

A technician helps to prepare CAR-T cells at the Dana-Farber Cancer Institute in Boston, Massachusetts.

IMMUNOTHERAPY

Gene therapy delivers hope

In CAR-T therapy, a patient's immune system is genetically modified to seek and destroy tumour cells. The radical treatment offers a final shot at a cure when other therapies fail.

BY BIANCA NOGRADY

First-in-human trials are risky. That's why they tend to involve the sickest of the sick — people whose disease has progressed beyond the reach of any existing treatment, and who have no other options. So it is a testament to the revolutionary nature of chimeric antigen receptor T-cell therapy (CAR-T therapy) that some of the people who took part in its first clinical trial are still alive 8 years later.

Stephen Schuster, director of the lymphoma programme at Penn Medicine at the University of Pennsylvania in Philadelphia, recalls that the first person who enrolled in the trial was in bad shape. The man had a genetically mutated lymphoma that had already defied numerous treatment attempts. Schuster thought nothing would save his life.

That pessimism turned out to be poorly founded. “Amazingly enough, he got

stronger and stronger and stronger,” Schuster recalls. “And then when I did a scan, he was in remission.”

In CAR-T therapy, a patient's immune T cells are genetically recoded to give them the ability to target their cancerous cousins, and two CAR-T products are now on the market. Tisagenlecleucel was approved by the US Food and Drug Administration (FDA) in August 2017 for paediatric and young-adult acute B-cell lymphoblastic leukaemia. It was the first gene therapy to be approved by the FDA and has since received approval for use against large B-cell lymphoma as well. Then, in October 2017, the FDA approved axicabtagene ciloleucel for use against certain adult large B-cell lymphomas.

But these treatments come at a financial and physical cost. In the United States, treatment with these CAR-T products starts at US\$373,000 for the single injection required. And as a gene therapy — the first of its

kind — CAR-T is presenting oncologists with unfamiliar side-effect challenges that they have been nervously feeling their way through.

THE FINAL RECOURSE

However, CAR-T therapy offers hope to people with B-cell lymphomas who have failed to respond to chemotherapy or stem-cell transplants (see page S48). Second- and now third-generation CAR-T therapies are making their way through clinical trials, and some think that the therapy will come to be used as a second- or even first-line treatment, rather than a last resort. These results mark “the end of the beginning” for CAR-T in lymphoma, says oncologist Caron Jacobson at the Dana-Farber Cancer Institute in Boston, Massachusetts.

The principle behind CAR-T therapy in B-cell lymphoma is simple: use a patient's own T cells to attack and destroy malignant B cells. But its developers faced two major

problems: finding a suitable target on malignant B cells for T cells to trace, and engineering the patient's T cells to attack the target with minimal collateral damage to other cells.

The target of choice so far has been an antigen (a molecule that causes an immune response) called CD19, which is found on the surface of B cells. "CD19 is nearly universally present on mature B-cell lymphomas, and it is necessary for the cell to survive," says Jacobson. It is also present on healthy B cells, so the therapy wipes out those cells as well. But the temporary loss of the B-cell population is something that people can survive with medical assistance; in most patients, cell levels recover within months.

To prime a patient's T cells to seek out CD19, the cells must first be harvested from the person's blood and separated by a mechanical process called apheresis. An inactivated virus, such as a lentivirus or retrovirus, is then used to insert a gene into the T cells. That gene codes for the CAR, which allows the T cell to target the CD19 antigen. Both treatments also contain co-stimulatory molecules that are designed to boost and sustain the T cells' attack on malignant B cells.

The treatment involves a single infusion of the genetically modified T cells. Then it's a case of waiting and hoping that the patient's immune system does the rest.

Phase II trials of CAR-T therapy have shown complete remission rates after 6 months of 30% for tisagenlecleucel (see go.nature.com/2p6oxqv) and 54% for axicabtagene ciloleucel (S. S. Neelapu *et al.* *N. Engl. J. Med.* 77, 2531–2544; 2017). Both trials were in people with large B-cell lymphomas that had progressed despite multiple rounds of chemotherapy and, in some cases, an autologous stem-cell transplant. In the axicabtagene ciloleucel trial, 52% of patients were still alive 18 months later.

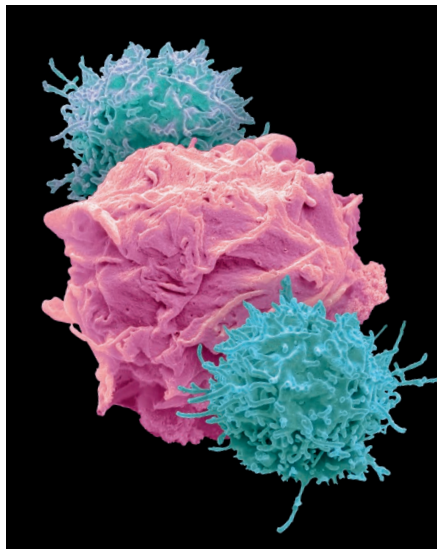
SUBDUING SIDE EFFECTS

When CAR-T therapy was first trialed, researchers had little idea of what side effects to expect. "It was kind of scary, finding our way through managing these unique cell-therapy-related syndromes," says Schuster. "They're different than the side effects that oncologists are used to managing, in the sense that they're more akin to what happens when somebody gets a serious infectious illness."

