



IMMUNOLOGY

CANCER KILLERS

Some advanced cancers can now be successfully treated by synthetic immune cells that are more powerful and longer-lasting than any found in the body

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Illustration by James Yang

TUMOR IMMUNOLOGISTS HAVE known for decades that the immune system can be an important ally in the fight against cancer. Most early attempts to recruit its potential proved disappointing, however. It turns out that investigators had not done enough to stimulate a key component of the immune system, a kind of master sergeant called the T cell. Without enhancing the ability of T cells both to identify and to attack cancer cells, researchers were, in effect, asking the immune system to go into battle with the biological equivalent of paper airplanes and pellet guns.

The first clues that T cells needed to be greatly fortified to fight cancer emerged in the 1980s. Researchers tried to strengthen the immune responses by drawing T cells from patients, multiplying them in the laboratory and then infusing the expanded number of cells back into the body. That approach helped some people but typically did not work for long: the cells tended to exhaust themselves and shut down soon after delivery.

Various groups of investigators then began addressing the problem in different ways. One strategy that we and our colleagues have developed is now showing exciting promise in clinical trials. Back in the mid 1990s, while trying to discover new treatments for HIV, two of us (June and Levine) created an improved technique to turbocharge T cells drawn from patients, making the cells more abundant, powerful and longer-acting than previous methods could achieve. Then, about a decade ago, a new way of genetically altering T cells became available that would allow them to efficiently home in on and attack certain kinds of cancer—such as leukemia and lymphoma—that originate in various types of white blood cells.

In the past few years these synthetic immune cells, known as chimeric antigen receptor T—or CAR T—cells, have been tested in dozens of studies collectively involving close to 1,000 patients with advanced cases of leukemia or lymphoma. Depending on the disease, half or more of those patients are now living longer than expected, and hundreds appear to be cancer free.

A consensus is building among cancer researchers that treatment with CAR T cells—either alone or in combination with other therapies—will eventually provide durable cures for certain blood cancers. The next hurdles will include confirming if this type of therapy can be effective against other kinds of tumors and better controlling the side effects, some of which can be fatal. But the success so far, which involved tackling a series of difficult challenges over the course of about 20 years, is heartening.

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TURBOCHARGE T CELLS

WHEN WE STARTED on the road that ultimately led us to CAR Ts, our first task—simply figuring out how to enhance the cell-killing powers of T cells from patients—was anything but simple. To become activated, T cells must receive signals from a different group of immune system players called dendritic cells. Only after receiving such instructions can T cells achieve their full potential: dividing and producing extra copies of themselves (all primed against the same target) and releasing chemicals called cytokines that boost the body's immune response even further. After a few days, the T cells quiet down, allowing the body—and the immune system—to return to normal.

In the mid-1990s, while working on HIV, June and Levine decided to improve on this natural process by stimulating T cells in the lab. Our goal was to take some T cells out of a patient, activate them, encourage them to multiply many more times than was possible within the body and inject them back into the same person—where we hoped they would boost the ability of the patient's immune system to fight HIV and the other infections that plague people with AIDS (the end stage of HIV infection).

But first we needed to find a good way to activate the T cells. In theory, we could expose them to dendritic cells that were also isolated from each patient, but dendritic cells vary substantially in number and quality, especially in people with HIV or with cancer. To get around the problem, we decided to develop artificial substitutes for the dendritic cells. Eventually we settled on tiny, magnetic beads that we coated with two proteins able to mimic and improve on the dendritic cells' stimulatory behavior.

Then we collected T cells from the blood of patients and energized them with our all-purpose beads. By the end of the five- to 10-day process, each of our patients' T cells had given rise to 100 more cells. Our microbead-based method is now one of the primary tools that investigators use to grow activated T cells for use in many different research experiments and clinical trials.

