



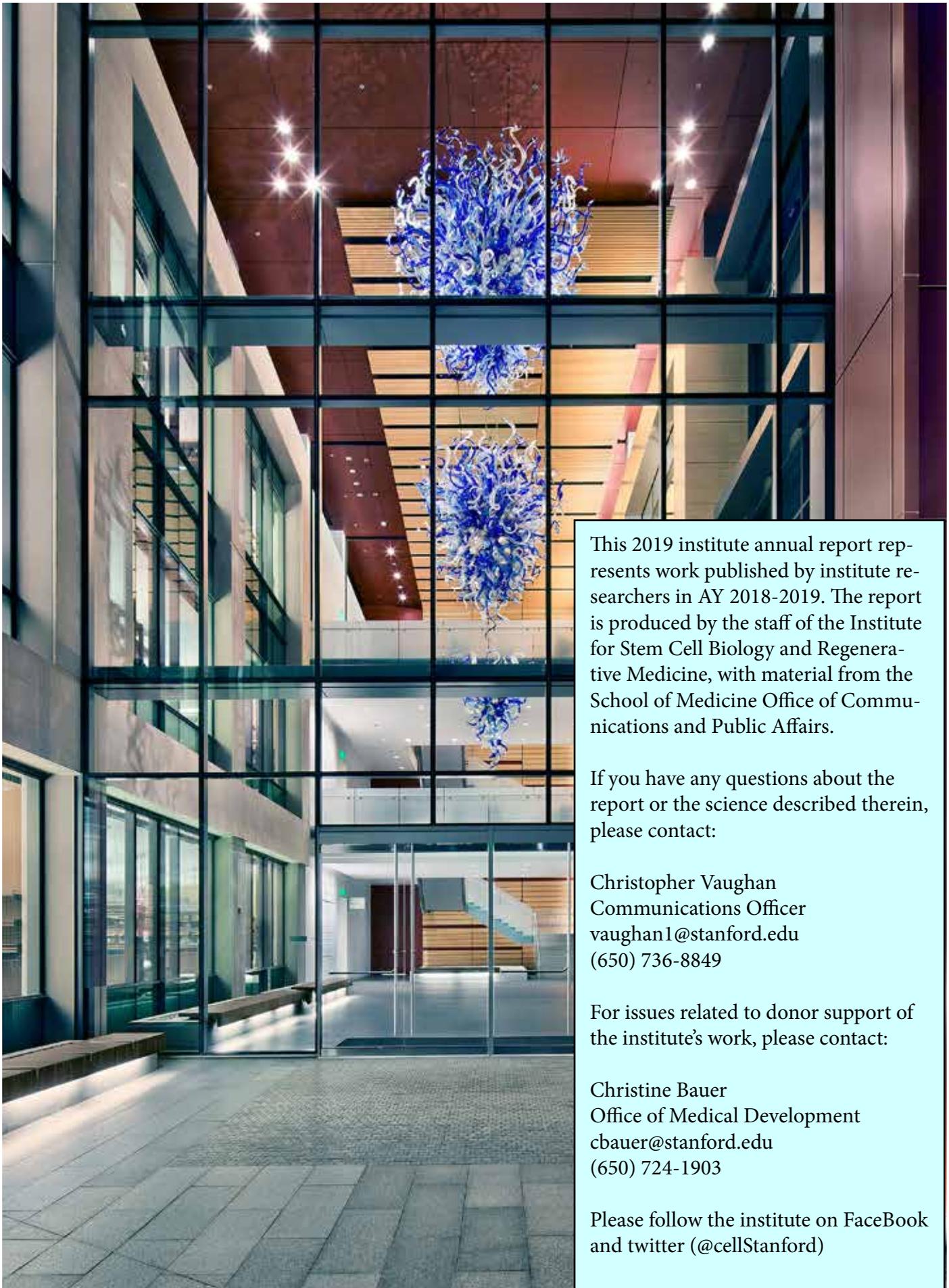
2019 ANNUAL REPORT

STANFORD INSTITUTE FOR STEM CELL BIOLOGY AND
REGENERATIVE MEDICINE



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This 2019 institute annual report represents work published by institute researchers in AY 2018-2019. The report is produced by the staff of the Institute for Stem Cell Biology and Regenerative Medicine, with material from the School of Medicine Office of Communications and Public Affairs.

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Message From The Director

This fall, I will turn 80, and the institute will have a symposium marking that event. Sometime in the next few years at the most, we will need to have a transition in leadership. We have been very successful in the fields of stem cell biology, in the quality of faculty we have gathered, in the careers we have started with students and postdocs at all levels, in the clinical translation we have carried forward, and in the continuing recruitment of stem cell scientists ...

... from all parts of the university into the institute. It is useful for us now to look more deeply at the foundations of our success to understand how we will continue to build a program of research and training with implications for all fields of medicine.

Nearly 17 years ago, we established the Stanford Institute for Stem Cell Biology and Regenerative Medicine, as well as the Stanford Cancer Center, with the intent to foster advanced stem cell and cancer stem cell research, training, and clinical

translation for all parts of Stanford University and the associated hospitals. With the help of many generous donors and the California Institute of Regenerative Medicine (established by the passage of Proposition 71 in 2004), we have built the Lorry I. Lokey Stem Cell Research Building. This building houses faculty, their labs, cutting-edge cell-sorting facilities, as well as equipment for DNA and RNA sequencing of the small numbers of stem cells and cancer stem cells that can be isolated. In addition, we also have an advanced, pathogen-free mouse facility, administrative space and hotel spaces for investigators bringing small collaborative research projects together. Our beautiful atrium and hallways house the Chihuly glass chandelier, figurative art, and an inspirational Oliveira sculpture that reminds us that our main objective is to establish stem cell science and medicine for the benefit of people stuck in the middle of hard-to-treat or incurable diseases. In creating the institute, then-dean Phil Pizzo



The Chihuly Chandelier

did not want just another department looking inward, but an interdisciplinary organization that looked outward, to spread our advances and ‘stem cell thinking’ to investigators and translational physicians throughout the university. So we recruited the best senior and most promising junior scientists in related fields to understand how the stem cells that we isolate develop, how they signal and are signaled by other cells, and how they change or retain gene expression to make fate choices to make more stem cells, or to differentiate to make the tissues and organs they inhabit and serve. We also

found how some mutations or inherited changes in gene expression in stem cells allow some of them to outcompete normal tissue stem cells to cause diseases, and others to develop into cancer stem cells. Along the way, brilliant discoverers of signaling molecules and their cognate receptors became stem cell signal transduction experts. Our faculty and collaborators in the Lokey discovered more and more efficient ways to analyze the gene expressions of single stem cells and found the mechanisms that open and close the suites of genes that determined cells’ fates during each cell division. They created a branch of stem cell science that has grown to include huge fields in advanced data sciences, embryonic and pluripotent stem cell biology (including the study of the generation of stem cells from normal, differentiated cells), cancer stem cell biology, disease causation, etc. Each of these became themselves a new branch of stem cell science and medicine. Young physician-scientists and budding PhD students and postdocs

joined teams to move these discoveries from the lab benches to clinical trials. We search for faculty who have proven, even early in their careers, that they have the kinds of thinking that opens a new field. Innovators who can do this are quite rare. This ability is not related, in our experience, to their classroom successes, or success on standardized tests. Just as important are those who do not necessarily have the ability to open a new field, but whose intellect and training and track record shows them to be most

proficient at entering a new field opened by an innovator, and digging into problems with a deep knowledge of published work in the field, and the ability to apply ‘reductionist’ science to get at mechanisms. We are quite fortunate to have a number of both types of scientists in our institute.

