Probing the mystery of drug resistance: New hope for leukemia's toughest cases

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Alejandro Gutierrez, MD. Credit: Michael Goderre / Boston Children'S Hospital

Three children Alejandro Gutierrez, MD, treated for leukemia during his fellowship at Boston Children's Hospital still haunt him more than a

decade later. One 15-year-old boy died from the toxicity of the drugs he was given; the other two patients went through the whole treatment only to die when their leukemia came back. "That really prompted a deep frustration with the status quo," Gutierrez recalls. "It's motivated everything I've done in the lab since then."

Gutierrez, now a researcher in the Division of Hematology/Oncology, has made it his mission to figure out why leukemia treatments cure some patients but not others. And in today's issue of *Cancer Cell*, he and 15 colleagues report progress on two important fronts: They shed light on how leukemia cells become resistant to drugs, and they describe how two drugs used in combination may overcome that resistance, offering new hope to thousands of children and adults with leukemia.

Cancer of the blood

The most common form of cancer in children and teens, leukemia affects the blood and the bone marrow, where most blood cells are made. There are many types of leukemia, affecting different blood cells in different ways, but they all begin with a genetic mutation that leads to rapid cell growth. As the leukemia cells multiply, they crowd out normal cells, preventing them from serving their roles in the body. Unless successfully treated, leukemia can lead to anemia, severe fatigue, a crippled immune system and eventually death.

There has been steady progress in treating leukemia in recent decades. Today some 64 percent of all patients—and about 80 percent of children—live five years or more after diagnosis. But some patients simply don't respond well to treatment.

"Our approach for these patients has been to crank up the intensity of therapy. We basically bring kids to the brink of death and then let them recover," Gutierrez says. "That approach has gotten us a long way, but I

really think we've reached the limit now."

Cracking the mystery of drug resistance

To go beyond that limit will require cracking the mystery of drug resistance, which occurs in about 20 percent of children with leukemia—and more than 50 percent of adults. It is the biggest reason leukemia still kills.

Like the well-known problem of microbial resistance to antibiotics, leukemia drug resistance is evolution in action. When a patient begins treatment, the medication often kills most of the leukemia cells. By chance, however, a small number of cells may have a genetic mutation that makes them impervious to the drug. Thanks to natural selection—"the survival of the fittest"—those resistant cells multiply, replacing their vulnerable brethren and eventually making the drug useless in that patient.

In a few cases, resistance is evident from Day 1, because all the leukemia cells are already equipped with resistance genes. "When you see one of these cases, it's very dramatic," Gutierrez says. "There's just no response to treatment."

Until now, how this happens at the molecular level has remained a puzzle. What specific genetic changes confer resistance on leukemia cells, and how do those changes enable them to shrug off the drugs? To figure that out, Gutierrez and his colleagues focused on one of the mainstays of childhood leukemia treatment, a drug called asparaginase, and its impact on an amino acid called asparagine.

Asparagus and amino acids

Asparagine (first isolated from asparagus juice) is one of the 20 amino acids human cells use as building blocks to construct the proteins that carry out most of the business of life. While certain amino acids must be absorbed, fully formed, from the diet, asparagine is one that normal, healthy cells can build from other molecules. But for reasons that aren't well understood, leukemia cells typically can't do this. They have to absorb asparagine from the bloodstream.

That's where asparaginase comes in. A natural enzyme, it breaks down asparagine in the bloodstream. Deprived of this vital amino acid, most leukemia cells die, while normal cells are largely unaffected. For this reason, over the last 40 years asparaginase has been increasingly used as a drug to treat leukemia.

Yet somehow, in about 20 percent of Dr. Gutierrez's pediatric patients, leukemia cells survive asparaginase treatment. How?

