HIDE AND SEEK

PIONEERING IMMUNOTHERAPY TO FIND AND KILL ELUSIVE CANCER CELLS IN CHILDREN

Pediatric cancer specialist Kara Davis was nervous. It was a spring morning in April and she was headed into the hospital to see 11-year-old Salvador De Leon. Sal had leukemia, and he wasn't doing well. After three grueling years of therapy, his most recent relapse left only one course of action: an experimental treatment to seek out and destroy the cancer cells that had eluded conventional cancer treatments. Davis knew that this approach could either cure him or kill him. The treatment, known as CAR-T cell therapy, relies on the use of a patients' own genetically modified immune cells to track down and attack the leukemia cells. Although some children with leukemia like Sal's have experienced stunning, years-long remissions after the therapy, about 30 percent of CAR-T cell recipients experience a temporary but potentially deadly side effect known as cytokine release syndrome.

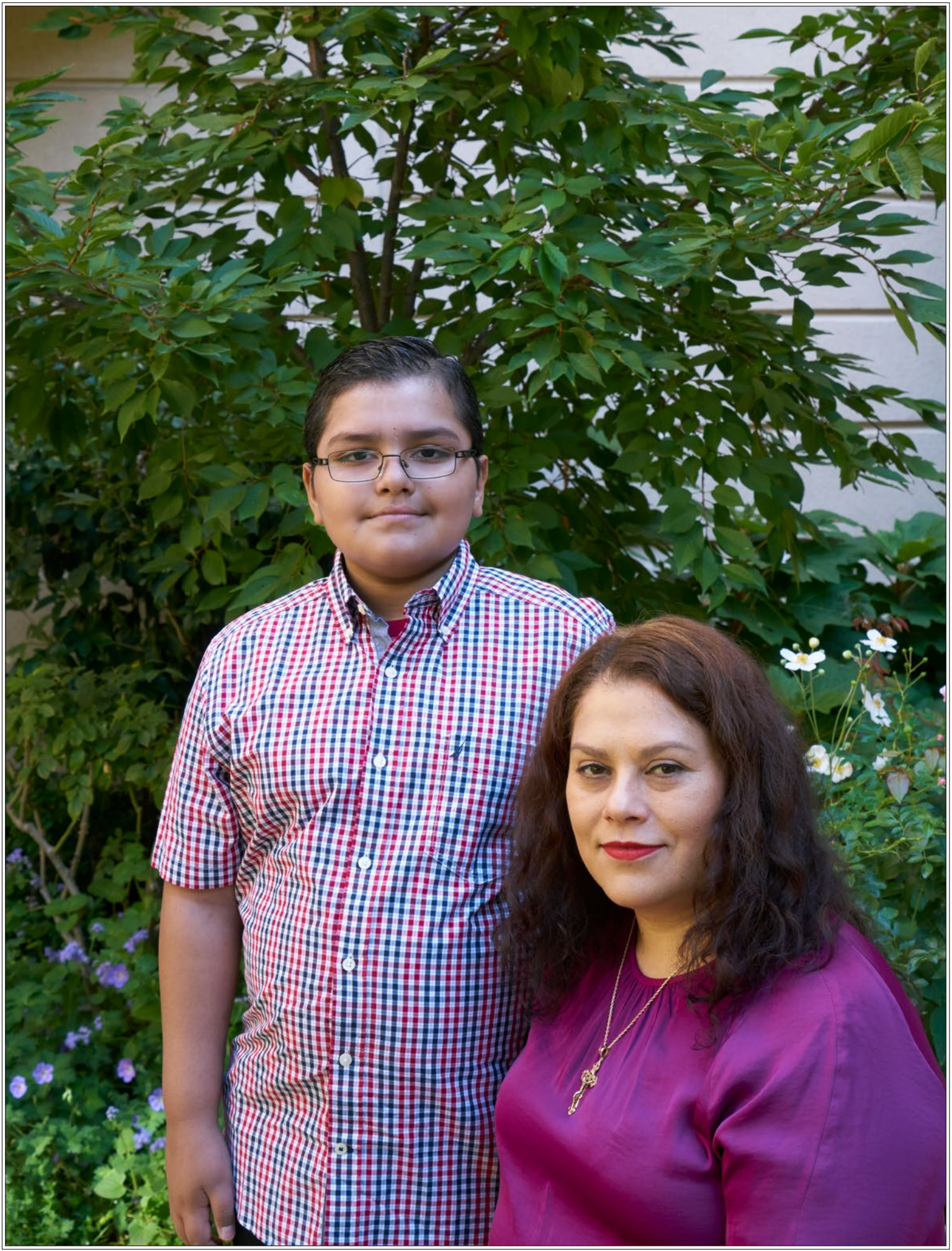
Davis, OD, an assistant professor of pediatrics at the Stanford School of Medicine, was concerned because Sal had reacted poorly to previous rounds of chemotherapy. Did this mean he was likely to struggle with the CAR-T therapy as well?

