The Dizzying Journey To a New Cancer Arsenal

Carl June and others are on a quest to make T cell therapy a cancer treatment success. Getting there is filled with highs and lows and lots of uncertainty

ONE JANUARY AFTERNOON IN 2011, oncologists Carl June and David Porter settled themselves at a table at Gia Pronto, the coffee shop in the atrium of the Perelman Center for Advanced Medicine. The glass and steel building sits at the nerve center of the University of Pennsylvania's (Penn's) massive medical complex in West Philadelphia, a few blocks from the Schuylkill River that cuts the city in two. Outside, construction cranes rise up, a sign of Penn's ongoing expansion.

June and Porter had a problem. In an exhilarating 6 weeks in the summer of 2010, they had treated three men with leukemia who were out of options. In a cell therapy experiment, the patients' own T cells were geneti-

strategy had worked beyond the doctors' wildest expectations, melting away pounds of tumor in each patient. In one case, the modified cells didn't grow well in the lab, and the patient, a 64-year-old scientist at a biotechnology company named Douglas Olson, received a mouse-sized dose. Now, he'd taken up running as a hobby and was teaching his grandchildren how to sail.

But generating the cells for all three patients had cost $350,000. The scientists were out of money and out of "vector," the disabled HIV viruses that they were using to insert new genes into T cells. They had applied to the National Cancer Institute (NCI) and elsewhere for funding to continue

in Olson and one other patient. The third man responded partly but later died of his disease. Funders deemed the therapy too experimental and too impractical. Everywhere, Porter and June were turned down.

"It was one of these best of all times, worst of all times," says June, who had assembled his small team, including Porter, more than a decade ago. They weren't the first to test this radical new approach in people, but their results were the most striking. "We knew something worked," even if the remissions ended tomorrow, June says. "We knew it wasn't an accident."

Sipping coffee, Porter and June weighed their next step. They were itching to test the

CREDIT: ERIC MENCHER/PENN MEDICINE

Experimenting. A life of twists and turns has Carl June pressing forward with a radical cancer therapy.

didn't have. "We basically decided that we would just publish with three patients," June says. Getting the word out, he hoped, could shift the dynamic in their favor. Porter was game to try, but skeptical that any reputable journal would accept a paper with an n of 3.

He turned out to be wrong. *The New England Journal of Medicine* welcomed a report about Olson and his mouse dose of T cells. *Science Translational Medicine, Science*'s sister journal, snapped up a manuscript detailing all three patients. The papers were published simultaneously on 10 August 2011. The university put out a news release that day. Its title: "Genetically Modified 'Serial Killer' T Cells Obliterate Tumors in

Porter was en route to vacation in western Maryland with his family when the embargo lifted. His phone started ringing. "I was in the car for 8 hours that day," he says. "I spent 8 hours straight on my phone, answering e-mail, answering phone calls. It was a story that took us all by surprise. It kind of went viral." June fielded 5000 requests from patients and their families for the therapy. Eight hundred media outlets worldwide covered the story.

NCI reversed course and awarded June's team nearly $500,000 a year for 4 years, in part to create engineered T cells for patients. Pharmaceutical companies began courting June and his colleagues. Almost exactly a year after publication, Novartis signed a multimillion dollar agreement with Penn, licensing rights to the therapy with the goal of getting it approved by drug regulators. Three patients, two of them still in remission today, proved to be the tipping point that June had imagined.

Two years later, nearly all of the thousands of cancer patients desperate for engineered T cells are a long way from getting them. For one, the therapy can tackle only a subset of blood cancers, and it remains highly experimental. About three dozen people at Penn have received it, along with more than 50 elsewhere. Not everyone is helped, and many of those who are suffer serious side effects. In those whose disease has disappeared, no one knows yet how long the calm will last. "The medical literature is just littered with examples of drugs that look great on your first 10 patients, and they don't pan out for one reason or another," Porter says.

History may urge caution, but it's hard not to be swept up in the moment. Despite the small numbers, many oncologists believe that what June's team and others now replicating it have seen is unprecedented. No cell therapy has proliferated in the body, endured, and slain cancer quite like this one.

A looming question now is how to move engineered T cell therapy forward—how to test it in more patients, at more centers, in different forms of cancer. Drug companies "don't care if it costs $500 million to develop the first vial, as long as you can make the second vial for $1," says Steven Rosenberg, an NCI surgical oncologist in Bethesda, Mary-

Online

sciencemag.org

Podcast interview with author Jennifer Couzin-Frankel (http://scim.ag/pod_6140).

not how T cell treatment works. Every batch is a distinctive drug, and right now, every step toward making it holds the chance of human error.

