAR-T therapy is not without its challenges. One issue is that donor T cells can identify the body of the person receiving the therapy as foreign and attack it, triggering graft-versus-host disease (GVHD), which can be fatal. The second major problem is that foreign T cells might be eliminated by the person's immune system before they can attack the cancer. In Grupp's experience, allogeneic cells "are almost universally gone in three to four weeks", he says. By contrast, Pule has detected autologous T cells in people two years or more after infusion.

> **"Redosing is part of the concept of allogeneic CAR-T."**

To improve the staying power of off-the-shelf CAR T cells, the biotechnology company Allogene Therapeutics in South San Francisco, California, has genetically modified CAR T cells to remove a protein known as CD52 from their surfaces. Antibodies that help to destroy cells that do carry the surface protein are then given to the person, depleting their own white blood cells that might otherwise kill the engineered CAR T cells. And to protect against GVHD, the T-cell receptor of the engineered cells can be altered, preventing them from attacking the person's cells.

In May, Allogene reported encouraging findings from a phase I trial of its allogeneic CAR T cells in 22 people with diffuse large B-cell lymphoma or follicular lymphoma (see go.nature.com/2fssusw). Tumours shrank in most people and around 40% of volunteers had a complete response to the treatment. "The overall response rate is somewhat in the same ballpark with what we see with autologous CAR-T products," says Neelapu, who led the trial. The number of CAR T cells expanded and peaked during the first 2 weeks, and persisted for up to 8 weeks, he adds. Moreover, there has been no sign of GVHD or neurological toxicity. "Safety-wise, it looks better than the currently available FDA approved products," he says. Cytokine release syndrome – a commonly observed side effect of CAR-T therapy in which proliferating T cells secrete inflammation-promoting cytokines – was experienced by one-third of people, but was reversible.

It might be that tackling cancers other than lymphoma, particularly solid tumours, will require staying power measured not in weeks but in months, or even years. However, another feature of allogeneic cells is that they can be created in batches, which allows for repeat dosing if their effects begin to wane. "Most of the data for solid tumours show CAR T cells getting exhausted after a single injection," says André Choulika, chief executive and founder of Cellectis in Paris, from which Allogene licenses some of its technology. "Redosing is part of the concept of allogeneic CAR-T." Sadelain notes, however, that although there are good reasons to be hopeful about allogeneic approaches, they are not yet validated in the clinic.

Home advantage

When Pule began working with CAR T cells almost a decade ago, creating them required two technicians to manipulate cells inside a sealed container. "At that point it looked like allogeneic was the answer," he recalls. But, in his view, advances in manufacturing processes are making autologous CAR-T therapy a viable default long-term option.

Today's closed manufacturing systems do not demand stringent clean-room requirements, and because the process of growing T cells is becoming more automated, it requires less technician input. This automation is accelerating the production of CAR T cells – Novartis is trying to reduce manufacturing time to two days. "These processes were cobbled together by academic investigators to be safe and reliable," says Sadelain. "We haven't yet seen the impact of industrialization." Software that automates the extensive documentation required for the production of the cells for every person is also emerging.

These developments should also reduce cost. "I'd be very surprised if autologous therapies were selling at more than $100,000 to $150,000 a treatment in 5 years' time," says Mark Lowdell, a cellular immunotherapist at University College London. And although there is no guarantee that the cost will drop, neither is it certain that allogeneic CAR-T will bring substantial savings.

Bruce Levine, a translational oncologist at the University of Pennsylvania, thinks that the cost of off-the-shelf CAR T cells will probably be lower than autologous versions – but not to the degree that some companies and researchers say. In an analysis last year[4] of the production cost of CAR-T therapy, he found that consumables comprised the "vast majority of the cost of a product". Allogene's off-the-shelf approach requires a viral vector to deliver

genes, which is one of the more expensive parts of the process. Multigene editing to get around rejection issues and the need to repeat treatment also add to the total cost. In the end, whether or not allogeneic CAR-T is cheaper might rest on the number of cells that can be produced in a single batch. "If you are making five or ten products per run, you probably are not saving any money. If you are making 100, you probably are," says Pule. In an early indication, Choulika says that Cellectis can already manufacture 100 doses of off-the-shelf CAR T cells from each donor.

Researchers are still debating the relative safety of allogeneic and autologous CAR T cells. Some critics of the off-the-shelf approach suggest that the requirement to weaken a person's immune system leaves them vulnerable to viruses. Pule has concerns that repeat dosing could eventually lead to the person's immune system reacting against the CAR itself. At the same time, other researchers suggest that autologous CAR T cells, which hang around for years, could turn against the person's immune system. "Once they destroy the tumour cells, they will start attacking normal B cells," says Lowdell. Recipients could become immune-deficient for life and require antibody replacement therapy.

Another way

As the debate continues, an alternative form of cell therapy is drawing attention. It has many of the benefits of allogeneic CAR-T, but with fewer drawbacks. Rather than administering T cells that form part of the adaptive immune system, NK cells are used.