The most common side effect, which occurred in 93% of participants in one axicabtagene ciloleucel trial and 58% of participants in a tisagenlecleucel trial, involves the cells releasing large numbers of immune-cell signalling molecules. Known as cytokine release syndrome, this condition presents as fever, low blood oxygen and low blood pressure — sometimes severe enough to require hospitalization.

Schuster says he has never had a patient die from cytokine release syndrome. Clinicians now know how to treat it with steroids and a medication that blocks the release of one of the cytokines implicated in the syndrome.

The other side effect that initially caused



T cells (blue) and a lymphoma cell (pink).

alarm is neurotoxicity, which presents as confusion, difficulty finding words and speaking, and sleepiness. Although worrying, these deficits are temporary, and resolve themselves within days or weeks of the initial infusion in most patients. But researchers still don't fully understand how they arise, says Sattva Neelapu, an oncologist specializing in lymphoma and myeloma at the MD Anderson Cancer Center in Houston, Texas. However, Neelapu notes that research reported last year (see go.nature.com/2seprog) suggests that the cytokines released by T cells might be linked to endothelial dysfunction and disruption of the blood–brain barrier.

INCREDIBLE EXPENSE

Another side effect of CAR-T therapy hits the wallet rather than the body: the cost of this treatment is exorbitant. Haematologist David Bishop at The Westmead Institute for Medical Research in Sydney, Australia, says that a major factor in the high cost is the need for viral vectors to effect the gene modification that makes CAR-T therapy possible. To ensure no undesirable viral components are included in the treatment, extensive and rigorous testing is required, which bumps up the cost considerably.

To address this, he and his colleagues are looking for cheaper methods of genetic modification. Their method uses so-called jumping genes, or transposons: mobile genetic elements that can be harnessed as a tool to cut DNA and insert new material. Instead of using viral vectors to insert the genetic material, researchers give the T cells an electric shock, which creates tiny holes in the cell membrane. The transposon system — in this case, the aptly named piggyBac — can then get inside and insert the designer CAR gene into the T-cell's DNA.

"It's a different way of genetic modification, and it turns out that it's far cheaper because making the transposon and transposase plasmids is really easy — you can just amplify them in bacteria and purify them," Bishop says. "What we're talking about is probably a 10- to 20-fold

reduction in the cost of making a CAR-T cell."

Transposon-based CAR-T therapy is being tested in phase I clinical trials in Australia in people with lymphoma and leukaemia. The trial is using T cells from matched sibling donors, rather than from the patients themselves. It's early days, but David Gottlieb, a haematologist at The Westmead Institute for Medical Research who is working on the trial, says the signs are encouraging. "We've treated a handful of patients on the first study and we've seen some excellent responses to the CAR-T cells," Gottlieb says, although some patients have experienced disease recurrence. "Of course, we're doing a dose escalation so these are in the early doses," he adds. "It's possible that those patients will respond to higher doses, and maybe would have responded in a more prolonged way to initial higher doses."

Researchers are also looking for more B-cell antigens that CAR-T cells could target. This could help to treat other lymphomas, lower resistance, improve efficacy and reduce side effects, Jacobson says. One such target is CD30. Barbara Savoldo, a paediatric haematologist and oncologist at the University of North Carolina at Chapel Hill, has been investigating CD30 as a target in Hodgkin's lymphoma and anaplastic large-cell lymphoma.

"There are old studies where they tried to knock down the [CD30] molecules and the tumour doesn't grow, so it's a good target because it's an essential molecule for the tumour

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to survive," Savoldo says. A phase I, dose-escalation study of a CD30-specific CAR-T therapy (C. A. Ramos *et al.* *J. Clin. Invest.* 127, 3462–3471; 2017) found the treatment to be safe and showed a clinical response, which gives researchers cause for hope.

Savoldo says the CD30 antigen could also be a target for other B-cell lymphomas and potentially T-cell lymphomas.

Haematologist David Porter at the University of Pennsylvania has been involved in CAR-T clinical trials from the early days of the therapy, and he is still struck by the speed with which CAR-T has progressed. "We treated our first patient with our CAR-T-cell product in August 2010, and seven years later, it was an FDA-approved product," he says. "That's just unheard of."

Many promising medical therapies end up falling by the wayside, but Porter says CAR-T therapy has delivered way beyond what was expected when it first arrived on the scene. "It has revolutionized the way that we treat certain malignancies," he says. "Cell therapy is now in the mainstream of cancer therapies." ■

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