Tinkering With T Cells

How researchers are tweaking engineered immune cells' cancer-targeting receptors—and why it matters

June 11, 2018 By Susan Keown

"There's 40 million T cells in this little thing," graduate student Alex Salter said as he held up a tightly sealed, plastic flask about the size and shape of a pocket paperback. The liquid inside sloshed gently as he stepped aside to let one of his colleagues in the busy lab at Fred Hutchinson Cancer Research Center go by. Chock-full of nutrients, the orange Kool-Aid-colored growth medium in the flask was clouded by the hordes of human cells that had been happily multiplying there for days.

These cells were Salter's babies. He had genetically engineered their forebears to carry different versions of a cancer-targeting molecular weapon, and he was readying to put his burgeoning T-cell army to the test in dishes full of breast cancer cells. He wanted to know: Which ones of these kill cancer the best, and why?

A type of immune cell, T cells are at the heart of a class of emerging therapies that seek to aim the power of the immune system at cancer. Two approved therapies for certain advanced blood cancers involve genetically reprogramming patients' T cells to target their tumors, and many more such strategies are in the pipeline, including at Fred Hutch. These therapies and others still in development have shown promise in eradicating even treatment-resistant cancers that have penetrated throughout patients' bodies.

But today's T-cell therapies are like the Wright Flyer was to aviation. While many problems have been solved to get to this point, there are so many innovations yet to come. And that's why T-cell tinkerers like Salter and his colleagues are hard at work.

The molecular weapons Salter is working with are artificial receptors, called chimeric antigen receptors, or CARs. Embedded in T cells, CARs and their natural cousins, T-cell receptors, or TCRs, are the tools that allow T cells to recognize cancer and trigger an immune attack.

T cells are only about as wide as a strand of spider silk. So it can be hard to fathom the teensy scale of TCRs and CARs, the finely constructed molecular machines that stud their surface. Salter is a member of the lab of Stanley Riddell, MD, at Fred Hutch, whose research group is one of many now learning how tiny variations in the complex structures of these receptors can have a big impact on patients: namely, whether an engineered T-cell product eliminates a cancer and,

crucially, how durably and safely it does so.

“Some of the design problems have been able to be solved,” Riddell said, citing a variety of improvements that scientists have made to the cancer-recognizing parts of CARs. But, he added: “There’s still many, many questions.”

Scientists around the world are working to save more lives by tinkering with T cells and their precision-engineered, cancer-fighting receptors. Let’s take a look under the hood of some of this work at Fred Hutch.

What are TCRs and CARs and how can they be harnessed in cancer therapy?

First, some basic biology: T cells are naturally armed with T-cell receptors. These TCRs are suites of molecules trained to ever-so-precisely recognize telltale markers, called antigens, that mark other cells as foreign or diseased. When a TCR recognizes its antigen partner, it sets off a tightly controlled series of signals inside the T cell, activating an immune response aimed at precisely eliminating a threat with minimal damage to healthy cells.

CARs are designed to do a similar thing — activate a T-cell response against disease. But there are a lot of differences. CARs are artificially created Frankenmolecules consisting of an extremely sticky cancer antigen–recognizing component on the outside of the T cell, stitched together with signaling components, borrowed from natural T cells, on the inside of the cell. CARs are less complex than TCRs, and they recognize a much more limited range of antigens. Unlike a TCR, however, a CAR is infinitely customizable, like a set of Legos whose parts can be swapped out in different ways to target a different antigen or achieve a different effect.

“TCR structure is pretty well-conserved,” Salter said — meaning that TCRs throughout the natural world are made from the same basic components — “but with CARs the world is your oyster. When you have companies to print new DNA, you can design whatever you want and test it, in vitro, in a matter of weeks.”

Designing better T-cell therapies with TCRs

That’s certainly not to say that researchers who design TCR-based T-cell therapies have little to do.

