
Photo by Flynn Larsen

# THE TRANSPLANT SORCERER

Judith Shizuru has long dreamt of using stem cells to perform—and transform—bone marrow transplantation. She recently took a big step toward that goal.

Back in the early 1990s, when Judith Shizuru was still doing double duty as a postdoctoral researcher and a medical student at Stanford, she would often chat with Irv Weissman about making graft vs. host disease (GVHD) a worry of the past. A potentially lethal complication of bone marrow transplantation, GVHD occurs when mature immune cells from a donor—which flood in with the blood-making stem cells in the foreign marrow—attack the tissues of the patient receiving the transplant. "We'd be saying, 'well, if you could just transplant pure stem cells, which are immunologically naïve, you won't get GVHD,' " Shizuru recalls.

These were not idle fantasies. Weissman, who is today director of the Ludwig Center at Stanford, had by 1991 isolated the hematopoietic stem cells (HSCs) they were talking about—the source of all types of blood cells. Yet, as they knew, that wouldn't be enough. At the other end of the transplantation process, the existing stem cells in the patient's bone marrow would still need to be vacated to allow the new ones from the donor to take root. This is achieved even today by subjecting recipients to a grueling, and sometimes lethal, regimen of chemotherapy and radiation.

In 2016, Shizuru hit a golden milestone in her quest to transform bone marrow transplantation. In a study published in *Science Translational Medicine*, Shizuru and her team reported that they had, using no chemotherapy or radiation, prepared mice for bone marrow transplantation and successfully completed the procedure with reasonable success using purified hematopoietic stem cells.

"It's been a long time getting to the point where we think we're going to be able to translate this concept into the clinic," says Shizuru. If their approach indeed translates, it has the potential to radically alter the prospects of people undergoing transplants of all sorts and patients with disorders ranging from autoimmune disease to cancer.

## BEGINNINGS

Shizuru grew up in Mountain View, California, a third-generation Japanese American and the fourth of five children. Her parents had both been interned in the Midwest during World War II and resettled in California. Shizuru was a good student, thanks in part to the tutelage of her siblings, and was accepted to Northwestern University.

She soon transferred, with a scholarship, to Bennington College in Vermont, where she could get the liberal arts education she wanted. Shizuru thrived at the school. "I grew up very blue collar, but in the Bay Area," says Shizuru. "My father was a postal worker, so we couldn't actually afford music lessons. But

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at Bennington playing music was encouraged. They gave me a violin and I had a wonderful violin teacher. So in the time I was there I learned to play, and had the joy of playing Bach, Mozart and more.”

When she moved back to Mountain View after college, Shizuru took a job as a lab technician in a transplantation laboratory at Stanford University Medical School, where she’d worked as a secretary during long winter breaks from Bennington. Her boss urged her to join the graduate school and introduced her to her first mentor, who was working on the transplantation of pancreatic islet cells as a treatment for diabetes.

When that mentor left Stanford, Shizuru joined the laboratory of the acclaimed clinical immunologist Garry Fathman, who supervised her graduate studies. Shizuru continued her postdoctoral research on islet cell transplantation at Stanford as a postdoc, and soon forged a working relationship with a group of leading women at the Juvenile Diabetes Research Foundation (JDRF). They not only sponsored her research but also encouraged her to pursue a medical degree. Shizuru took their advice. With support from the JDRF and plenty of hands-on help from her long-time friend and lab assistant, Cariel Taylor, Shizuru conducted her postdoctoral studies while completing medical school at Stanford, followed by a residency at the University of California, San Francisco.

It was in medical school, says Shizuru, that she became convinced that the path to inducing immunologic tolerance of transplanted tissue—so that recipients do not reject their new organs—went through stem cell research. “Stem cell transplantation is like the Holy Grail,” says Shizuru. “It redefines the universe of self and non-self in the body and it’s a potential treatment for a variety of autoimmune diseases.”

Her research at Ludwig Stanford is still dedicated to the basic immunology of bone marrow and HSC transplantation, and the application of that research to medicine.

## ROUTE TO STEM

Shizuru’s long collaboration with Weissman—whom she has described as “the Picasso of biomedical research”—dates back to the early 1990s. So she was one of the first to find out when a graduate student in his lab figured out which antibodies could be used to safely deplete HSCs from the bone marrow of immune-compromised mice. Shizuru leapt at the opportunity to translate the discovery for clinical application.

