



Irv Weissman

LUDWIG WORKING FROM HOME

“All four of the **‘don’t eat me’** signals that we know of were discovered from my lab, and they were all funded by Ludwig.”

The stem cell pioneer

FOLLOWING THE END OF HIS MEDICAL school training in 1965, Irv Weissman faced what should have been a life-changing decision: continue with an internship and medical residency or become a full-time researcher.

As it happened, the choice was a no-brainer. Weissman had, in fact, already made his decision by age 10, when he got hooked on science after reading *Microbe Hunters* by Paul de Kruif and deciding he'd like nothing more than to follow in the footsteps of the book's protagonists. "The people in the book not only made discoveries about microbes, they immediately applied those discoveries to medicine," says Weissman, who is today director of the Ludwig Center at Stanford University. "I knew then that's what I wanted to do."

It didn't take him long to get started. While still in high school, Weissman talked his way into a laboratory run by a physician in his hometown of Great Falls, Montana, and was soon contributing to experiments that would ultimately help pave the way for the first successful skin and organ transplants. Then, as a researcher at Stanford, he led the first isolation of a tissue stem cell—the hematopoietic stem cell—and went on to describe the steps by which it generates all blood cells. The discoveries he made along the way, and continues to make today, promise to transform transplantation medicine and the treatment of ailments ranging from autoimmune diseases to cancer. They include his characterization of "don't eat me" signals exploited by cancer cells to evade immune attack, a body of work that is already being applied by a company he co-founded to translate that work into a

While in medical school, Weissman recruited other students to work with him, researching how the immune system develops to distinguish “self” from “non-self.”

cancer therapy. In 2019, he and his colleagues reported in *Nature* a fourth such signal and showed how its blockade could prompt an immune attack on malignancies, including ovarian and triple-negative breast cancers.

The novice

Weissman was 15 when he first heard about Ernst Eichwald, a doctor and researcher who had recently moved from the University of Utah to work as a pathologist at Montana Deaconess Hospital in Great Falls. In what Weissman often describes as the most important event in his life, he approached Eichwald to ask for a job. “He was partially deaf and spoke with a thick German accent, and our conversation went nowhere until I said I’d work for free,” Weissman says. “We settled on \$25 a month.”

As Eichwald’s research assistant, Weissman

learned not just the fundamentals of science, but also how to puzzle things out for himself. Eichwald once told him, for example, that about a quarter of the skin grafts he had conducted on his mice had been rejected, and asked Weissman to speculate on the cause. “At first, I thought it had something to do with the age of the mice, but that wasn’t it,” Weissman says. “Eventually, I asked, ‘Were the skin grafts from males rejected by females?’ and he said ‘Yes.’ That experience helped me realize that I can think and do science.”

After high school, Weissman continued working in Eichwald’s lab as a college student attending what is now Montana State University (MSU). By that point, he was conducting his own experiments to understand why adult mice rejected tissue from nonmatching donors, while fetal mice exposed to blood-forming cells from adult mice of a different strain accepted transplanted tissue from that strain for the rest of their lives.

After graduating from college, Weissman joined the medical school at Stanford, drawn there by its unique five-year medical program. “Stanford divided the two years of basic science that every medical student takes into three years,” Weissman says. “That meant that, every day, we had half a day free.” At the end of his first year at Stanford, Weissman joined the lab of Henry Kaplan, a professor of radiology. In an unusual move, Kaplan gave the young Weissman a shared lab of his own and the support of a research assistant.

By his junior year, Weissman had recruited other medical students to work with him, researching how the immune system develops to distinguish “self” from “non-self.” In 1964, he spent nine months in the UK working in the lab of immunologist Jim Gowans at Oxford University. While there, Weissman performed a landmark experiment in which he showed that the thymus, rather



Photo by Flynn Larsen

than merely producing hormones to aid immune cell development at a distance, actually matured T lymphocytes before sending them out to lymphoid organs.

The experience at Oxford affirmed for Weissman that he wanted to pursue a career in scientific research. “That important discovery that the thymus was the place that made T cells made me decide that, as much as I loved medicine, I wasn’t going to do an internship and residency,” Weissman said.

A hard lesson

Over the next two decades, Weissman and his lab at Stanford identified where many of the different cell types of the immune system are made and how they work. In 1988, he isolated purified hematopoietic stem cells for the first time from mice. Shortly after, his lab replicated the achievement with human

tissue, and went on to trace the steps leading from the stem cell to each of the many types of mature cells found in blood, and identify how they run awry in many blood diseases and cancers.

These discoveries opened up the possibility of using a patient’s own stem cells to regenerate tissues, organs and cells damaged by disease. But in 2001, the Bush administration placed strict limits on the use of federal funds for human embryonic stem cell research. In response, Weissman worked with real estate developer Robert Klein to write a proposition to provide \$3 billion for stem cell research in California. In 2004, 59% of California voters approved Proposition 71: the California Stem Cell Research and Cures Initiative, leading to the establishment of the California Institute for Regenerative Medicine (CIRM).



Photo by Flynn Larsen

CIRM's funding mechanism was set up to avoid a painful business lesson Weissman learned in the 1990s, after forming a company called SyStemix Inc. to test the use of purified blood-forming stem cells to reconstitute the immune system of cancer patients. The company's clinical trial was abruptly ended in 2000 after the pharmaceutical company Novartis bought Systemix and shut down its stem cell programs. "To this day, the stem cell transplants we did as part of Systemix's clinical trial is the only instance of people being cured of metastatic breast cancer, but it's not a standard practice of medicine," Weissman says.