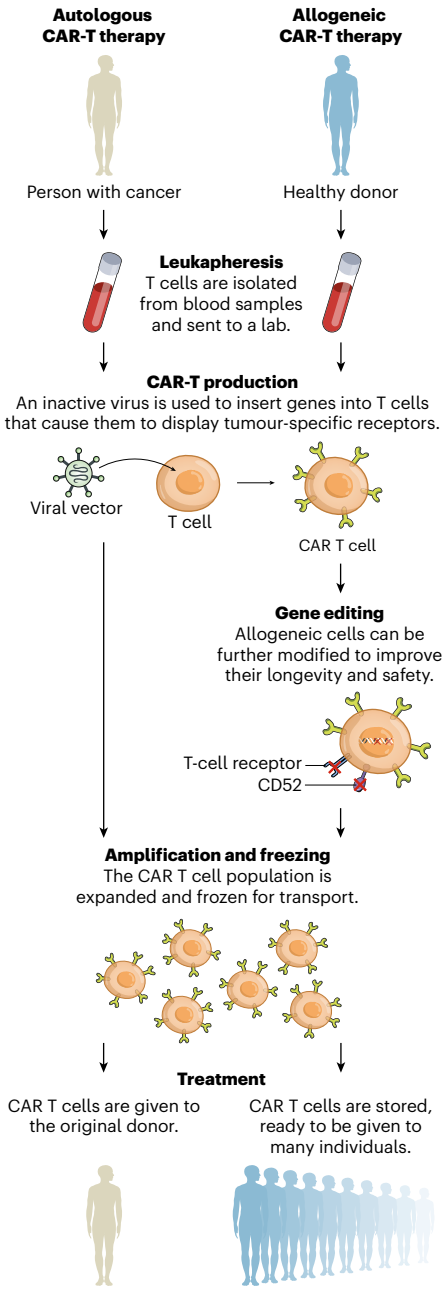
NK cells are equipped with receptors on their surface which look for signs of danger or stress that might indicate a tumour or a cell infected with a virus. When they find such a cell, NK cells can attack it directly and call T cells to the site to help. And, crucially, donor NK cells cannot cause GVHD, because they do not express T-cell receptors — removing the need for costly immune suppression.

Katy Rezvani, an oncologist at MD Anderson Cancer Center, led a trial earlier this year in which NK cells collected from cord blood were engineered with CARs that allowed them to target the CD19 protein found on some tumour cells[5]. The NK cells were also made to express IL-15, a cytokine that encourages proliferation and persistence of NK cells. The resulting CAR NK cells were then given to 11 people with relapsed or treatment-resistant non-Hodgkin's lymphoma or chronic lymphocytic leukaemia.

Eight people had a response to the treatment within a month. At follow-up about 14 months later, 7 people had complete remission and 1 had partial improvement.

A NEW PATH TO CELL THERAPY

Autologous CAR-T therapy alters a person's own T cells. Some researchers, however, advocate an allogeneic approach, in which T cells are sourced from a healthy donor and engineered to work for many people.



As a first-in-human trial, Rezvani says she was nervous about potential toxicity effects, especially because inserting the gene that encodes IL-15 was a new approach. But the volunteers did not experience cytokine release syndrome or neurotoxicity. The cells also remained in the body for at least 12 months — something that many in the field had not expected of cells that normally disperse quickly after doing their job. "Before I saw Katy Rezvani's work at MD Anderson, I really didn't believe that CAR NK cells were a thing," says Grupp. "You need cells that will proliferate in a patient's body to get a successful treatment response, but she has shown that they can do that." The technique is still in its infancy — Sadelain notes that only around 20 people have been infused with CAR NK cells so far — but it is now being rapidly expanded.

Some researchers are trying to make CAR NK cells more effective. The pharmaceutical company Editas Medicine in Cambridge, Massachusetts, is using the gene-editing technology CRISPR to remove a receptor on NK cells for TGF-β — a signalling molecule produced by some tumours that can shut down immune cells. The hope is that removing the receptor will make the CAR NK cells more-potent tumour-killing machines. This might mean they can target solid cancers, which has so far proved difficult with CAR-T therapy. "Gene-edited NK cells will be one of the key medicines in future off-the-shelf CAR therapies, especially for solid tumours" says Rick Morgan, a senior vice-president at Editas Medicine, which is collaborating with Sandhill Therapeutics in Dallas, Texas, to develop NK-cell therapies.

Rezvani is also looking at ways to get CAR NK cells to work against solid tumours such as glioblastoma. Meanwhile, Sadelain has been exploring whether stem cells could be a perpetual, standardized source of either NK or T cells for use in oncology. "We are talking about the potential to grow one clone, to produce cells for thousands of patients" says Sadelain. Fate Therapeutics, an immunotherapy company in La Jolla, California, has a number of cell products derived from stem cells in clinical trials.

A leading approach to cell therapy for cancer has yet to emerge, but work on allogeneic CAR T and CAR NK cells is gathering pace. "Three or four years ago I would have been very sceptical of the allogeneic approach, but we have had the data from Katy Rezvani and some Allogene data," says Levine. "It looks promising." Whether autologous cells are abandoned altogether in favour of a theoretically more cost-effective off-the-shelf approach, or whether the approach becomes one part of a broader treatment landscape, remains to be seen. In the end, it might not matter. "Patients and payers don't care if it is autologous or allogeneic," Pule says. "So long as it works."

Anthony King is a science writer in Dublin.

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