Searching for the resistance gene

To find out, Gutierrez embarked on an ambitious study with help from collaborators from Boston Children's Hospital, the Broad Institute and the Dana-Farber Cancer Institute. The paper's lead author is Laura Hinze, a visiting fellow from Hannover Medical School in Germany. Hinze came to Boston Children's in the fall of 2017, intending to stay for only a few months. "But the project turned out very well, so I decided it made sense to stay and complete it," she says.

The Gutierrez-Hinze team applied the gene editing technology known as CRISPR to a group of human leukemia cells that were resistant to asparaginase. One by one, they knocked out all the cells' genes. Next, they tested the gene-altered cells to see which ones were now killed by the drug. In this way, they identified the genes responsible for the resistance.

What they discovered is that drug-resistant leukemia cells overcome the lack of asparagine in the bloodstream by cannibalizing proteins inside the cell, breaking them down to free up this one scarce amino acid.

Just as a camel's hump provides a store of fat the animal can draw on when food is scarce, leukemia cells treat their own proteins as a resource to tap "if they're starving and need more building blocks," Gutierrez says. "They're harvesting asparagine in order to survive treatment of cancer."

Early mornings, late nights with 140 mice

The key to this process is an enzyme called GSK3, which controls the breakdown of proteins inside the cell through a key signaling pathway. Knowing this gave Gutierrez and Hinze an idea: "If we could target his pathway, we could, in theory, kill leukemia cells with minimal effect on normal cells."

To test this theory, they turned to Florence Wagner, Ph.D., of the Broad Institute and Kimberly Stegmaier, MD, of the Dana-Farber Cancer Institute, who had developed a drug that blocks the action of GSK3.

Armed with this GSK3 inhibitor, Hinze injected drug-resistant leukemia cells into 140 mice and then treated them with different drugs twice a day for 12 days. "She spent many early mornings and late nights treating those mice," Gutierrez says.

Hinze divided the mice into four groups. One group got no treatment; the second got asparaginase alone; the third got only the GSK3 inhibitor, and the fourth got asparaginase and the GSK3 inhibitor together.

The results were striking: The first three groups of mice all died soon after—in some cases before—their treatments were complete. But the

mice given the two drugs together lived four times as long—and might have lived longer if the treatment had continued.

Two factors better than one

Gutierrez calls the experiment a dramatic example of two factors together having an effect that neither can achieve alone—a phenomenon scientists call "synthetic lethality."

"Asparaginase alone doesn't do anything to resistant cells, and likewise, the GSK3 inhibitor alone doesn't do anything to them," Gutierrez says. "But when you put them together, the cells are dead."

Just as important, the two-drug regimen had little effect on normal cells in the mice, suggesting it may be well tolerated as a treatment.

Gutierrez, who still spends about one day a week treating patients, is anxious to test this two-drug approach. He hopes to launch a clinical trial as soon as a human version of the GSK3 inhibitor is available. Several pharmaceutical companies are already developing such drugs, but Gutierrez could not say when a trial might begin. "It's hard to predict and completely out of our control," he said. "But hopefully in the near future."

Broader implications

Gutierrez hopes this discovery will lower resistance to asparaginase among the children who come to Dana-Farber/Boston Children's with leukemia. Beyond that, he believes his team's approach will inspire other researchers to seek out the causes of resistance to other leukemia drugs, with each new discovery improving the odds of survival for patients not helped by current therapies.

The discovery may also offer a less toxic alternative to leukemia patients who are cured by current treatments. That's important because some leukemia drugs can have serious side effects decades later.

"Sometimes you'll get through this whole thing, your leukemia will be cured, and then five, ten, 20 years later you'll have a late effect—heart failure, for example," Gutierrez says. "So for patients who are cured, I hope this will actually be able to replace some of the most toxic elements of standard therapy."

Finally, Gutierrez notes that the team doesn't fully understand why the two-drug treatment hits leukemia cells so hard while largely sparing normal cells. "We're actually very interested in that question," he says. "And if we can understand that, I think there's a good chance this approach will be broadly useful in many other types of cancer that are related to leukemia."

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