Stem cells in bone marrow sit in specialized physical niches, and for a new stem cell to take hold, the old one has to be nudged out. “It’s like musical chairs: the seats are occupied,” Shizuru explains. “You have to get

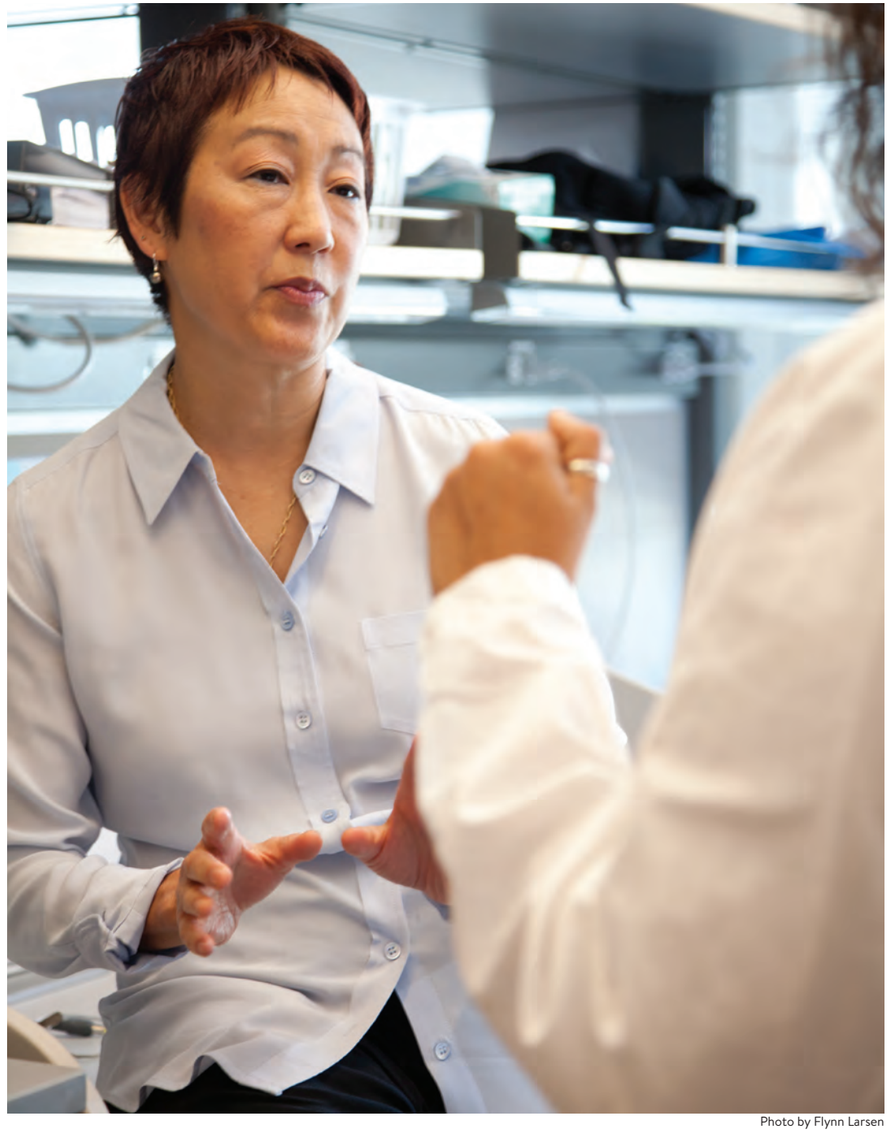

Photo by Flynn Larsen

the host stem cells out so the donor cells can take.”

This need is known simply as “making space” and there are currently only two ways to do it: chemotherapy and radiation, and both are DNA-damaging. Such measures are dangerous, which is why bone marrow transplantation carries a roughly 20% risk of killing the patient. “If there were a way to get rid of the host stem cells safely, that would make the whole procedure safer,” says Shizuru.

The work on c-Kit followed two parallel paths. One was to see whether antibodies to human c-Kit could be used to prepare patients with severe combined immune deficiency (SCID) for HSC transplantation. Known popularly as the “bubble boy disease”,