But how best can we select and provide the best environment for graduate students and medical students who can become these two types of faculty in the future? My own bias is that we primarily teach the key experiments in our field rather than the facts of the field, and that in our labs and weekly seminars by students and fellows, we first let them show their ability to discover, rather than train them by micromanagement.

We have built this institute and our associated graduate and translational programs with a minimum of bureaucracy, instead asking at each step, “what can we do to lower the barriers to successful discovery and translation? In doing so we often find



Universal Woman by Nathan Oliveira

ourselves at odds with those who use their positions to favor their own bureaucracy. We are not always popular.

Leadership transition can occur by selection from within or by an international search. No such search will be successful if there are not additional resources available, including space and billets. An internal candidate will not likely have the negotiating strength to command the kinds of resources even to modernize the space we have, much less add space for faculty. In consultation with Dean Minor, we will form a Scientific Advisory Board (SAB) to advise us and advise the dean. The names of candidate

members are now in front of us, and the number of votes for each candidate, will determine the order in which we will recruit them. The SAB hopefully will come and spend 1.5–2 days with us. Each member of the institute will present their vision and accomplishments to the SAB. I urge faculty who have something to contribute to rearrange their commitments in order to present to the SAB the real vision of how we developed, who we are, and where we hope to go. The more they know, the more likely the ISCBRM will be able to survive and thrive for decades to come. We can take pride that we started this field, and are among the best in it. I am grateful for the opportunity to help develop and guide the Institute for Stem Cell Biology and Regenerative Medicine at Stanford University.

– Irv Weissman



Translational Stem Cell Research

A major focus of the institute has always been the **translation** of stem cell research into **therapies** that can be used to treat human disease. Stem cell therapies offer the promise of **one-time treatments** that can **cure for life**.

This year, the institute made a significant advance in this area, conducting a clinical trial in the use of **antibodies** to prepare children with the “Bubble Boy” severe combined immune deficiency for **blood stem cell transplants** with much lower toxicity than caused by the standard chemotherapy or radiation. And institute researchers published data that in mice a cocktail of antibodies can entirely replace chemotherapy or radiation to prepare immune competent recipients for purified blood stem cell transplants between immunologically unmatched subjects.

Antibody could increase cure rate for blood, immune disorders

The institute is pioneering methods that could make blood stem cell transplantation safer, opening up its use as one-time treatments that provide lifetime cures for autoimmune disorders, immunodeficiency disorders and many other conditions.



Judy Shizuru, MD, PhD

Antibody-based treatments can gently and effectively eliminate diseased blood-forming stem cells in the bone marrow to prepare for the transplantation of healthy stem cells, according to a study in mice by researchers at the Stanford University School of Medicine.

The researchers believe the treatment could circumvent the need to use harsh, potentially life-threatening chemotherapy or radiation to prepare people for transplant, vastly expanding the number of people who could benefit from the procedure. “There are many blood and immune disorders that could be cured by a transplant of healthy stem cells,” said institute member Judith Shizuru, MD, PhD, professor of medicine at Stanford. “But the pre-treatments necessary to get the healthy cells to transplant effectively are so toxic that we can’t offer this option to many patients. A treatment that specifically targets only blood-forming stem cells would allow us to potentially cure people with diseases as varied as sickle cell disease, thalassemia, autoimmune disorders and other blood disorders.” Shizuru is the senior author of the study, while

postdoctoral scholar Wendy Pang, MD, PhD, and assistant professor of pediatrics Agnieszka Czechowicz, MD, PhD, share lead authorship of the work. Czechowicz became member of the Institute for Stem Cell Biology and Regenerative Medicine in 2018.

The study is one of two recently co-authored by Shizuru, Czechowicz and research associate Hye-Sook Kwon, PhD, indicating that an antibody targeting a protein called CD117 on the surface of blood-forming, or hematopoietic, stem cells can efficiently and safely eliminate the cells in mice and non-human primates. CD117 is a protein found on the surface of the stem cells. It regulates their growth and activity; the antibody, called SR1, binds to the protein and prevents its function.

The use of antibodies against CD117 to eliminate blood-forming stem cells is based on studies conducted by Czechowicz, then a graduate student in the laboratory of study institute director and co-author Irving Weissman, MD.

The results of these studies showed the antibody treatment was safe in non-human primates,

setting the stage for a clinical trial of the antibody at Stanford and the University of California-San Francisco in children with an immune disorder called severe combined immunodeficiency (SCID). Hematopoietic stem cells (HSCs) are found in the bone marrow. They give rise to all the cells of the blood and immune system. Blood cancers, such as leukemia, arise when the stem cells or their progeny begin dividing uncontrollably; other genetic conditions such as sickle cell anemia or thalassemia occur when the hematopoietic stem cells generate malformed red blood cells or hemoglobin. Often the best chance for a cure for these and other diseases is to eliminate the patient's own defective hematopoietic stem cells and replace them with healthy stem cells from a closely matched donor. But in order to do so, the patient must be able to withstand the pre-treatment, known as conditioning. Most conditioning regimens consist of a combination of chemotherapy and radiation in doses high enough to kill stem cells in the marrow.

Shizuru and her colleagues studied a mouse model of a class of human diseases called myelodysplastic syndromes, or MDS. People with MDS are unable to make mature, properly functioning blood cells and the only cure is a stem cell transplant. The disease primarily affects older adults, who are more likely than younger people to have additional, complicating medical factors and who are less likely to withstand the conditioning regimen.

"Many of these people are elderly and unable to qualify for a transplant," Pang said. "But there is no other cure for MDS."

Because there are many different types of MDS, the patients are assigned risk levels based on disease type, blood test results and the presence or absence of specific mutations in the affected cells. According to the World Health Organization, patients with low-risk MDS have a median survival rate of 5.5 years; those with high-risk disease have a median survival of 2.2 years.