REDESIGN THE T CELL

THE BODY FACES TWO major challenges in mounting an immune response to cancer. One is that malignant cells spring up from our

IN BRIEF

Synthetic immune cells, known as chimeric antigen receptor T, or CAR T, cells have proved remarkably effective

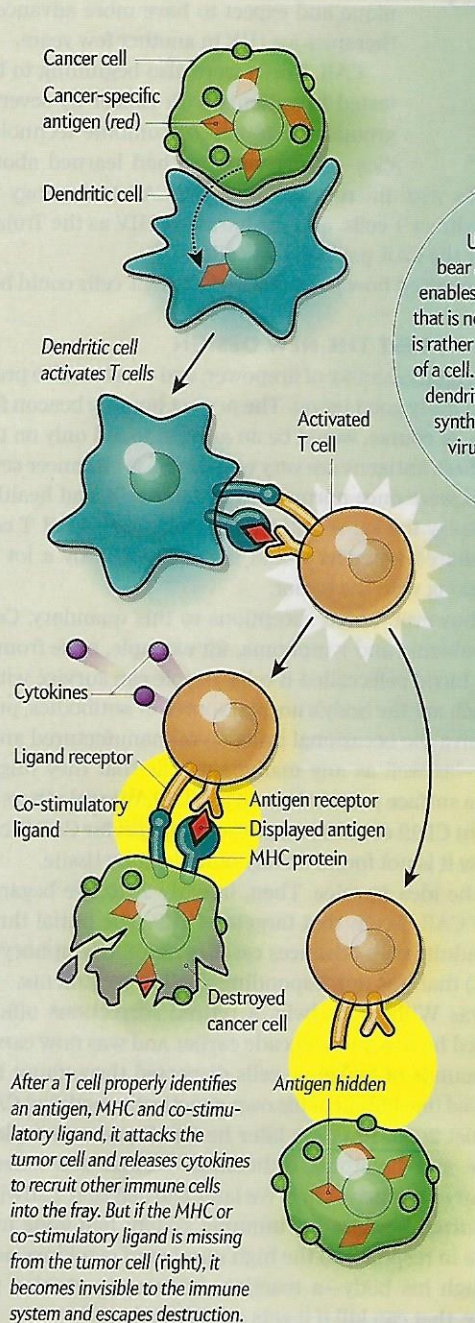
at treating leukemia and lymphoma. **CAR T cells boost and enhance** the body's ability to fight malignant cells.

But they can trigger unwanted side effects and, in some cases, death. **Researchers are now designing new**

CAR T cells they hope will treat other forms of cancer and cause fewer deleterious side effects.

Normal Immune Response Is Complicated

Although a healthy immune system can recognize and destroy cancer cells, the process is complex and prone to breakdown. So-called dendritic cells absorb and process some of the proteins found either on the surface or inside of a malignant cell. Then, the next time the immune defender meets other immune cells called T cells, it "presents" them with bits of those proteins, known as antigens. This action prompts the T cells to do two things: (1) search out and identify any cells that contain both the antigen that had been presented by the dendritic cell and another protein called an MHC and (2) attack the antigen-bearing cell if it also possesses yet a third protein, called a co-stimulatory ligand.



Synthetic Immune Cells

Researchers have developed a variety of experimental treatments in recent years to boost the immune system's ability to identify and destroy malignant tumor cells. Among these therapies, delivery of synthetic immune cells, known as CAR T cells, has proved particularly effective for the treatment of advanced cases of leukemia and lymphoma. Built into each custom-designed CAR T cell are two powerful shortcuts, depicted here, to soup up the immune response.