Just steps away from the Riddell Lab is the equally busy research lab of Phil Greenberg, MD. The Greenberg group’s research is aimed at the same goal as Riddell’s: using genetically engineered T cells to kill cancer. But Greenberg’s team focuses on TCRs, the cousins of CARs that arose not in a lab a couple decades past, but in our extinct ocean-dwelling ancestors 500 million years ago.

The delicate and complex activity of TCRs, honed over eons, is a source of awe for Greenberg Lab research associate Thomas Schmitt, PhD, who studies TCRs and works on improved T-cell therapies.

“The immunological synapse of a T cell, I don’t know how many different molecules are in here,

but even just the TCR/CD3 structure contains six different molecules," Schmitt said. (CD3 is the name of a molecule that is a central part of the TCR signaling complex.) "And it's all basically evolved to give this exquisite sensitivity."

In ongoing clinical trials, research teams genetically engineer T cells with specific TCRs selected for their ability to recognize a cancer-associated antigen. Some of these experimental therapies have shown early promise in trials for patients with certain advanced cancers, and many more are still under development in the lab, aimed at some of the most deadly cancers, like ovarian and pancreatic cancer.

When choosing the TCR to engineer into T cells as a cancer therapy, a research team can opt to use a naturally occurring TCR or artificially modify one to increase its specificity for its target, for example.

Choosing a TCR is not a simple task. While evolution long ago settled on a standard structure for the overall TCR complex, nature has also come up with an ingenious mechanism for generating near-infinite variability in one tiny part of the TCR — the part that actually recognizes antigen. This random process ensures that your immune system is equipped to take on the dizzying array of pathogens and mutant cells you might encounter over your lifetime. And it means that there may be more than a hundred million unique TCR sequence combos in just one person. Multiply that by the number of people in the world. And then consider that this mind-boggling diversity includes many slightly different TCRs that recognize the exact same antigen with different levels of specificity or stickiness.

Hitting these balances just right is a focus of intense research by scientists who engineer TCRs, like Schmitt and his mentor Greenberg. A T-cell therapy may not effectively eliminate cancer if the TCR doesn't bind strongly enough to its target — and thus doesn't trigger a strong kill signal to the T cell. Conversely, binding too stickily can trip the T cell's natural shutdown mechanisms. And if the same target antigen is also present on healthy cells, or if the TCR reacts unexpectedly to a slightly different antigen on noncancerous cells, it could trigger a deadly immune attack on healthy tissues. (Some participants in a few past TCR clinical trials have unexpectedly gotten very sick or died from these issues. Since then, researchers have developed additional methods aimed at improving safety.)

Schmitt is working on new ways to generate or identify natural TCRs whose unusually high (but not too high) stickiness for their target antigens could make for an especially tenacious cancer fighter. Recently, he developed a new lab method for generating T cells with especially sticky TCRs for a particular target. And he and colleagues are applying a high-tech genetic sequencing method developed at the Hutch to sort through huge numbers of T cells from normal blood donors to identify an extremely rare TCR that binds tightly to one particular cancer antigen of interest.

In search of better therapies, T-cell tinkerers like Schmitt are constantly iterating, incorporating what they've learned from earlier designs and emerging insights about fundamental T-cell biology.

"Everyone's trying to take the basic research as it comes out and apply it to making this therapy

work as well as it can," he said.

Changes and challenges in CAR design

Even as researchers like Schmitt fine-tune nature's TCR design into a better cancer therapy, their less-than-three-decades-old cousins, the CARs, have undergone rapid evolution in design. Given the infinite variability of these artificial receptors, research teams have made some big changes to their structures over the years with the goal of developing better, safer therapies or treatments for new types of cancer.

Researchers throughout the world have added more T-cell stimulators on the inside-the-cell end of the CAR so that the T cells activate more vigorously when the CAR recognizes an antigen. Many groups have worked on modifications to the antigen-binding component of the CAR to, for example, increase its stickiness for its target. Scientists have been working on a variety of extra components for switching CARs off or on under certain circumstances — so that the CAR is only activated when it senses a tumor, for example, or so that doctors could intervene to switch it off if a patient's CAR T cells are causing dangerous immune overactivity. Riddell's group, for one, created a tiny tag that can be added to CARs to help track and purify them. And one of the Riddell Lab's big contributions to CAR structure was to optimize the length of the tiny chain that stitches parts of the CAR together, which, it turned out, has big effects on the CAR T cell's ability to kill tumor cells.