SR1, the anti-CD117 antibody Pang and Czechowicz studied, recognizes CD117 on the surface of hematopoietic stem cells isolated from either healthy donors or from patients with MDS. They found that the antibody blocked the growth of both healthy and diseased stem cells in a laboratory setting. Then, the researchers investigated the effect of SR1 treatment on mice that were engineered to have a dual blood

systems consisting of both human and mouse hematopoietic stem cells.

They found in the mice that SR1 quickly and efficiently eliminated both healthy human hematopoietic stem cells and cells isolated from low-risk MDS patients. In those animals with diseased human stem cells, SR1 pre-treatment significantly improved the ability of healthy hematopoietic stem cells to engraft after transplantation.

"SR1 directly targets the disease-initiating cells for elimination in the mice, even though these cells typically have a significant competitive advantage," Pang said. "This is the first antibody directed against CD117 that has been proven to clear both normal and diseased human cells from the recipient. We are very pleased with the results."

Although SR1 is also able to significantly reduce the number of high-risk MDS cells from the mice, the researchers found that the effect was transient: The diseased cells eventually returned. However, when anti-CD117 antibody treatment was used in combination with transplants of normal human blood stem cells, normal human blood formation was restored and high-risk MDS cells remained stably suppressed throughout the course of the experiment.

"Based on the results of this study and others, we have received approval from the Food and Drug Administration to move forward with a clinical trial for MDS patients using a version of SR1 appropriate for a trial in humans," Shizuru said. "We are very hopeful that this body of research is going to have a positive impact on patients by allowing better depletion of diseased cells and engraftment of healthy cells."

Antibody-drug combo may remove need for tissue-matching in organ transplantation

Researchers' experimental approach for preparing mice for blood stem cell transplantation may one day make it possible in humans to safely transplant organs or cells from any donor to any recipient.

For those hoping for a new heart, liver, bone marrow or other organ, the wait for a compatible organ has always been part of the excruciating drama of transplantation.

If an organ isn't tissue-matched — that is, if it doesn't bear an immunological resemblance to the patient's own tissue — the patient's body will likely reject it. Even when the organ is a close match, there are enough differences that the organ recipient will likely have to take anti-rejection drugs, possibly for life. These drugs have toxic side effects and leave patients vulnerable to infections.

All of this may change in the future because of a set of collaborative discoveries by institute member Agnieszka Czechowicz, MD, PhD and her colleagues at Stanford, Harvard, Boston Children's Hospital and the National Institute of Allergy and Infectious Diseases.

Czechowicz began the work as a graduate student in the laboratory of institute director Irving Weissman, MD. She continued her research during her residency and fellowship at the Dana Farber Cancer Institute/Boston Children's Cancer and Blood Disorders Center before returning to Stanford as a faculty member.

In a pair of papers published in *Nature Communications*, the researchers describe how an antibody-drug conjugate seeks out and eliminates blood-producing stem cells in mice. In the first study, Czechowicz and her colleagues found that the antibody-drug conjugate could effectively and specifically eliminate blood-producing stem cells in a mouse without damaging side effects. In the second study, the researchers found that this same antibody-drug conjugate, in combination with a short-course of immune suppression, could also be used to replace some of a mouse's blood-producing stem cells with donor stem cells that do not match those of the recipient. "The result is a chimera — a mix of original and transplanted blood stem cells — in the recipient," Czechowicz said. Mice with these mixed blood and



Agnieszka Czechowicz, MD, PhD

immune cells did not develop any complications and were able to accept a skin transplant from the stem-cell donor even many months later, the researchers found.

"Using this technique to make recipients tolerant to donor organs is incredibly exciting," Czechowicz said. "It indicates that we could have a relatively safe method of inducing tolerance without the need for chronic immune suppression, and do that without needing to match donors and recipients for tissue type. This approach could be transformative for the transplant field."

Antibody-drug conjugates are one way to eliminate chemo-radiotherapy to enable pure blood stem cell transplants. Weissman and Czechowicz have taken diverging paths since their initial experiments 12 years ago in which, starting with immune deficient mice, the team used an antibody to CD117 on blood-forming stem cells to allow purified HSC to engraft. But it didn't work in immune competent mice unless an antibody to the CD47 'don't eat me' molecule on HSC was added, and then stem cell grafts from identical twin mice allowed HSC to engraft them. This is moving toward the clinic, increasing the opportunity for engrafting gene modified HSC. Moving to a "matched" donor, which occurs in 25% of siblings, required the elimination not only of the recipient blood stem cells, but also their immune T cells that can reject the donor blood stem cell graft, as the team reported in 2016. In that study, anti-CD47 plus anti-T cell antibodies plus anti-CD117 antibodies worked. But in these times when the average family has 1-2 children, the probability of a match is not great. Within any family, the mother shares half of the matching genes,

as does the father, or any child of the recipient, plus half the siblings. This half-match is called a haplo match. As an example, if the father is AB at the important matching gene, and the mother CD, the child will be AC, or AD, or BC, or BD. The AC and AD children share the father's A gene, while the BC and BD children share the father's B gene. But two kinds of immune cells, natural killer cells (NK) and scavenger macrophage cells, could reject the HSC graft even if the T cells have been eliminated. Benson George and Weissman have recently solved that case (see story below). Antibodies to NK cells and antibodies to an immune-stimulating molecule have allowed not only AB HSC to engraft antibody-conditioned BC recipients, but heart transplants from the AB are not rejected by the 'chimeric' immune system derived from donor and from recipient blood-forming stem cells. Their paper, published in June 2019 in *Cell Stem Cell* have set the stage for clinical translation where virtually all humans can be the recipient of an HSC graft from a family member, and also tolerate a tissue or organ graft from that donor.

Antibody treatment allows transplant of mismatched stem cells, tissues in mice

If the antibody treatment is eventually found to be viable in humans, it could increase the numbers of people who benefit from hematopoietic stem transplants, Stanford researchers said.

A combination of six antibodies can successfully prepare mice to accept blood and immune stem cells from an immunologically mismatched donor, according to a study by institute Director Irv Weissman, MD, and his colleagues.

The recipient animals can then accept an organ or tissue transplant matching that of the donor stem cells without requiring ongoing immune suppression.

The recipient animals can then accept an organ or tissue transplant matching that of the donor stem cells without requiring ongoing immune suppression.

If the findings are replicated in humans, the work could transform the treatment of people with immune or blood disorders while also vastly increas-

ing the pool of available organs for those who need transplants.