CAR T Cell Therapy Is Streamlined

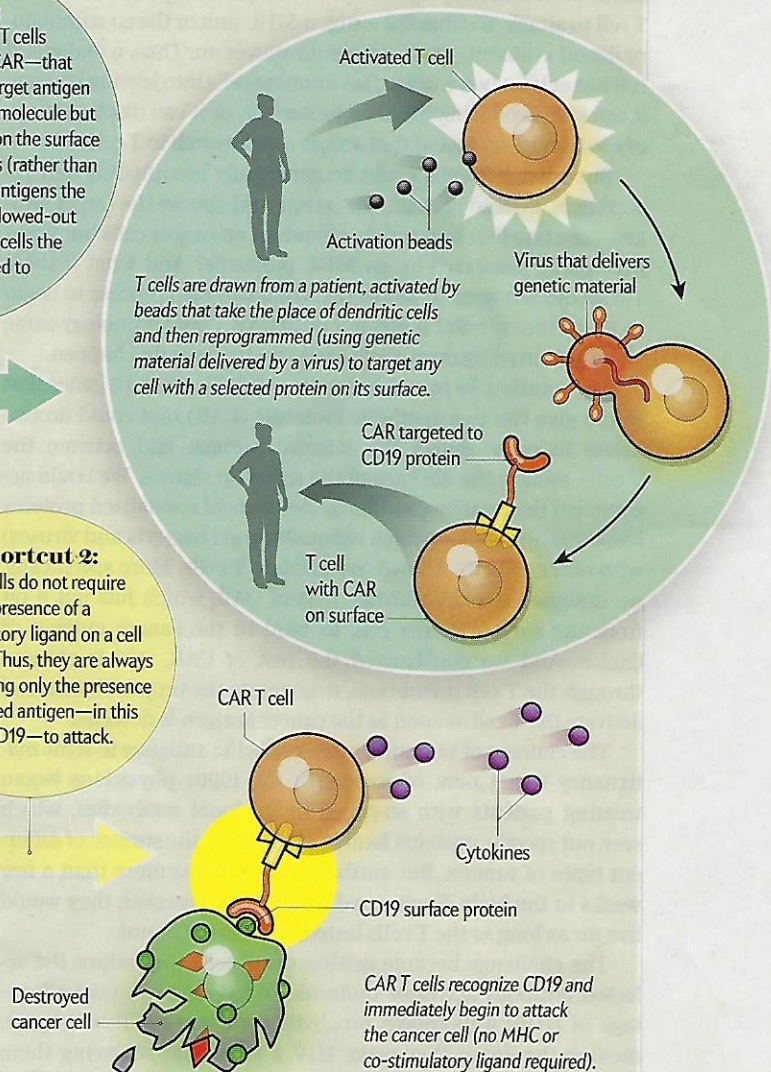
CAR (for chimeric antigen receptor) T cells are much more potent than anything the body could produce on its own. Whereas typical T cells normally call off their attack after a few weeks, investigators have genetically engineered CAR T cells so that they will remain active for months if not years against targets of the researchers' own choosing, such as a protein called CD19.

Shortcut 1:

Unlike most T cells, CAR T cells bear an antigen detector—CAR—that enables them to recognize a target antigen that is not attached to an MHC molecule but is rather simply sitting by itself on the surface of a cell. In addition, researchers (rather than dendritic cells) decide which antigens the synthetic T cells target. A hollowed-out virus is used to deliver to T cells the genetic material needed to make the CAR.

Shortcut 2:

CAR T cells do not require the presence of a co-stimulatory ligand on a cell to attack it. Thus, they are always "on," requiring only the presence of a selected antigen—in this case, CD19—to attack.



own cells. Because our immune system has evolved so as not to attack our tissue, it often has trouble distinguishing cancer cells from normal cells. The second challenge is that many cancer cells exploit various tricks to thwart an immune response. They have learned how to hide from the immune cells, as well as how to interfere with an effective immune response.

As part of the mechanism for protecting healthy tissue from “friendly fire,” a T cell inspects a cancer cell for the presence on its surface of two requisite molecules before it will attack. One consists of a large protein complex, known as an MHC molecule, that cradles a protein fragment, or antigen—the target “presented” to the T cells by dendritic cells. The second required molecule—a so-

Unlike regular T cells, CAR T cells attack a cancer cell immediately after detecting their target.

called co-stimulatory ligand—provides the on signal that tells the T cell to attack. If either the antigen-MHC unit or the co-stimulatory ligand is absent, the T cell simply moves on. Thus, a malignant cell has at least two ways to fool immune cells into leaving it alone: it can stop producing MHC on its surface, or it can display a form of co-stimulatory ligand that acts as an off switch to T cells.

But what if T cells could be genetically modified so that researchers, instead of dendritic cells, could choose the target antigen—say, one that is naturally abundant on cancer cells but is not necessarily presented by an MHC molecule? And what if these T cells did not need to follow the usual two-step process to begin to attack tumor cells? It was not until CAR T cell technology came along that investigators could easily try to make this happen.

The solution, in principle, was to outfit T cells with genes that would give rise to a synthetic molecule (CAR) that could do two things at once: detect the selected antigen and activate the T cell—even in the absence of the usual on signals. We could accomplish this goal by combining elements of specialized proteins known as antibodies (which normally target bacteria and viruses) with other proteins known to stimulate T cells. More specifically, we designed the antibodylike part of CAR, which juts out a bit from the surface of the cell, to bind to the cancer antigen of choice. And we constructed the rest of CAR, which plunges through the T cell membrane, to generate the proper signals and activate the T cell as soon as the cancer antigen is detected.

The concept of targeting cancer-specific antigens to fight malignancy is not new, of course. In the 1990s physicians began treating patients with so-called monoclonal antibodies, which seek out specific proteins found primarily on the surface of different types of tumors. But antibodies do not last more than a few weeks in the body. Engineered into T cells, however, they would live for as long as the T cells lasted, for years at a time.

