How Does Leukemia Escape From Immunotherapy?

Single-cell sequencing tech enables deep dive into mysterious results, sets stage for future improvements.

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Editor's note: This story was originally published on April 16, 2018, when Dr. Kelly Paulson presented the team's work at the annual meeting of the American Association for Cancer Research. It has been updated to reflect the study's publication in a peer-reviewed journal, including some new research results.

When an experimental new cancer treatment shows promising results for many patients, these successes are exciting. But cancer researchers say that the most important insights — those that will lead to better therapies for even more patients — come from studying the failures.

A new study by a team from Fred Hutchinson Cancer Research Center and colleagues does just that, highlighting one way that an aggressive leukemia can wriggle its way free of targeted attack by a high-tech strategy for immune-based therapy. With these insights in hand, the researchers have designed what they hope will be an improved version of the experimental therapy that will help more people in the future.

The study was published on Feb. 9 in the journal Science Translational Medicine.

"It's important for our clinical trial participants, for our patients and our families to know that, even if the trial doesn't go the way that we want it to for that particular patient, we don't stop working," said Dr. Kelly Paulson, who worked on this project while she was a senior fellow at Fred Hutch and is now the interim lead of the Center for Immuno-oncology, Paul G. Allen Research Center at Swedish Cancer Institute. "That we don't stop asking what we can do better next time. That we don't stop working hard to understand how we can fight cancer better, and how we can make the next treatment better and stronger."

In an immunotherapy trial, more promising results for some than others

The research project was initiated in the lab of Fred Hutch immunotherapy scientist Dr. Phil Greenberg. It was subsequently led collaboratively by a larger team of cancer immunotherapy researchers: Greenberg, Dr. Thomas Schmitt (associate in clinical research in the Greenberg Lab), Dr. Aude Chapuis, graduate student Miranda Lahman, postdoctoral fellow Dr. Denise Buenrostro,

Paulson (all three of the Chapuis Lab at Fred Hutch), and Dr. Nathalie Vigneron of the Ludwig Institute for Cancer Research in Brussels.

Their study was a deep dive into the cells of certain patients enrolled on an ongoing early stage trial of an experimental immunotherapy at Fred Hutch. In this small trial, people who'd undergone bone marrow transplant for a type of advanced, aggressive leukemia receive an infusion of donor T cells genetically engineered with a special receptor. This special T-cell receptor enables the immune cells to recognize cancer cells with a telltale molecular signature.

Every participant whose cancers were in remission at the time they received the engineered cells has continued in remission for several years — "truly wonderful," said Paulson, given how aggressive their cancers were. But the engineered cells did not help improve the survival of patients whose cancers were not completely in remission after transplant when they received the special T cells, the scientists reported.

Why? The research team dived in to find out.

A cancer's escape

The scientists focused on certain patients whose cases were especially mysterious: like that of one man, who was only in his early twenties when he enrolled on the trial after his acute myeloid leukemia came back. On the trial, he received genetically engineered T cells after his second bone marrow transplant failed to put his cancer into full remission.

With the engineered cells, his cancer did go into remission, but only for 12 months. And the reason his cancer came back wasn't obvious. His genetically modified, cancer-targeting T cells were still circulating en masse throughout his blood. And his leukemia cells still displayed the molecular signature — a protein called WT1 — that the modified cells recognized.

The team harnessed a powerful technology called single-cell RNA sequencing, or scRNA-seq, to help them understand what was going on. scRNA-seq enables researchers to look at the identity and activity of many different cells at once. In scRNA-seq, sequencing technology records the gene activity of the thousands of diverse cells contained within a small sample of tissue — a blood sample, say. Then, sophisticated statistical techniques sort through the billions of sequences the technique generates to create maps showing which kinds of cells are present and what each one is doing.

By analyzing samples from the man's remission and his relapse, the researchers found that the genetically modified, cancer-targeting T cells stayed present, but they had lost their activity. "It suggested to us that the T cells weren't seeing the leukemia cells very well anymore," Paulson said.

Digging a bit deeper, they found out the likely reason for this: The man's relapsed cancer cells had a slightly modified version of the molecular machinery cells use to process the proteins like WT1 that reveal their identity. With this tweak to their processing machinery, the cancer cells were still

displaying their WT1 signatures. But they were displaying them in a different way — a way that the T cells had not been engineered to recognize.

And that's why the modified T cells were inactive at the time of relapse, the team realized. From the T cells' perspective, it was as if the cancer cells floating alongside them in the blood weren't even there.

"It's patients like that that show why you need to find more [treatments]," Paulson said.

Lahman said that now that the researchers know just how important this processing machinery is in the immune system's ability to kill cancer, it's changed the way they develop new T-cell therapies.

"One of the big things that we've take away from this is understanding the upstream processing of our immunotherapy targets is really important when we pick our targets," she said. Now, she explained, "when we search for these T-cell receptors, the way the target is processed is a question that we ask very early on."

The next iteration of a T-cell therapy on the horizon?

Schmitt said that "it is well-appreciated" that tumors can change to avoid attack by genetically modified T cells. Many of the details are still to be determined, he added, but this will change. "Single-cell RNA-seq will help elucidate the precise mechanisms and frequency of such events," he said.

Back in the Greenberg Lab, Schmitt looked for different versions of the cancer-targeting receptor used in this trial. What he was searching for were T-cell receptors that could recognize WT1 processed with a variety of cell machineries.

Of the dozens of different T-cell receptors Schmitt synthesized, one stood out in the team's testing.

Armed with this new T-cell receptor, T cells could recognize and kill tumor cells no matter which machinery the cancer cells use to process and present WT1. In lab dishes and in lab mice, the team found that the T cells engineered with this new receptor killed not only leukemia cells but other cancers with the WT1 marker, including pancreatic and triple-negative breast tumor cells.

And, they could even kill the man's leukemia cells — the same ones that his immune system was once powerless to stop.

(Independently — and published in same issue of Science Translational Medicine — a research team from the lab of Dr. Chiara Bonini at the Istituto di Ricovero e Cura a Carattere Scientifico Ospedale San Raffaele in Milan, Italy, identified a T-cell receptor that recognizes the same portion of WT1 as the Hutch team's T-cell receptor.)

Later this year, the researchers hope to open at Fred Hutch a small, early stage clinical trial that

would test the safety of engineering their new T-cell receptor in people with a range of cancers that have the WT1 marker, as the first step toward its continued development as a possible new treatment. The trial would enroll people with several cancer types: acute myeloid leukemia prior to blood stem cell transplant, ovarian and other gynecologic cancers, mesothelioma and colorectal cancer, Chapuis said. It would also incorporate another innovation: Two different kinds of patient T cells will be reengineered on the trial, both killer and helper T cells. (The earlier trial engineered only killer T cells, but researchers have since learned that helper T cells are important in T-cell therapy, too.)

But, meanwhile, work continues in the lab. Besides the man who was the focus of this study, there were others for whom the experimental treatment didn't work as hoped. And the researchers want to know why.

"We're interested in uncovering the other potential mechanisms of escape," Lahman said. "We're still learning more that can improve our therapy in the long run."

Like the man featured in the scientists' recent study, many of these patients have died of their cancers. But their cells, stored safely in research freezers, still live, holding information that could help others.

"We have not forgotten about them," Buenrostro said. "There is a lot of hard work that has been put in, but there's still a lot of work to be done. You answer one question and you get the answer, but then you get multiple questions on top of it. It's a never-ending process."

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