"I was very worried," Davis recalls. "But there really weren't any other good options for Sal."

BY KRISTA CONGER

PHOTOGRAPHY BY LESLIE WILLIAMSON

Salvador De Leon and his mother, Maria De La Cruz



When she broached the subject of the new treatment with Sal's family, his mother, Maria De La Cruz, didn't hesitate. "If it has any chance of saving his life, we will do it," she recalls saying. "We will do whatever it takes."

CAR-T cell therapy is a new form of what's known as cancer immunotherapy, and it's been uncommonly successful. So successful, in fact, that in August the Food and Drug Administration fast-tracked its approval of a CAR-T cell treatment for children like Sal with relapsed or unresponsive acute lymphoblastic leukemia. Marketed by Novartis, it's the first cell-based gene therapy approved by the FDA for use in humans.

It's also big money. In the same week of the FDA approval, the pharmaceutical giant Gilead Sciences purchased Kite Pharma for nearly $12 billion to gain control of its version of the CAR-T cell therapy. It seems the move paid off. In October, the FDA approved the Kite-developed therapy for treatment of adults with certain types of lymphoma.

Currently the CAR-T cell therapy must be custom-made for each patient, and is estimated to cost about $475,000 per child. But the results have been astounding. Some desperately ill children have been seemingly cured of their cancer with just one treatment.

"This is without a doubt a watershed moment in the history of cancer therapy," says Stanford's Crystal Mackall, MD, a cancer immunotherapy expert and former head of the National Cancer Institute's pediatric oncology branch.

But the treatment isn't perfect. It kills healthy B cells as well as their cancerous peers, which compromises a patient's immune system. It's unknown exactly how long the genetically engineered cells stay in the body — or even how long they should stay. It's unbelievably expensive (one watchdog group claims it's on track to be the most expensive drug ever marketed). And it so far has been relatively ineffective against solid tumors.

Now researchers at Stanford, including Mackall, Davis and their colleagues, are investigating ways to make CAR-T cell therapy faster, cheaper, safer and more broadly applicable to other types of cancers. They're experimenting with combination therapies that target more than one molecule on the leukemia cells. They're also looking for new targets on cells in solid tumors, and brainstorming ways to reduce the cost. And, of course, they're closely following the progress of the kids like Sal in ongoing clinical trials at Stanford.

Although they are quick to point out the potential caveats of the CAR-T cell treatment, it's hard not to be moved by the excitement in their voices. "Prior to CAR-T cell therapy, you would not even use that word, 'cure,'" says Davis. "Instead I'd suggest other treatment options that might give the family a bit more time together."

Ronald Levy, MD, a pioneer in the field, concurs.

"I've been working in the field of cancer immunotherapy for 40 years, and there's never been a more exciting time," says Levy, who is the Robert K. and Helen K. Summy Professor at Stanford. "Some of the responses we're seeing with this treatment are nothing short of miraculous. The world of cancer immunotherapy has changed forever."

On that April morning, Davis, a mother of two children about Sal's age, was desperately hoping to change Sal's world. As his modified T cells were infused through an IV in his arm, Sal's care team monitored him for any negative reaction.

"But he just breezed through," says Davis. "He did so well, in fact, that I began to worry about the other possibility: that maybe the cells just weren't working. So we all just held our breath for the next month."