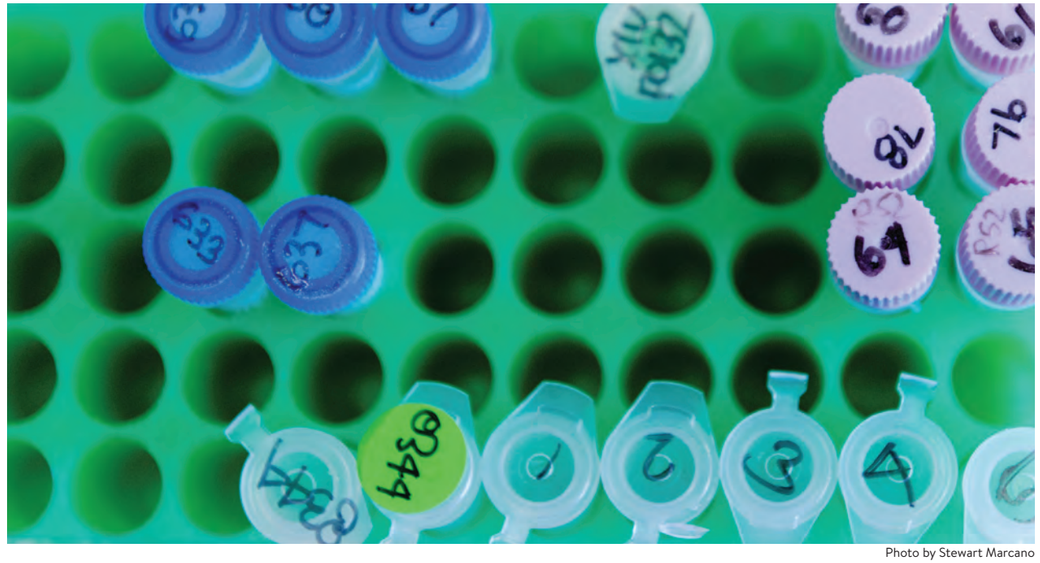

Photo by Stewart Marcano

SCID is a rare congenital disorder that leaves patients without a functioning immune system. This means that SCID patients, from an immunological perspective, reflect the conditions of the mouse experiments that initially suggested c-Kit antibodies could be used to make space for new stem cells. But first Shizuru's team needed to find human c-Kit antibodies suitable for clinical use.

A Google search revealed that the biotech Amgen already had a human c-Kit antibody, and a former postdoc of Shizuru's was on the team developing it as a treatment for a lung disease. Amgen agreed to begin a collaboration with her lab focused on transplantation. After showing that the human c-Kit depleted HSCs in mice with human immune systems and in a large animal model (monkeys), Shizuru's team obtained permission from the US Food and Drug Administration to start a clinical trial for children with SCID. It is now recruiting patients.

## STEMMING REJECTION

But could c-Kit antibodies be used to prepare people with functional immune systems for bone marrow transplantation? This was the focus of a second path of research undertaken primarily by postdoc Akanksha Chhabra in Shizuru's lab in collaboration with MD/PhD candidates Aaron Ring and Kipp Weiskopf, both of whom have since completed their training at Weissman's lab and moved on.

Turned out c-Kit antibodies alone worked only tepidly in mice with competent immune systems. This is because T cells interfere with both the making of space by host HSCs and the engraftment of the new ones. Weissman's team, however, had a potentially useful antibody, one that is now being tested in clinical trials as a cancer therapy. The antibody targets a cell-surface protein named CD47 that is expressed by HSCs as well as cancer cells. CD47 transmits a "don't eat me" signal to the immune system's macrophages, and blocking it invites macrophages to gobble up targeted cells.

Shizuru and her team wondered whether they could clear up space in immune-competent mice if they used anti-c-Kit antibodies to tag HSCs and then hit them with anti-CD47 antibodies.



As they reported in *Science Translational Medicine* in 2016, their hunch was right: the combined treatment led to a greater than 10,000-fold reduction in the number of HSCs in the mice. "It was spectacular," Shizuru recalls. "It was the kind of data you get to see just once in a lifetime." But it still wasn't enough. "To me, as a transplant physician, this doesn't matter if you don't get engraftment," explains Shizuru.

To get that, the researchers would have to deal with the T cells in the host that were hampering engraftment. Chhabra accomplished that by adding a couple of antibodies to the mix that selectively target the two problematic types of T cells. The researchers then purified HSCs from the donor, leaving behind the donor's T cells as well, and tried out the transplantation.

Not all of the host's HSCs were replaced by the donor's, but the procedure worked better than expected, and it was utterly devoid of the toxic therapies that have long made bone marrow transplantation an option of last resort. "This was the proof of concept that you can use an all-antibody approach to get engraftment of stem cells," says Shizuru.

Further, Shizuru points out, the levels of stem cell engraftment achieved could be sufficient to treat genetic diseases like sickle cell anemia and SCID, in which even a partial restoration of functional blood-based cells can significantly improve a patient's condition. In the longer term, the procedure could have important implications for the treatment of a broad variety of cancers, since first line therapies can devastate the immune system.

Ludwig is currently supporting the Shizuru lab's exploration of their HSC transplantation approach to treat myelodysplastic syndrome, a blood disease that can progress to malignancy. Meanwhile, Shizuru and her team are trying to figure out ways to target c-Kit alone to accomplish HSC transplantation across unrelated animals. "We're trying immunotoxins linked to the antibodies and exploring other approaches to deplete c-Kit expressing stem cells," she says.

"A few years ago I told my lab that if in my lifetime I can get the mouse c-Kit antibody alone to work in a normal immune-sufficient mouse, I can die a happy woman," says Shizuru. "But we were able to accomplish this goal in just a few years, using a combination of anti-cKit and anti-CD47 antibodies. So now I'm going to have to raise the bar and tell them: 'if in my lifetime we can replace toxic drugs and radiation and still get pure stem cells to engraft in people, I will die a very, very happy woman.' "

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