As academic cancer researchers and companies work to expand the therapy's reach, June and his colleagues are in the public eye. Along with the accolades are critics charging that they've claimed more than their share of scientific credit and lawsuits alleging violations in agreements with collaborators. They are deeply driven to save lives; cancer looms large in June's own autobiography. But at stake, too, for the researchers and their institution, is money and scientific glory, and the chance to combat cancer with immunology on a grand scale.

T cells remodeled

The backbone of June's work was forged in the mid-1980s by an Israeli immunologist. Zelig Eshhar was on sabbatical in Palo Alto, California, when he began toying with an unorthodox question: whether T cells, the sentries of the immune system, could be coaxed to destroy different targets. To accomplish this, Eshhar knew that he needed T cells to recognize and latch onto molecules that they normally ignore. And the only way to make that happen was by inserting foreign DNA into T cells, to alter the receptors they produced.

Eshhar returned home to the Weizmann Institute of Science in Rehovot, Israel, and got to work. Failure after failure followed. The technology to insert DNA was rudimentary. Then, in the late 1980s, Eshhar triumphed, adding a combination of gene sequences

> "We knew something worked. … We knew it wasn't an accident."
>
> —CARL JUNE, ABRAMSON CANCER CENTER UNIVERSITY OF PENNSYLVANIA

into a type of immortalized T cell that more readily accepts foreign DNA and endowing the cells with new targets they could kill. "The moment we realized it was working … we became, I don't want to say obsessed, but really invested," he recalls.

Eshhar's feat was only the first step. To treat a disease like cancer, researchers

fied T cells would destroy healthy tissue, too. They also had to ensure that the cells multiplied inside the body and persisted, wiping out every trace of cancer and preventing it from coming back.

Slowly, a handful of researchers picked up on Eshhar's accomplishment and carried it forward. At Memorial Sloan-Kettering Cancer Center in New York City, cell therapist and oncologist Michel Sadelain set to work introducing genes into human T cells. "It took me 3, 4 years to better transfer genes into more than 0.5% of the culture," Sadelain says. "Today, we can take a high school kid [and] in an afternoon, they know how to take T cells and blast genes in all of them." Sadelain pushed for a name, and "chimeric antigen receptor" cells, or simply CAR cells, stuck.

While Sadelain focused on cancer from the start, June got there circuitously. His career trajectory tracked Cold War history, and the reason for that was Vietnam. In 1971, when he was 18, a lottery gave June a near-certain chance of being drafted. He abandoned plans to enroll at Stanford University and applied to the U.S. Naval Academy in Annapolis, Maryland. The war ended 2 years later, but June remained with the military, which financed his medical education. With fears of nuclear attacks running high, he trained as an oncologist and a bone marrow transplanter to treat those exposed to high doses of radiation. In 1989, the Berlin Wall collapsed. The Cold War ended soon after. The military "didn't care about bone marrow transplants after that," June says. He needed a new passion.