The challenge became getting the T cells to produce the selected antibody-activator molecule. We decided to take advantage of HIV’s well-known proclivity for infecting T cells by removing the genes that make HIV a killer and replacing them with genes that contained the necessary information for building our antibody-activator chimera. We then allowed these now harmless HIV particles to infect the T cells that we had removed

from our patients. The altered viruses acted like a Trojan Horse to deliver the genes into the T cells; the cells took it from there, producing CAR and fitting it onto the cells’ surface. Using this and other techniques, several different groups of investigators, including our own, have refashioned T cells so that they can attack tumor cells after recognizing only a single protein on the cells’ surface. (No MHC or co-stimulatory ligand required.) Furthermore, this new custom-tailored T cell can be designed to go after exactly whatever antigen—or perhaps even combination of antigens—investigators choose.

In the mid-1990s and early 2000s, collaborating with others, we learned how to turn T cells drawn from HIV patients into CAR

T cells and tested these in human clinical trials. We continue to improve our technique and expect to have more advanced therapies for HIV in another few years.

CAR T cells were also beginning to be tested in patients with cancer by several groups. We sought to combine technologies—taking what we had learned about

activating T cells with microbeads, with the CAR technology to redesign and redirect T cells, and the harmless HIV as the Trojan Horse to deliver the CAR payload to T cells.

We soon discovered how powerful these CAR T cells could be.

TEST THE NEW DESIGN

NOW WE HAD THE RIGHT AMOUNT of firepower, and we were also pretty sure we had a fairly good target. The perfect homing beacon for our CAR T cells, of course, would be an antigen found only on tumor cells, but these antigens are very rare. Because all cancer cells arise from what were once normal cells, tumor cells and healthy cells mostly display the same antigens. Developing a CAR T cell against these shared antigens would inevitably destroy a lot of healthy tissue along with the tumor.

There are, however, noted exceptions to this quandary. Certain types of leukemia and lymphoma, for example, arise from a group of white blood cells called B cells. People can survive without B cells, which are the body’s normal source of antibodies, provided they receive the occasional infusion of manufactured antibodies. B cells—as well as any malignant cells that they might become—bear a surface protein known as CD19. We and others in the field thought CD19 could be an attractive target for CAR T cell therapy because it is not found on any other healthy tissue.

We tested the idea in mice. Then, in early 2010, we began a clinical trial of CAR T cells that targeted CD19. The initial three patients were adults with advanced cases of chronic lymphocytic leukemia (CLL) that was not responding to other treatments.

The first was William Ludwig, a retired corrections officer who had learned he was sick a decade earlier and was now carrying over five pounds of leukemic cells dispersed throughout his body. He received one billion of his own genetically modified CAR T cells in August 2010. Ten days later he developed a fever, low blood pressure and breathing difficulties—serious side effects that landed him in intensive care. We later learned that Ludwig’s symptoms occurred because his immune system had gone into triple overdrive in response to the high number of cytokines now coursing through his body—a reaction, known as cytokine release syndrome, that can kill if it gets out of hand.

Fortunately, Ludwig came through, and one month later his

doctors could find no evidence of leukemic B cells in his body. This outcome was so extraordinary and unexpected that clinicians performed a second biopsy, which confirmed the results. We then treated the two other patients, who also had extraordinary responses. More than six years later Ludwig and one of the other patients are still alive and free of leukemia. Further testing showed that the CAR T cells multiplied in the bloodstream and bone marrow, where blood cells are made; each CAR T cell that had been infused (or its daughter cells) in these three patients was ultimately responsible for killing between 1,000 and 93,000 tumor cells. When the CAR T cells were isolated from blood samples months later, they still retained the ability to kill leukemic cells bearing the CD19 molecule in the lab. In effect, these long-term sentinels had become a “living drug” that continued to patrol the body, hunting for any potential recurrence.

EXPAND THE REPERTOIRE

AS SIGNIFICANT AS OUR INITIAL RESULTS WERE, we were out of money and unable to try our experimental treatment on any more patients. Review panels at federal research agencies deemed the therapy too risky and thus not worth further funding. Nevertheless, we submitted two papers describing the first three patients that were quickly accepted and published simultaneously in August 2011 in the *New England Journal of Medicine* and *Science Translational Medicine*. Extensive media coverage followed, as did inquiries from biotechnology start-ups and companies that were interested in licensing the technology from the University of Pennsylvania, where we work.