SAL'S JOURNEY BEGAN IN THE SPRING OF 2014, WHEN HE WAS 8 YEARS OLD. The inveterate Oakland A's fan and video game lover had been struggling with what seemed to be allergies and was having trouble sleeping. Eventually, De La Cruz began to suspect there was something more seriously wrong.

"He was really tired, so I decided to take him to see the doctor," she recalls. "The next thing I knew, the doctor was asking me if I knew what leukemia was."

About 10,000 children age 14 and younger are diagnosed with cancer each year in the United States; acute lymphoblastic leukemia, or ALL, accounts for about a third of the total. Fortunately, it is one of the most treatable pediatric cancers. Ninety percent or more of children with the disease respond well to chemotherapy and quickly achieve remission. Many are cured completely. But the situation is much more dire for those who either don't respond to treatment, or whose cancer recurs. About 30 to 50 percent of these children die within five years. These statistics, coupled with the prevalence of the disease, place ALL on the top of the heap of deadly cancers in children even though most patients are cured.

Unfortunately, Sal's cancer cells harbored a dangerous swap between the DNA at the tip of chromosome 22 and the tip of chromosome 9, creating a hybrid known as a Philadelphia chromosome. The swap married portions of two important genes — leaving one, an important regulator of cellular growth, permanently stuck in the "on" position. Relatively rare in children with ALL, the presence of the Philadelphia chromosome leaves patients less able to achieve remission with standard chemotherapy and subject to quick relapse if

remission is achieved. Five-year survival rates of these relapsed patients are only about 10 percent.

"This situation is very difficult to treat," says pediatric oncologist Catherine Aftandilian, MD. "For these kids to have their best shot, we have to give very intense chemotherapy. Nearly all these patients end up in the intensive care unit as a result of the treatment."

Genetic missteps like the Philadelphia chromosome are one reason children's tumors tend to be better than adults' at hiding out in normal tissue, escaping the hordes of immune cells that patrol our bodies looking for trouble. That's because kids' cancer cells have had less time to accumulate the many genetic mutations that build up over the course of a lifetime of cigarette puffs or regular exposure to ultraviolet rays. Each of those changes stands a chance of creating a new target upon which the immune system can pounce.

Instead, cancer cells in children often arise as a result of one or two powerful mutations. These alone are sufficient to send a cell spinning off the normal developmental track and into out-of-control cell division. But these lone-wolf mutations don't always create the types of red flags our immune system is looking for.

"In many ways childhood cancers are the most elemental forms of cancer," says Mackall, who is a professor of pediatrics and of medicine, as well as associate director of the Stanford Cancer Institute and director of the Stanford Center for Cancer Cell Therapy. She also leads the Stanford-based center of the Parker Institute for Cancer Immunotherapy.

"A child's cells, which have tons of development and expansion potential, can go from being healthy to full-bore cancer seemingly overnight. And these cancers tend to grow quickly and aggressively. But, because these cancer cells are genetically more similar in terms of mutations to normal developing tissue than adult cancer cells are, it is harder for the immune system to recognize them as dangerous."

As a result, even some very promising immunotherapies in adults have been relatively unsuccessful in children. It's no good trying to amp up a nonexistent immune response, for example. Instead it has been necessary to craft a whole new approach.

IRONICALLY, THE ROOTS OF CANCER IMMUNOTHERAPY ARE AS OLD AS THE PYRAMIDS. The ancient Egyptians recognized a relationship between bacterial infection and cancer, and even deliberately cultivated infections in tumors in the hopes of causing regression of the mass. Throughout the centuries, doctors have attempted to fight fire with fire, balancing the risk of deadly infection with the near-certain death from cancer.

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William Coley, MD, a physician at New York City's Hospital for Special Surgery in the late 1800s, was one of them. After losing a patient to what was probably a rapidly spreading sarcoma, and noting that others battling an unrelated infection survived, he devised a concoction of bacteria he hoped would provoke a cancer-fighting immune response. He began marketing the injectable treatment as "Coley's toxins" in 1899, and these toxins continued to be used intermittently through the mid-1900s as a modestly successful treatment for some types of cancers.