The work builds on a series of recent studies conducted at Stanford that may pave the way for this type of stem cell transplant, known as a hematopoietic stem cell transplant, to safely treat a variety of disorders. The technique is now primarily used to treat cancers of the blood and immune system.

"Radiation and chemotherapy are the current standard for preparing patients for a bone marrow transplant," said Weissman. "For the past decade, we have been working to step-by-step replace these nonselective and dangerous treatments with targeted antibodies. This study is an important milestone that began with our isolation of purified blood stem cells 30 years ago."

Weissman is the senior author of the study, which was published in *Cell Stem Cell*. Graduate student Benson George is the lead author.

“This study indicates that it’s possible to perform these transplants in mice in a much gentler way without requiring a complete match between the donor and the recipient stem cells,” George said. “It also opens the door to increasing the availability of solid organs for transplant.”

“We wanted to eliminate three major barriers: the toxicity of the conditioning procedure, the need to have an immunologically

matched donor and the difficulties in transplanting purified hematopoietic stem cells,” George said.

The researchers found that treating mice with a combination of six specific antibodies safely and efficiently eliminated several types of immune cells in the animals’ bone marrow and allowed haploidentical pure hematopoietic stem cells to engraft and begin producing blood and immune cells without the need for continued immunosuppression.

The degree of difference in a haploidentical transplant is similar to what naturally occurs between parent and child, or between about half of siblings.

“This finding suggests that, if these results are replicated in humans, we could have a child with sickle cell anemia in the clinic and, rather than considering stem cell transplant as a last resort and contingent on finding a perfectly matching donor, we could instead turn to transplant with stem cells from one of the child’s parents as a first-line therapy,” George said. Additional experiments showed that the mice treated with the six antibodies could also accept completely mismatched purified hematopoietic stem cells, such as those that might be obtained from an embryonic stem cell line.

After transplantation with the mismatched stem cells, the recipient mice developed blood and immune systems that contained cells from both the donor and the recipient. This allowed them to sub-

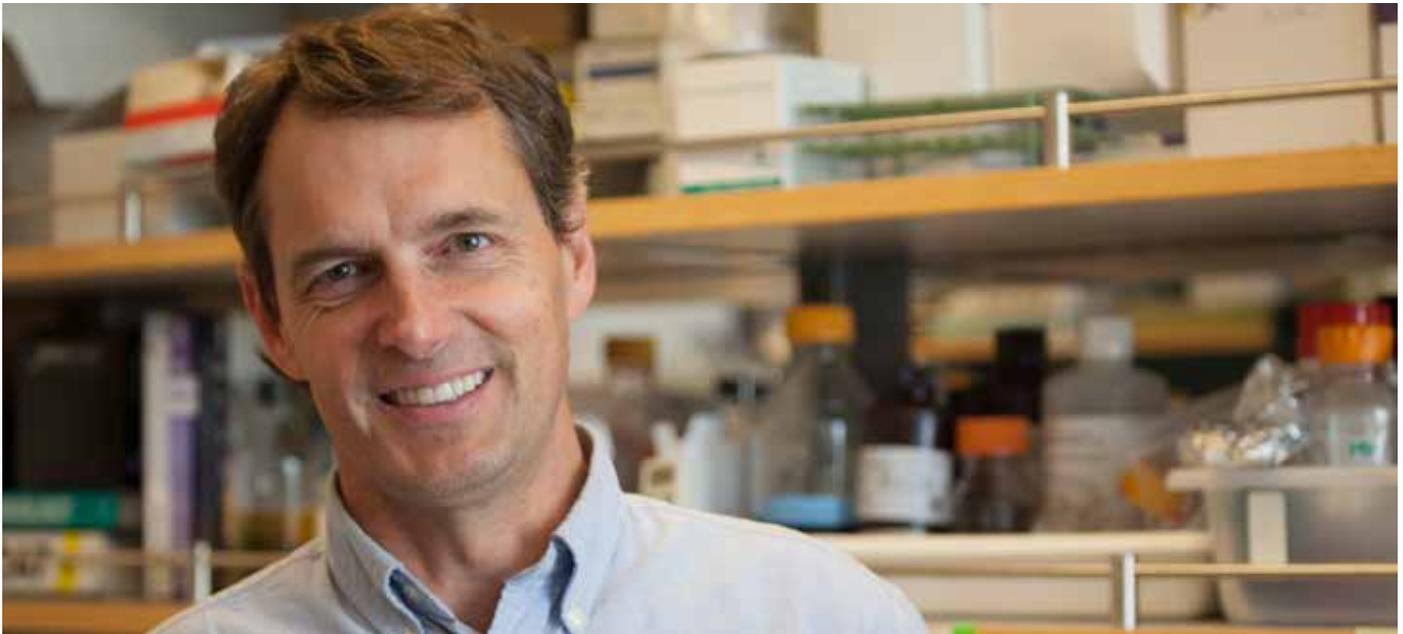


MD/PhD student Benson George (left) and research associate Devon Cajuste

sequently accept transplants of heart tissue from animals genetically identical to the donor animals. “The immune systems exist together in a kind of a symbiosis,” George said, “and they view both the donor and recipient tissue as ‘self.’ This suggests that it may be possible to make haploidentical stem transplants both safe and achievable in human patients without the need for either conditioning with radiation or chemotherapy or subsequent immunosuppression.”

The researchers are next planning to conduct similar antibody-mediated conditioning followed by transplant with mismatched hematopoietic stem cells in large animal models.

If the technique one day clears the hurdles necessary to prove it is safe and effective in humans, the researchers envision a time when people who need transplanted organs could first undergo a safe, gentle transplant with hematopoietic stem cells derived in the laboratory from embryonic stem cells. The same embryonic stem cells could also then be used to generate an organ that would be fully accepted by the recipient without requiring the need for long-term treatment with drugs to suppress the immune system.



Matthew Porteus, MD, PhD

Scientists use CRISPR for possible ‘Bubble Boy’ therapy

In preclinical trials, Stanford scientists and their collaborators harnessed the gene-editing system CRISPR-Cas9 to replace the mutated gene that causes the devastating immune disease.

Very rarely, a boy is born with a mutation that renders his immune system barren — devoid of any and all immune cells. The disease, X-linked severe combined immunodeficiency, or SCID-X1, often is referred to as the bubble boy disease. It affects only males and is lethal if not treated in the first year of life, or if the boy lives in a sterile environment. Now, scientists at the institute and their collaborators have used the gene-editing system CRISPR-Cas9 to devise a new treatment to replenish immune cells in mouse models of SCID-X1. The results are promising, the scientists said, because they believe the treatment could potentially work in humans, as well.

SCID-X1 affects about 1 in 50,000 male births. Those with the disease suffer from a debilitating mutation in a single gene, IL2R gamma. When this gene is defective, the immune system never develops.