With that experience in mind, Weissman

stipulated in Prop 71 that the state agency should fund not only stem cell science, but its development for medical applications as well—through early clinical trials. In the 16 years since Prop 71 passed, CIRM has funded or supported research that has led to more than 60 clinical trials to study the use of stem cells to combat a host of diseases, including diabetes, spinal cord injury, various cancers and—most recently—COVID-19.

"I hope this will be an enduring legacy by the California voters—a new way to advance discoveries through clinical trials without having the risks that both venture capital and big pharma now avoid," Weissman says.

Weissman, for his part, has made enormous headway with his Stanford colleagues in harnessing stem cells to transform bone marrow transplantation, which currently requires the use of harsh chemo- and radiotherapy to destroy the recipient's existing, and diseased, blood stem cells. In 2019, for example, he and his colleagues described in *Cell Stem Cell* a gentle method—involving treatment with six antibodies—by which mice could be transplanted with blood stem cells from an immunologically mismatched donor. Further, they showed that recipient animals could then accept an organ or tissue transplant matching that of the (mismatched) donor stem cells without requiring ongoing immune suppression. If the findings are replicated in humans, the work could transform the treatment of immune and blood disorders, and vastly expand the pool of available organs for transplantation.

The 'don't eat me' era

During their investigations of hematopoietic stem cells and human leukemia stem cells in acute myelogenous leukemia, Weissman and his team had noticed that a protein, CD47, was expressed at higher levels in the leukemia stem cells than in normal bone marrow hematopoietic stem cells, or in their multipotent daughter cells—the stage at which most leukemia stem cells are found. They began investigating it in earnest after a Swedish group showed that red blood cells in mice that didn't exhibit this surface protein were removed by macrophages, immune cells that gobble up potential threats. CD47 was, in effect, a "don't eat me" signal.

In 2009, Weissman and his team reported in *Cell* that CD47 overexpression is linked to worse outcomes in acute myeloid leukemia and suggested it is a potential therapeutic target for the cancer. Their subsequent studies showed that the protein is abundantly expressed in nearly every human cancer. With support from Ludwig Cancer Research, Weissman's group also developed an antibody that blocked CD47 and showed that it

Weissman and colleagues have made enormous headway in harnessing stem cells to transform bone marrow transplantation.

restored the ability of macrophages to engulf cancer cells and, in immune deficient mice transplanted with human primary leukemias, lymphomas and other cancers, inhibit or eliminate a variety of tumors.

In a 2013 study published in the *Proceedings of the National Academy of Sciences*, his group showed that after engulfing the cancer cells, the macrophages presented molecular markers, or antigens, of the cancer cells to killer T cells, recruiting them into the attack on cancer cells as well. An antibody targeting CD47 is now in clinical trials—alone and in combination with other immunotherapies—in people with several types of blood and solid cancers. In March 2020, a biotech company, Forty Seven Inc., that Weissman co-founded to develop CD47-based immunotherapies was purchased by the pharmaceutical company Gilead for \$4.9 billion.

A fourth signal

Meanwhile, the finding that not all patients respond to anti-CD47 antibodies motivated Weissman to look for alternative don't-eat-me signals that might also stump macrophage attack. The hunch paid off. Over the next several years, the team uncovered two other such signals exploited by cancer cells. One of them is PD-L1, a protein that also scuttles T cell attack and is targeted by

“We don’t know yet, but you could imagine that some macrophages will have all four ‘don’t eat me’ receptors, some will have three, some two, and some only one.”

checkpoint blockade immunotherapies; the other is a protein associated with the major histocompatibility class 1 complex that cells use to present antigens to T cells. In 2019, Weissman’s group reported in *Nature* that a fourth protein, CD24—which ordinarily plays a part in controlling the severity of certain immune responses—also transmits a don’t eat me signal to macrophages.

“All four of the ‘don’t eat me’ signals that we know of were discovered from my lab, and they were all funded by Ludwig,” Weissman says. So far, it seems that only CD47 is found on the surface of all cancer cells—something that is not true for the other three signals. “We were lucky we discovered CD47 first, and I emphasize the word ‘lucky,’” Weissman says.

In their 2019 paper, Weissman and his team showed that blocking the CD24 signal in mice implanted with human breast cancer cells allows immune cells to attack the cancers. The team also found that ovarian and triple-negative breast cancer, both of

which are very difficult to treat, are especially vulnerable to macrophage attack when their CD24 signals are blocked.

Interestingly, CD24 and CD47 operate in seemingly complementary ways. Some cancers, like those of the blood, appear to be highly susceptible to CD47 blockade, whereas others, such as ovarian cancer, are more vulnerable to CD24 blockade. Weissman suspects the same is likely true for the other “don’t eat me” signals. “We don’t know yet, but you could imagine that some macrophages will have all four ‘don’t eat me’ receptors, some will have three, some two, and some only one,” he says.

Thus, it might be that most cancers will be susceptible to attack by blocking one of these signals, and that cancers may be even more vulnerable when more than one signal is blocked. “Let’s imagine that you have an ovarian cancer and you have 50,000 CD24 molecules per cell and 80,000 CD47 molecules per cell,” Weissman says. “Even if we block all of the CD47 molecules, that cell still has a lot of don’t-eat-me signals left, so the chances that it will be eaten are not great.”

Weissman envisions a future where doctors will be able to fine tune a cancer patient’s immune response with a precisely tailored cocktail of antibodies that can revive the macrophage attack. “We know from previous experiments that we have to block at least 80 percent of all of the signals for the cancer cells to be eaten,” Weissman says. Doctors might even check whether the composition of the “don’t eat me” signals changes over the course of therapy, and then tweak the cocktail as needed.

Such precision therapy would likely require the screening of tumor-associated macrophages for the corresponding receptor of each signal. “It is my fond desire that we will get to that point,” Weissman says. If he’s on the case, we probably will. ■



Photo by Monty Rakusen