Eventually one of our grant applications came through, which allowed another trial to begin in 2012, this time in children. Then we decided to form an alliance between the University of Pennsylvania and Novartis to finance development and the future submission of our results to the FDA for commercial approval. News of the partnership triggered a licensing and investment frenzy, with many medical centers around the world forming new biotechnology companies dedicated to producing new variations of CAR T cells. Our latest results in children show an overall survival rate after 12 months of 62 percent, compared with less than 10 percent after a year using standard treatments.

Over the past few years many groups—including Memorial Sloan Kettering Cancer Center, Seattle Children's Hospital, the Fred Hutchinson Cancer Research Center allied with Juno Therapeutics, the National Cancer Institute allied with Kite Pharma, and others—have reported astonishing responses in advanced cases of leukemia and lymphoma. At our center, we have treated 300 patients with CAR T cells targeting B cell malignancies. The response rates vary by disease: about half of our patients with advanced chronic lymphocytic leukemia show marked clinical improvement (based on the decrease in leukemic cells in their body, among other factors), whereas about 90 percent of children with acute lymphoblastic leukemia have shown a complete response—no evidence of cancer cells—one month after treatment.

No one really knows why CAR T cell therapy does not work for everyone with CD19 malignancies. Some relapses seem to occur because the infused CAR T cells did not multiply in the patient or because new leukemic cells evolved that did not produce the CD19 molecule and thus were unaffected by treatment. Even so, the magnitude of the response for these malignancies is unprecedented. Two companies are expected this year to ask the FDA to

approve CAR T cells for the treatment of cancer: Novartis, for pediatric acute lymphoid leukemia and later for lymphoma, and Kite for a type of lymphoma.

Many challenges remain. As a research community, we are still developing ways to manage and possibly to prevent the most severe side effects. Although fatalities among patients are generally rare, a number of people with acute lymphoblastic leukemia have died from treatment-related problems, which may stem in part from the fragile health of these patients, as well as from differences in the design of CAR T cells at different institutions.

We are now in the “Model T” stage of CAR T cell development. Making it more widely available to patients with B cell cancers and other tumors is a priority, and a number of recent scientific and technological advances will be tested in clinical trials over the next several years. To treat cancers other than B cell malignancies, investigators will probably need to identify and target certain combinations of antigens that are more commonly found on cancer cells than healthy tissue. One of us (Posey), for example, is trying to develop an immune-based treatment for breast and pancreatic cancer. These and other so-called solid tumors are even better at hiding from and suppressing the native immune system than leukemia and lymphoma, which are more accessible because they circulate in the blood. To smoke out such cells, Posey is designing a CAR T cell that will search for two targets instead of just one: the first is a certain sugar molecule that is found solely on the surface of cancer cells and that allows those cells to reproduce faster than normal cells do; the second is a protein found on both cancerous and healthy cells. In theory, this specific combination of sugar and protein targets should occur in abundance only on cancer cells, which should limit this particular CAR T cell's ability to harm normal tissues.

Progress is rarely linear, of course. Disappointments, failed hypotheses and setbacks are inevitable. But there is no doubt in our mind that the success we have already seen in advanced leukemias and lymphomas justifies future research into the development of yet more CAR T cells. ■

DISCLOSURE: Like many cancer researchers, the authors have some commercial ties to for-profit companies. Avery D. Posey, Jr., has intellectual property licensed to Novartis and to Tmunity Therapeutics, which develops anticancer therapies. Carl H. June and Bruce L. Levine receive royalties and laboratory funding from Novartis based on an intellectual-property licensing agreement and alliance with the University of Pennsylvania. Novartis and the University of Pennsylvania have applied for drug patents based on some of the work summarized in this article. June and Levine are co-founders of and have equity in Tmunity Therapeutics and also receive consulting fees from and advise several other companies involved in cell therapy and cancer research. These relationships are managed in accordance with University of Pennsylvania policy and oversight.

MORE TO EXPLORE

Fire with Fire. Video on CAR T therapies. Directed by Ross Kauffman. Red Light Films, 2013. www.youtube.com/watch?v=h6Sz1ZzfPd4

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FROM OUR ARCHIVES

Blocking HIV's Attack. Carl June and Bruce Levine; March 2012.

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