The standard treatment for patients with SCID-X1 is a bone marrow transplant, which supplies them with stem cells that will give rise to a working immune system. But the transfer process is tricky and not guaranteed to work. So, Matthew Porteus, MD, PhD,

professor of pediatrics, came up with a new idea: correct the genes in the patients’ own cells.

Through CRISPR-Cas9, Porteus and his team have done just that. Using cell samples that came from people with SCID-X1, the researchers genetically altered the class of stem cells that give rise to blood and immune cells. Their approach got the gene working again.

Each mouse that received the edited cells began generating new immune cells and displayed no detectable adverse side effects. “To our knowledge, it’s the first time that human SCID-X1 cells edited with CRISPR-Cas9 have been successfully used to make human immune cells in an animal model,” said postdoctoral scholar Mara Pavel-Dinu, PhD.

A paper describing the work was published in *Nature Communications*. Porteus is the senior author, and Pavel-Dinu is the first author.

Gene-based therapy for SCID is not new. In the 1990s, scientists began to dabble in gene therapies that used a virus to deliver a new, functional IL2R gamma gene. “It was very effective, but about 25 percent of the patients developed a leukemia



other big thing here is safety,” Porteus said. “We don’t see any abnormalities in the mice that receive the treatment. More specifically, we also performed genetic analysis to see if the CRISPR-Cas9 system made DNA breaks at places that it’s not supposed to, and we see no evidence of that.” That’s crucial, Porteus said, because it ensures that other healthy

“We’ve shown that this is a novel and effective strategy to potentially treat this disease.”

genes aren’t being erroneously tampered with. Translating lab research to a patient population takes time, Porteus said, but he’s optimistic that if larger mouse studies are successful, the CRISPR-Cas9 gene therapy could be piloted in human patients in the next year or two through the Stanford Center for Definitive and Curative Medicine. Institute Co-Director Maria Grazia Roncarolo, MD, also participated in the research.

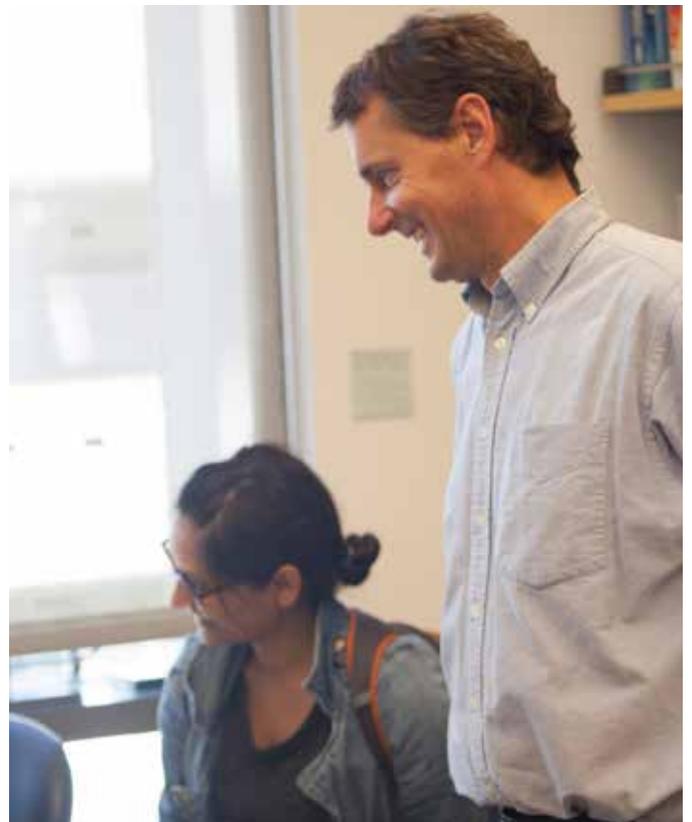
because the virus integrated into an erroneous gene,” Porteus said. “It showed both the promise of what gene therapy could do and highlighted the area that needed to be improved.”

Porteus’ approach uses CRISPR-Cas9 to create a double-stranded break in DNA to insert a healthy copy of the IL2R gamma gene in the stem cells that create immune cells.

Using the gene-editing system, scientists tweaked cells from six people with SCID-X1 and then transplanted those cells into mouse models of SCID-X1. Those mice were then not only able to make their own immune cells, but many of the edited cells retained something called “stemness,” meaning that they maintained their ability to continually create new cells.

“The idea is that these modified stem cells will give rise to the blood system and the immune system for the entirety of the patient’s life, which we hope is 90 or more years,” Porteus said. “And we see evidence for that in our study.”

“We’ve shown that this is a novel and effective strategy to potentially treat this disease, but the



Anti-CD47 cancer therapy safe, shows promise in small clinical trial

An immunotherapy conceived at the Institute for Stem Cell Biology and Regenerative Medicine appeared safe in an early clinical trial. Half of the participants responded positively to the treatment, aimed at triggering macrophages to engulf cancer cells, researchers reported.

A novel immunotherapy appears safe for use in patients with a type of blood cancer called non-Hodgkin's lymphoma, according to a phase-1 multicenter clinical trial led by a researcher at the Stanford University School of Medicine.

Although some patients showed signs of a transitory anemia or reactions at the injection site, there were few other significant side effects to the treatment, the researchers said.

The therapy combines an experimental antibody developed by researchers at the institute and a commercially available anti-cancer antibody called rituximab. The experimental antibody, known as Hu5F9-G4, blocks the protein CD47, a "don't eat me" signal that inhibits immune attacks on cancer cells. The antibody combination was used to treat people with two types of non-Hodgkin's lymphoma: diffuse large B-cell lymphoma and follicular lymphoma. Half of the 22 people enrolled in phase 1 of the trial had a positive response to the therapy, and about one-third went into complete remission from their cancer.

"It was very gratifying to see how the treatment was well-tolerated and showed a clinically meaningful response," said Ranjana Advani, MD, professor of medicine at Stanford.

The group described their results in *The New England Journal of Medicine*. Advani is the lead author. The senior author is Sonali Smith, MD, a professor of medicine at the University of Chicago. The trial was funded by Forty Seven Inc., the company that licensed the patent from Stanford to produce Hu5F9-G4, and by the Leukemia and Lymphoma Society.

In 2010, researchers led by institute director Irv Weissman, MD, Ravi Majeti, MD, PhD, and Sidd Jaiswal MD, PhD, showed that nearly all cancer cells cover themselves with a protein known as CD47, which acts as a "don't eat me" signal to immune cells called macrophages.