Over the next decades, though, the concept of immunotherapy began to fall out of favor as radiation and chemotherapy became more prevalent. And a growing understanding of the immune system and its need to distinguish "self" vs. "non-self" during development made it seem unlikely that the body would have the wherewithal to kill off tumors arising from its own tissue. A few researchers, however, continued to argue for the concept of "immune surveillance," which suggested that immune cells patrolled the body to identify and eliminate potentially cancerous cells by recognizing abnormal proteins or targets on the cells' surfaces. These researchers explored ways to enhance this immune response in the clinic, at first by administering signaling molecules called cytokines to stimulate the proliferation of immune cells called T cells, and later by trying to genetically modify T cells to attack cancers.

A dramatic discovery by Stanford's Levy in 1976 hinted at the possible payoff of harnessing the immune system in this way. Levy was studying a type of blood cell cancer called B cell lymphoma. B cells are white blood cells — cells of the immune system — that make antibodies to bind to invaders like bacteria or other pathogens. A protein complex called the B cell receptor is randomly generated from short protein segments every time a new B cell is born. That makes the B cell receptor on the cancerous cells a potent, cancer-specific target for the immune system. On Thanksgiving Day, 1976, Levy showed that it's possible to create large quantities of antibodies to recognize and tag cancerous B cells for destruction.

The method worked, and some patients treated with the antibodies were cured. However, generating a unique batch of antibodies for each lymphoma patient proved too cumbersome. The scientists discovered that a less-specific target found only on B cells also worked well without requiring analysis of each patient's tumor. In 1997, the resulting drug, Rituxan, became the first FDA-approved monoclonal antibody for cancer treatment. Currently, several hundred thousand people each year receive the drug.

Antibodies alone have drawbacks, however. Although they float freely through the body, they serve primarily as red flags to trigger other cells of the immune system to kill their target. In contrast, T cells are efficient killing machines. But T cells are finicky. They only recognize proteins that are displayed in a particular way on a cell's surface. This safeguard keeps them from killing indiscriminately.

In 1989, Israeli scientist Zelig Eshhar, PhD, hit upon the idea of engineering a T cell with an antibody on its surface — a kind of a T cell-B cell hybrid that would combine the precision targeting of an antibody with the raw killing power of an activated T cell. Researchers at the University of Pennsylvania, Memorial Sloan Kettering Cancer Center, St. Jude Children's Research Hospital and the National Cancer Institute spent the next two decades optimizing the approach, which they termed chimeric antigen receptor T cells, or CAR-T, for use in humans. By 2010, the first case reports were trickling out: A lymphoma patient saw improvement; two of three people with leukemia went into remission.

"This is entirely unique," says Mackall. "It's something we cooked up in the lab. We've taken a powerful cell, and tricked it to go after a tumor by recognizing something it would normally ignore. And it turns out it works very well."

In some ways, Sal was extremely lucky. Stanford's early participation in the CAR-T trial was not a given. Shortly before his diagnosis in 2014, Davis, who was working primarily in the lab at that time, had a casual conversation with a Novartis liaison about the possibility of obtaining an experimental drug for her research into the biological causes of leukemias like ALL. She was hoping to identify markers on the surface of the cells that could be used to track the disease's origin and progression — perhaps helping to identify those patients most likely to relapse.

"She said 'Hey, you might be interested in this clinical trial we're running,'" says Davis. It was the CAR-T trial targeting a protein on the surface of B cells called CD19, and Stanford became one of only five participating sites in the country.

As the administrators and physicians plowed through the months of paperwork necessary to enroll patients in the trial, however, Sal became very ill. In addition to the effects of the chemotherapy, he battled multiple infections that kept him in the ICU for over a month in November and December of 2014.

"We didn't know if he would make it through that period," says Aftandilian.

Sal was eventually discharged and seemed to be doing better. But the leukemia wasn't totally eradicated. Lumbar punctures revealed the presence of a few rogue leukemia cells in his spinal fluid, and by April 2016 he had officially relapsed. A stem cell transplant was his next best option. Patients undergoing transplants receive high doses of chemotherapy to obliterate their cancer cells, but in doing so their own immune system is also destroyed. It's then replaced with the blood- and immune-forming stem cells from a healthy donor.