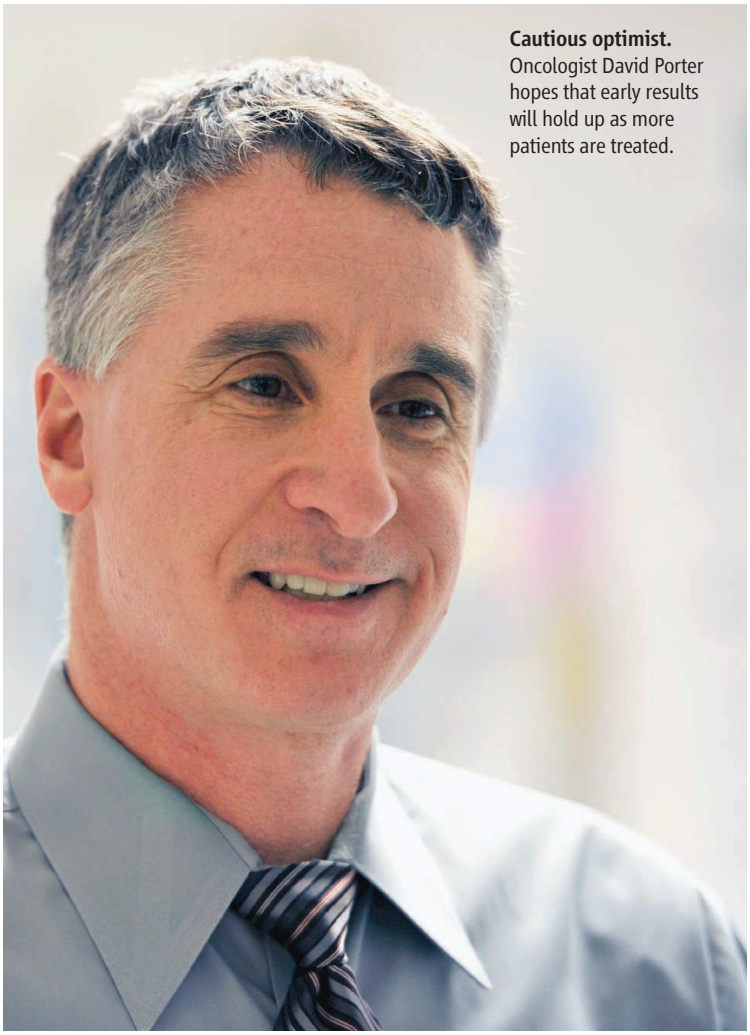
The Navy didn't fund cancer research, so June, then at the Naval Medical Research Institute in Bethesda, turned to HIV. The decision proved prescient, as he learned the ins and outs of T cells and the immune system, knowledge that would later serve him well. He spent a decade training T cells to flourish in HIV patients, whose own T cells are destroyed by the virus. An immunologist in the lab,

turning them on outside the body to help them destroy their targets.

Then in 1995, June's personal and professional lives abruptly converged. His wife Cynthia was diagnosed with ovarian cancer. The couple had a 3-year-old daughter and two teenage sons. "I saw for the first time what it was like to be on the other side of the bed," he says. June was a believer in manipulating the immune system to treat cancer, but suitable immunotherapies weren't ripe at the time.

Cynthia June was 46 when she died in 2001, shortly after her husband left the Navy and the family relocated to Philadelphia. Their daughter was 9 years old. "It took a long time to recover," he says, speaking slowly as he thinks back on those years. "A lot of people helped me out."

The ripples hit those around June, too. "We knew Cindy, we had socialized with her," Levine says. "We saw what happened and what it did to Carl. Those are hugely



Cautious optimist. Oncologist David Porter hopes that early results will hold up as more patients are treated.

© PEGGY PETERSON PHOTOGRAPHY/PENN MEDICINE

Advances and acrimony

At Penn, June continued his HIV work but also threw himself into cancer, motivated by his wife's death and by a belief that the pieces were falling into place to successfully treat patients with CAR cells at last. He was enthusiastically welcomed by two oncologists: Porter, who cares for adults with blood cancer at Penn's Abramson Cancer Center, and Stephan Grupp with the Children's Hospital of Philadelphia (CHOP), who showed up at June's office door one day and asked to collaborate.

A handful of researchers elsewhere were also in the race to bring CAR therapy to people. In addition to June, they included Sadelain at Sloan-Kettering, Rosenberg at NCI, and Malcolm Brenner at Baylor College of Medicine in Houston, Texas. All were converging on the same cancer target, a marker called CD19. The only cells sporting CD19 are B cells, which proliferate dangerously in B cell leukemias. This was valuable for two reasons. The marker was a promising bull's-eye, because it was all but universal on these cancer cells. And although B cells are an important component of the immune system, they are not needed for survival—which was reassuring, because attacking CD19 would surely destroy healthy B cells, too.

How to design the very best CAR against CD19 was the big question. CARs "come in multiple flavors," Sadelain says. There are different ways to engineer a new receptor that will latch on to CD19. One important ingredient is the "co-stimulatory signal," which is embedded in the CAR cells to activate them and keep them alive in a patient. Sadelain's group, like the others, studied a slew of possibilities in mice and settled on one, called CD28, which looked the most promising. Rosenberg and groups at two other centers picked CD28 as well. All four had clinical trials up and running when June's trial opened.

June chose a different co-stimulatory signal, called 4-1BB, in part to distinguish

was a strong candidate in mice but not quite as impressive as CD28. A group at St. Jude Children's Research Hospital in Memphis, Tennessee, led by an oncologist named Dario Campana, had designed the first CAR construct with 4-1BB. Unlike the other groups, June's also used a disabled HIV virus to genetically engineer the T cells and a different recipe for growing them in the lab.