Weissman and his colleagues later developed an antibody called Hu5F9-G4 that blocks the CD47 protein, prompting macrophages to engulf and devour cancer cells. Rituximab is an antibody that the team, with Mark Chao MD, PhD and Ash Alizadeh, MD, PhD, has shown to amplify positive "eat me" signals.

The combination of rituximab and Hu5F-G4 has previously been shown to work well in fighting human cancers in animal models, but this is the first published result of a clinical trial of this therapy in humans. The clinical trial builds upon previous studies of CD47 and its role in cancer that were

Of the 22 patients enrolled in the trial, 11 showed a clinically significant reduction in their cancers. In 8 of those patients, all signs of cancer were eliminated.

conducted in Weissman's laboratory and funded by the California Institute of Regenerative Medicine and the Ludwig Institute for Cancer Research.

For this clinical trial, participants were administered a combination of Hu5F-G4 and rituximab at 10 clinical centers. All the patients in the study had failed to respond to or relapsed after at least two previous types of therapy. Hu5F-G4 was administered to the patients at slowly increasing dosages to test for adverse reactions to the antibody. Of the 22 patients enrolled in the trial, 11 showed a clinically significant reduction in their cancers. In eight of those patients, all signs of cancer were eliminated, Advani said. Three other patients in the trial did not respond to the treatment and died due to disease progression.



Michael Stornetta participated in the clinical trial of the anti-CD47 antibody against his cancer.

Although there are many things that can kill cancer cells, the real test of a therapy is whether it can kill the cancer cells without harming normal cells. Advani said she was particularly pleased that the researchers observed only minor side effects in the

By strange coincidence, the same day that he learned the treatment was working, he also learned that his house burned to the ground.

participants.

“It’s very exciting to have a potentially new class of immunotherapy like this,” said Advani, who is the Saul A. Rosenberg, MD, Professor of Lymphoma. “For the first time we have an antibody that activates macrophages against cancer and appears to be safe

for use in humans.” Clinical trial participant Michael Stornetta, a retired Santa Rosa businessman who said he had never previously been sick with anything worse than colds, flus and the usual childhood maladies, was hit with follicular lymphoma over five years ago. He said that after attempting multiple therapies with “varying degrees of success,” he was referred to the Hu5F9-G4 trial at Stanford.

In October of 2017, he drove with his wife and son to Stanford to view the first scans that would reveal whether the experimental treatment was working. The scans showed that his cancer was significantly

reduced. By strange coincidence, the very day that he learned that the treatment was working, he also learned that his house had burned to the ground in the Sonoma County fires. “It felt like a miracle on one side and devastation on the other,” Stornetta said.

Weissman and institute member Ravindra Majeti, MD, PhD, professor of medicine at Stanford, are co-authors of the paper. Weissman is a founder of Forty Seven, and he and Majeti are board members of the company. Other Stanford-affiliated authors are instructor of medicine Mark Chao, MD, PhD, and clinical research coordinator Thu Tran. Chao is a founder of Forty Seven and its vice president of clinical development.

Researchers at the City of Hope, Sarah Cannon Research Institute/Tennessee Oncology, the University of Alabama-Birmingham, Washington University in St. Louis, Levine Cancer Institute, the University of Chicago, the National Cancer Institute, the Dana Farber Cancer Institute and the University of Oxford also contributed to the study.

Surgical adhesions can be treated, prevented in mice

An immunotherapy conceived at the institute appeared safe in an early clinical trial. Half of the participants responded positively to the treatment, aimed at triggering macrophages to engulf cancer cells, researchers reported.

A cellular culprit — as well as a possible treatment — for a common, sometimes life-threatening post-surgical complication has been identified by researchers at the institute.

The condition arises when abnormal fibrous connections called adhesions form after abdominal surgery, tethering our normally slippery organs together or anchoring them to the abdominal wall. Symptoms can include chronic pain, female infertility, bowel obstruction and, occasionally, death. According to the National Institutes of Health, the annual cost of treating post-surgical adhesions in the United States surpasses \$1 billion.

“This is a very common surgical complication, but it’s not been well-studied,” said Jonathan Tsai, MD, PhD, a former member of the Weissman lab at Stanford and now resident physician at Brigham and Women’s Hospital in Boston. “Until now, it wasn’t even known what cell type was involved in originating the adhesions. Now we’ve come up with a way to isolate the injured tissue before they form the adhesions, and identify the molecular pathways involved.”

The researchers developed and studied a mouse model of adhesion formation to identify the cell responsible for the initial steps. They also showed that an antibody-based therapy could break down those that had already formed. The hope is that similar techniques could help treat post-surgical adhesions in humans.

Tsai is the lead author of the work, which was published in the journal *Science Translational Medicine*. Yuval Rinkevich, PhD, a former postdoctoral scholar, and institute director Irving Weissman, MD, share senior authorship of the study. Institute member Gerlinde Wernig, MD, an assistant professor of pathology, and others also contributed to the research.

The researchers found that a combination of two antibodies — one that targets the cells responsible for adhesion formation and another that silences a

“don’t eat me” signal that cancer cells use to evade the immune system — could significantly reduce the severity of established adhesions in the animals.

“Although we used a mouse model to study adhesion formation,” Weissman said, “we found similar characteristics in adhesions from patients, which makes us think this approach could be translated into the clinic.”

Normally the surface of our abdominal organs and the lining of our abdominal cavity are covered with a slippery membrane called mesothelium. The mesothelium allows our organs to glide smoothly past one another when we bend, twist or run. When the mesothelium is disturbed, fibrous connections form between neighboring surfaces, ranging in severity from single threads to vast, immobilizing webs.

The NIH estimates that about 93 percent of abdominal surgeries result in adhesions and that about 20 percent of surgical patients will be re-hospitalized for adhesion-related complications.

Although the complication is common, it’s not well-understood. Researchers have identified some cell types involved in later steps of the process, but it’s not been known which cell type is responsible for the initial steps. It appears to arise in regions where blood flow is restricted, such as in the tiny pinches of tissue caused by surgical sutures. As a result, less oxygen is delivered to cells in the region — a condition known as hypoxia.

Tsai used a mouse model of the condition to trace the formation of adhesions and the resulting patterns of gene expression in the mesothelium.

“We found that adhesions arise from cells of the mesothelium after injury,” Tsai said. “By tracing the patterns of gene expression, we were able to come up with a cellular ‘family tree’ for these fibrotic tissues



Gerlinde Wernig, MD, PhD

and identify the biological pathways involved.” Tsai and his colleagues found that, in mice, cells of the mesothelium respond to hypoxia by making a protein called HIF1alpha. This in turn promotes the expression of other proteins essential for the formation of adhesions. When the researchers treated the animals with a small molecule that inhibited the activity of HIF1alpha, the resulting adhesions were significantly less severe.