Salter doubleclicks a file on his lab computer. A window pops up that's filled with a wall of A's, G's, T's and C's: the letters of the DNA code for one particular CAR he's working on. Long strings of letters are highlighted — bright blue, yellow, pink — indicating what component of the CAR they encode. A few letters are marked to indicate changes he's made — say, swapping an A for a G — whose effects on T cells' cancer-killing abilities he's now testing in his dishes.

Some of the hints Salter has gathered that guide the changes he's making and testing come from research that's been published by other scientists. Other hints come from work he's done with Fred Hutch's Amanda Paulovich, MD, PhD, to carefully map out the signals through which CARs tell their T cells to attack. By deciphering how various CARs send different signals in different ways, Salter has learned how engineering specific changes to CAR structures can affect how well a CAR T-cell therapy might work for a patient.

"Most of the stuff that's done clinically [in CAR T-cell therapy] is very simple: You've taken the same backbone and just put a different head on it to target a different cancer," Salter said. "Where my research has come into play is saying maybe that's too simplistic for what we need to be doing. We need to understand a lot more about how these receptors function."

'The next big challenge' for T-cell therapy: Solid tumors

The two currently approved T-cell therapies program CARs into the T cells of people with certain leukemias and lymphomas, and many of the most promising results emerging from clinical trials — both those using CARs and those using TCRs — have been in blood cancers like leukemia. But solid tumors like cancers of the lung, breast and ovary, for example, offer particular challenges for T-cell

therapies. Many solid cancers, which top lists of the most common and deadly malignancies, lack a single cancer-specific antigen target, and they typically have special powers for shutting down immune attack.

“There’s so much complexity in that [solid] tumor microenvironment that’s beyond just having an antigen and having a T cell that recognizes it,” said Schmitt, the TCR researcher. “I think there’s so many other barriers that are going to have to be overcome there. So I think that getting this to work in solid tumors, whether with CARs or TCRs, is going to be the next big challenge.”

There’s some creative engineering going on in the lab to solve some of these problems. For example, some of Schmitt’s colleagues in the Greenberg Lab are engineering workarounds for one common defense mechanism in solid tumors and some other cancers: triggering kill switches on T cells. The team’s strategy is to trick tumors into activating T cells instead by reprogramming T cells with an artificial molecule that looks, to the tumor, like a kill switch — but which is instead wired to send T-cell stimulation signals. So, the more the tumor tries to stop the T cell, the more active the T cell gets.

CAR T-cell tinkerers also are coming up with some interesting tricks to outwit solid tumors. As Salter fiddles with his computer files at his lab workstation, his labmate Shivani Srivastava, PhD, sits just two feet away, arguing good-naturedly about some aspects of her experiments with her and Salter’s mentor, Riddell.

A postdoctoral researcher, Srivastava is working on what’s known as a “logic-gated CAR,” an even more complex design that could help target wily solid tumors that lack a single good antigen to distinguish them from normal, healthy cells. Up on her computer screen glows a cartoon of a logic-gated CAR design she’s been testing in mice with breast cancer. In this cancer, having both of two particular antigens (but not just one alone) marks a cell as likely cancer. So in the design she’s testing, CAR “B” pops up in the T cell only if CAR “A” detects its target antigen. The T cell only kills the cell if both CARs A and B engage both of their targets.

Results in lab models look really good so far, she said. “This was the first time we were able to find something [in our model] that looked like it preserved the anti-tumor effect without any toxicity,” she said.

Srivastava, like the other T-cell tinkerers she works with in the lab and down the hall, is hopeful about what their research is yielding. The promise, after all, of their work on these tiny cancer-targeting receptors is huge: more people’s lives saved, more safely, from deadly cancers.

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