As it turned out, combatting cancer was in the details. The first to publish an anti-CD19 CAR therapy success was Rosenberg's team in 2010. They used the CD28 strategy, and one patient with a form of lymphoma achieved a long-lasting partial remission. But it was Penn's results in the three men with leukemia, with a 4-1BB CAR, that transfixed the cancer community and the wider world.

"It made a believer out of a lot of people who were pretty skeptical," says Ravi Bhatia, who treats blood cancers at City of Hope in Duarte, California, and counts himself among past doubters. His own hospital had been studying CAR cell therapy for several years, but there and elsewhere the transplanted T cells had quickly disappeared from the bloodstream. "That," Bhatia says, "was a big concern."

June, Porter, Levine, and Grupp—who was gearing up to treat the first children—sought to stay anchored amid the hype. "You try and keep your feet on the ground and say, 'We still have work to do,' " Levine says. The competition was fierce and not always friendly. In the pages of *The New England Journal of Medicine*, Rosenberg's group and June's sparred over whether Rosenberg's CAR therapy success, published 12 months before June's, was due to engineered T cells or attributable to chemotherapy that the patient received first, to make room for new cells. "There's acrimony out there," Sadelain says.

> "You try and keep your feet on the ground and say, 'We still have work to do.' "
>
> —BRUCE LEVINE, UNIVERSITY OF PENNSYLVANIA

The most bitter came in July 2012. St. Jude sued the trustees of the University of Pennsylvania for breaching materials transfer agreements signed with St. Jude in 2003 and 2007, when Campana had shared his CAR materials with June.

Penn shot back with a lawsuit of its own, arguing that June's CAR cell construct was different than Campana's. Less than 3 weeks after that suit, in August 2012, Novartis and Penn unveiled an alliance to commercialize the T cell treatment. The company said that it

In the months that followed, the legal dueling continued. Then in March of this year came a turning point: St. Jude's application for a patent on Campana's T cell construct, with its 4-1BB signaling domain, was approved.

Three days later, Penn sued St. Jude again, claiming that the Campana patent was invalid. That lawsuit exposed an undercurrent of concern over who owned what. Penn's lawyers are seeking "a judicial determination" that they are not infringing on the St. Jude patent.

> "The medical literature is just littered with examples of drugs that look great on your first 10 patients, and they don't pan out."
>
> —DAVID PORTER, ABRAMSON CANCER CENTER UNIVERSITY OF PENNSYLVANIA

Neither June nor Campana, who is now at the National University of Singapore, would comment on the lawsuits. Novartis spokesman Scott Young wouldn't say much either—the company is not a party to any of the three suits—but he stressed in an e-mail message that "we have complete confidence in the viability of our collaboration with UPenn."

Jumping the hurdles

That collaboration is now moving swiftly ahead. At Novartis, dozens of people are strategizing over how to manufacture personalized T cells for patients. Novartis needs to determine how long the cells can hold up outside the body, because that determines how many costly cell-processing facilities the company must open worldwide. It has to automate its method of growing and manipulating the cells as much as possible to reduce costs and the chance of human error. It has to consider whether the time from "vein to vein," when the cells are removed until they're put back in, can be shortened. It now stands at about 3 weeks.

"All of this has to be thought through very carefully, not only for the U.S. but also on a global scale," says Manuel Litchman, who is overseeing the therapy's development program for Novartis Oncology. In the cramped lab at Penn, Levine is busy training Novartis employees. Company officials

an immunotherapy manufacturing facility in Morris Plains, New Jersey, which had been owned by a company, Dendreon, making a prostate cancer vaccine. "It's not going to look that different in Morris Plains than it looks in Bruce's lab," Litchman says. "It's just going to be replicated many, many times over, to fill up the suites there."

One top priority is consistency. Every batch of T cells will be different because each originates with a different patient. But other scientific and manufacturing variables—the vector that inserts the foreign DNA, techniques to grow the cells, how they're transported—can make the outcome unpredictable.

June's group learned this the hard way: After the fanfare around their first three patients, they treated three more in January 2012 with a new vector lot. None responded. "I was just stumped out to the max," June says. He had no idea what had happened and still can't say whether something went awry with the vector material or whether the outcome was due to random fluctuations in the therapy's success. "All we knew was, it worked three times, and then it didn't work three times." All three of those patients later died of their disease.

Next in line was patient 7, who turned out to be another roller coaster. She was Emily Whitehead, a 6-year-old with end-stage leukemia whose parents turned to June's cell therapy as a last-ditch hope. The experimental treatment sent her body into a deadly immune overdrive. She spent 2 weeks on a ventilator in the CHOP intensive care unit while doctors tried everything they could think of to save her.