Possible role for macrophages

They also found that treating the animals with antibodies that bind to mesothelin, a protein specific to injured mesothelium, significantly reduced the severity of adhesions that had already formed.

Combining anti-mesothelin antibodies with an anti-CD47 antibody had an even greater effect, suggesting that roving immune cells called macrophages, which gobble up sick or dying cells, may also play a role in removing abnormal fibrous tissue.

By tracing the patterns of gene expression, we were able to come up with a cellular ‘family tree’ for these

fibrotic tissues.

“When the mesothelium is irritated, it begins to express mesothelin, which is normally expressed only very early in development,” Weissman said. “This triggers proliferation of the cells and initiates an inflammatory cascade that brings in immune cells and proteins that glom everything up with fibrous tissue. But these cells also have CD47 on their surface, and we’ve found that anti-CD47 can synergize with anti-mesothelin to remove these adhesions after they’ve been formed.”

Finally, the researchers studied samples of adhesions that had been removed from patients. They found that the human tissue expressed many of the same genes and used similar biological pathways as those the researchers identified in the mice. Tsai and his colleagues are hopeful that similar antibody-based treatments may help prevent or treat the formation of adhesions in people.

‘DNA origami’ triggers tissue generation in early development

In trying to decipher the “DNA origami” responsible for the generation of transplantable human skin, institute researchers have uncovered a master regulatory hierarchy controlling tissue differentiation.

A developing embryo faces the difficult task of concocting myriad tissue types — including skin, bone and the specialized glop that makes up our internal organs and immune system — from essentially the same set of ingredients: immature, seemingly directionless stem cells. Although some of the important players that provide direction to this transformation are known, it’s not been clear exactly how they work together to accomplish this feat. Now, institute researchers have identified a key regulatory hierarchy in which proteins called morphogens control gene expression by directing the looping of DNA in a cell. This looping brings master regulators called transcription factors in contact with specific sets of genes necessary to make particular tissue types.

Varying concentrations and types of morphogens cause different looping events, directing different cell fates much in the same way that railroad workers

control the direction and eventual destination of a train car by connecting different portions of track. Although the researchers were particularly interested in learning more about how to stimulate the production of a type of skin cell called keratinocytes to treat epidermolysis bullosa, a blistering skin disease with few treatments, they believe their findings may have implications for the derivation of other therapeutically useful tissue types.

“For the first time, we were able to see how morphogens and master transcriptional regulators work together to make specific cell types,” said institute member Anthony Oro, MD, PhD, professor of dermatology. “We’ve always wondered how a transcription factor required for the production of vastly different cell types knows which genes to make into proteins in which situation. Now we’ve answered that question: morphogens help the master transcription factors hook up to the right targets.

Changing the concentration or type of morphogen, or even the order in which they are added to a cell, causes dramatically different outcomes.”

A paper describing the research was published in the journal *Nature Genetics*. Oro, who is also the Eugene and Gloria Bauer Professor, is the senior author. Postdoctoral scholar Jillian Pattison, PhD; former postdoctoral scholar Sandra Melo, PhD; and SCBRM graduate student Samantha Piekos share lead authorship.

Morphogens are responsible for the body patterning that ensures, for example, that a fly’s wing ends up on its thorax rather than the top of its head. They were the first important class of proteins identified in the early days of developmental biology, in part because their effect on a developing embryo is so dramatic. Subsequent studies showed that they work through the process of diffusion and can have different effects based on their concentration throughout the embryo. Cells that are near other cells making and releasing the morphogen are exposed to a much higher concentration than those farther away; as waves of varying morphogens overlap and interact, they direct the proper placement of legs, wings and the head, for example.

Soon, researchers also identified other types of proteins called master transcriptional regulators that bind to DNA to control the expression of specific genes throughout the cell. But they quickly learned that each of these regulators could spark the formation of vastly different cell types, and it was unclear how each regulator knew to favor the development of one tissue type over another.

Oro and his colleagues were studying the effect of two well-known morphogens involved in skin development — BMP4 and retinoic acid — on the activity of a master transcriptional regulator called p63 that is responsible for tissue types as diverse as skin, thymus and the lining of the esophagus.

In particular, they were interested in the process by which human embryonic stem cells can be triggered to develop into keratinocytes to form sheets of skin to repair the blistering and open wounds seen in people with epidermolysis bullosa. Previous attempts, although somewhat successful, yielded impure populations of cells that are difficult to use therapeutically. In search of a more reliable way to produce the cells, they wondered if they could generate keratinocytes by exposing the stem cells to a defined combination of morphogens

and transcription factors. To do so, however, they experimented with when, and how much, of each component to add and watched how the cells reacted.

The researchers found that, although p63 is required to make skin cells from embryonic stem cells, it is not sufficient. In the absence of BMP4 or retinoic acid, nothing happens, even if p63 is snugly bound to its landing pad on the DNA. However, when BMP4 or retinoic acid is added, the DNA conformation changes, and p63 begins transcribing skin-specific genes. This dependence of p63 activity on the presence of morphogens was unexpected and telling.