Immunotherapy experts Kara Davis (left) and Crystal Mackall

Sal had his transplant in June of 2016. But even that was unsuccessful.

"I remember so clearly getting the phone call about six months later telling me that Sal had relapsed," says Af-

tandilian. "I was with another patient at the time, but I hurried downstairs as quickly as I could. His family was devastated, as were we."

CAR-T CELL THERAPY IS TIME-CONSUMING AND EXPENSIVE — the price tag of hundreds of thousands of dollars per patient limits access. Because Sal was participating in a clinical trial, there was no charge to his family. But researchers increasingly worry about how institutions, insurance companies and families will bear the cost of removing, genetically engineering and growing each patient's T cells in the laboratory — particularly now that the FDA has approved the CD19-targeted CAR-T cells for clinical use.

Recently some companies suggested a middle ground: Families would be charged for the treatment only if it works. And, they point out, if successful, each patient may need only one treatment of the modified cells, with minimal additional medications to support the immune system.

"I think we'll see a rapid evolution in the cost of the technology," says Mackall. "Did we ever imagine that we would one day have cellphones that can do what they do for the amount we pay now? This is a new field, and it's only going to get more affordable."

Time and access are other sticking points. As demand for the treatment increases, it is becoming more difficult for existing, approved cell-manufacturing facilities to keep up. Currently it takes about two to four weeks from the time a patient's cells arrive at the facility until the genetically engineered T cells are ready for infusion back into the patient — time in which physicians must battle to keep their acutely ill patients alive. In Sal's case, this turnaround time, coupled with a technical glitch, nearly killed him.

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"We collected his cells at the end of January," says Davis, "and we kept him on a low dose of chemotherapy to keep his cancer at bay. But when his cells arrived at the manufacturing facility in New Jersey, they had been thawed. The container had somehow been damaged during shipping."

"I was furious," says Aftandilian. "I wanted to go out onto the tarmac and yell at the baggage handlers responsible for transporting the cells. They could have easily cost Sal his life."

At the end of February, the team tried again; in early April the modified cells were infused and Sal's physicians were nervously watching him for any signs of … well, anything.

"We were all so worried," says Aftandilian. "I kept waiting every day for him to get a fever and he just didn't." After a month, Sal had another check of his bone marrow to search for the presence of any leukemia cells, and his care team finally let out their collective breath.

The cancer cells were gone.

"It was truly amazing," says Davis. "Maria had tears in her eyes when we told her." It's been seven months, and Sal is back at school. "Right now he is cancer-free," says De La Cruz. "I look at him and he seems just fine."

IT'S TOO SOON TO TELL FOR SURE WHETHER SAL HAS BEEN CURED of his cancer. But other children have remained in remission for years. And researchers are working to improve cancer immunotherapy options for children and adults with all types of cancer. For example, Mackall is supervising a clinical trial in which the CD19-targeted CAR-T cells are combined with another type of CAR-T cell trained to seek out and attack another B cell marker called CD22. And Levy is investigating ways to combine the CAR-T cells with other immunotherapy approaches that block naturally occurring immune system checkpoints that prevent the immune system from tackling the cancer.

"The future is going to be in combinations of therapies that work together," says Levy. "Right now, CAR-T therapy is a salvage therapy. It's just a slice of the cancer immunotherapy pie, but it's a big pie." Levy envisions the possibility of genetically engineering the T cells within a patient's body, eliminating the need to manipulate them in the laboratory and making the treatment faster, safer and cheaper because it would no longer have to be customized for each patient.

Meanwhile Davis and immunology graduate student Zinaida Good are looking for markers of B cell leukemia cells that correlate with the likelihood of a patient's relapse after initially successful treatment. Recognizing patients likely to relapse could allow them to skip the grueling treatments and try immunotherapy sooner. "When I tell people that I treat kids with cancer, they often say, 'How can you do that, it must be the saddest job in the world,'" says Davis. "But it's not that to me at all. It's a very hopeful job, particularly now." **SM**

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