"We thought it was over," June says. He drafted an e-mail message to Penn's provost: "It is with regret that I inform you that our first pediatric patient on the CART19 trial will likely die," he wrote. "There is nothing to do at this point other than hope for a miracle." June pledged to "conduct a full investigation." It turned out that he didn't need to, and the e-mail was left unsent.

As doctors parsed Emily's lab results, they found that her revved up T cells were causing overproduction of a molecule called interleukin-6. She was saved, in a tale that

Survivor. Eight-year-old Emily Whitehead was the first child on the experimental Penn protocol, and she's now cancer-free 1 year later.

because his daughter Sarah had been diagnosed with rheumatoid arthritis shortly after her mother's death. Grupp happened upon it independently, when a colleague found it by Googling on his iPhone.

Emily remains in remission more than 1 year later, her hair long enough now for pigtails and her 8th birthday behind her. In her DNA, Grupp discovered a gene mutation that predisposes to a hyperactive immune response, which could help explain why the therapy sickened her as it did. Grupp has since switched to giving other children a tenth of the T cell dose that Emily received, although "in my heart of hearts I'm not sure the dose matters that much," because the cells multiply with abandon inside the body. All those on the Penn trial became deeply attached to Emily after her harrowing experience. Levine displays pictures of her in his office. June, who remarried and now has a 10-year-old daughter of his own, chokes up when he speaks of Emily and her family.

For the Penn team, Emily and the other patients are teaching laboratories, showing what the engineered T cells can do. "I've never been involved in anything like this in

ries from patients and families, Grupp was taken aback by parents reporting their child's progress on Facebook before he'd shared the news with the wider scientific world. "I am in a position of having my results publicly disclosed without having them subject to peer review," he says. "That's the aspect of this I was least prepared for," and it's one that makes him "extremely uncomfortable."

> "When I'm doing informed consent with these families, the first thing I say is, 'Forget everything you've read about this.' Nothing could possibly be as promising as the various articles about this make it seem."
>
> —STEPHAN GRUPP, CHILDREN'S HOSPITAL OF PHILADELPHIA

Grupp has treated 14 children with acute lymphoblastic leukemia so far. Of the five reported at scientific meetings or published, four went into remission but one of those later relapsed. Porter's most recent data on adults, presented at a meeting in May, includes 10

For every T cell infused, between 1000 and 93,000 leukemia cells die, showing just how dramatically the engineered T cells are multiplying inside the body. The group is still studying why their T cells proliferate like this, although they suspect that it's partly due to the 4-1BB construct that Campana pioneered. As expected, healthy B cells are destroyed, and the long-term effects of that remain uncertain. The expense of CAR treatment has plunged, but it still costs $20,000 to $40,000 to generate the cells. That doesn't include supportive care in the hospital after patients receive them.

In March, Sadelain reported on five patients with acute leukemia in *Science Translational Medicine*. That disease is more aggressive than chronic leukemia in adults, and oncologists were heartened by what they read: Four of the patients went into remission, a necessary precursor to getting a bone marrow transplant, which they then received. Three are still alive at least 5 months after treatment. "That it was verified at another center, at Memorial, was very important," says Bhatia at the City of Hope. It was "not just something strange that happened" in the people treated at Penn.

Still, physicians like Porter and Grupp are mindful that this isn't life-changing for everyone. "When I'm doing informed consent with these families, the first thing I say is, 'Forget everything you've read about this,' " Grupp says. "Nothing could possibly be as promising as the various articles about this make it seem." Only four people, including Emily, have been followed for more than a year. A looming question is whether CAR therapy can work in solid tumors, and June and others are opening clinical trials to try and find out.

Nearly 3 years after the summer that changed everything, the Penn group is still working flat out to keep up: enrolling as many patients on the trials as they can, working with drug regulators to discuss how best to study the cells with an eye toward approval, collaborating with Novartis to train their employees and streamline the cell-generating process. "I'm tired," says Porter, and he sounds it. June, a serious bike racer and runner, has scaled back his hobby, though he did manage to fit in a 34-mile ultramarathon last weekend. "I didn't used to work as many hours as I do" now, he says. "I mean, I used to work, but I'd take more time off." He's eagerly waiting for the handoff, the day when Novartis starts processing T cells and making CARs. Neither June nor Novartis can say when that will be, but for June, it will mark a return to normalcy.