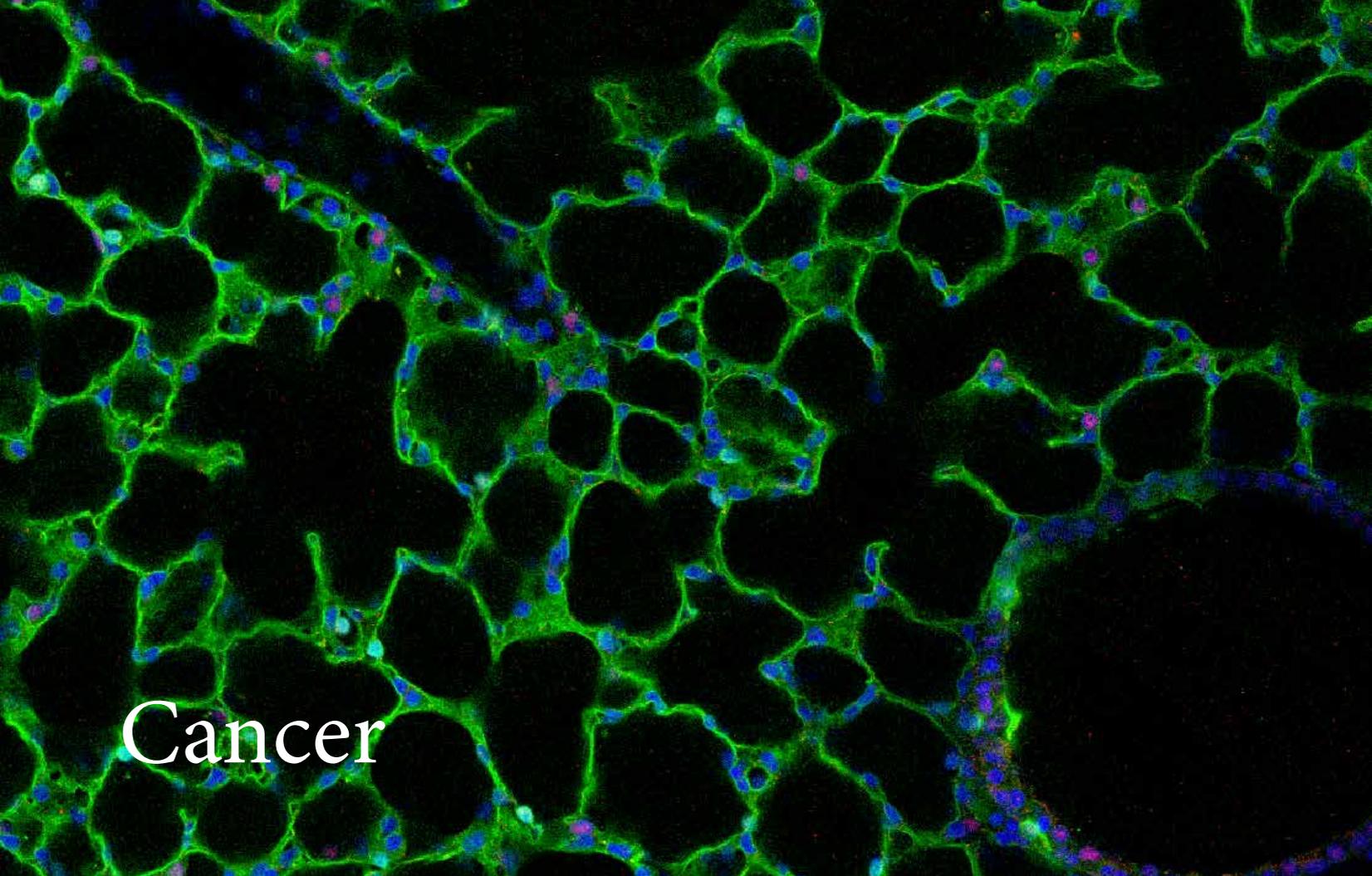


Samantha Piekos

“Basically, p63 binds to the DNA, and then sits back and waits, twiddling its thumbs, until it is connected to specific genes by the morphogen-caused folding,” Oro said. “Or sometimes the DNA folds weeks or months in advance, and this foreshadowing sets up a particular differentiation plan, poisoning the chromatin to assume a specific fate when the transcriptional regulator is added.”

Additionally, the researchers discovered that exposing the stem cells to retinoic acid and BMP4 together also triggered the expression of p63, indicating a complex and synergistic feedback loop that controls skin development.

“Now we have the tools necessary to understand how the DNA folds and unfolds in response to changing conditions,” Oro said. “Deciphering this chromatin origami is critical to learning how to make specific cell types for use in tissue replacement therapies. We know now that certain combinations and concentrations of morphogens cause the cells to fold their DNA in a certain way, while another stimulates the DNA to assume an entirely different conformation. Making specific cell types is not a random event, and we can work to harness and accelerate this process to generate all kinds of transplantable tissues.”



Cancer

The application of stem cell biology to **cancer research** is having a profound impact on our understanding of how cancer arises and propagates.

This year, in addition to new and continuing **clinical trials** of innovative cancer therapies, institute researchers have advanced our understanding of how cancers arise, how to diagnose and analyze them, and how better to treat them.

Liquid biopsy predicts lymphoma therapy success within days



Maximilian Diehn (left) and Ash Alizadeh led a study that found a blood test could show whether patients with a type of blood cancer were responding well to initial treatment or needed more aggressive treatment.

Changes in circulating tumor DNA levels quickly predict how patients with diffuse large B cell lymphoma are responding to therapy, according to a Stanford-led study. Currently, patients wait months for the results.

A blood test can predict which patients with a type of cancer called diffuse large B cell lymphoma are likely to respond positively to initial therapy and which are likely to need more aggressive treatment, according to a multicenter study led by researchers at the institute.

The study validates the clinical usefulness of tracking the rise and fall of circulating tumor DNA, or ctDNA, in the blood of patients before and after therapy. It suggests that clinicians may soon be able to determine how a patient is responding to treatment within days or weeks of starting therapy rather than waiting until therapy is completed five to six months later.

“Although conventional therapy can cure the majority of patients with even advanced B cell lymphomas, some don’t respond to initial treatment,” said associate professor of medicine Ash Alizadeh, MD, PhD. “But we don’t know which ones until several months have passed. Now we can predict nonresponders within 21 days after the initiation of treatment by tracking the levels of ctDNA in a

patient’s blood. We can look earlier and make a reliable prediction about outcome.”

The study was published in the *Journal of Clinical Oncology*. Alizadeh shares senior authorship with associate professor of radiation oncology Maximilian Diehn, MD, PhD. Instructor of medicine David Kurtz, MD, PhD, and postdoctoral scholar Florian Scherer, MD, are the lead authors. Institute member and assistant professor of biomedical data science Aaron Newman, PhD and others also took part in the research.

Diffuse large B cell lymphoma, a blood cancer, is the most common type of non-Hodgkin lymphoma. Because it is highly biologically variable, patients vary widely in their response to treatment. Although most people are cured by conventional therapy, about one-third are not. Being able to predict early in the course of treatment those who will need additional or more aggressive therapies would be a significant boon to both clinicians and patients. Circulating tumor DNA is released into the blood by dying cancer cells. Learning to pick out and read

these DNA sequences among the thousands or even millions of other noncancerous sequences in the blood can provide valuable insight into the course of the disease and the effectiveness of therapy. Recently, Diehn and Alizadeh showed that ctDNA tracking can also predict lung cancer recurrence weeks or months before any clinical symptoms arise.

“Combined with our recent study on lung cancer, our new findings speak to the power and likely utility of using ctDNA to assess how well cancer treatments are working in an individual patient. We are very hopeful that the approach will ultimately be extensible to most if not all cancer types,” Diehn said. If we can identify those people who are responding extremely well, we could spare them additional treatments.

In this study, the researchers tracked ctDNA levels in 217 people with diffuse large B cell lymphoma who were treated at six medical centers — three in the United States and three in Europe. For each patient, they compared levels of ctDNA before treatment began with the levels after the first and second rounds of conventional chemotherapy. They then correlated those changes with each patient’s outcome. They found that ctDNA was detectable prior to the initiation of therapy in 98 percent of the people studied. And, as would be expected, the amount of ctDNA in the blood dropped in all patients once treatment began. But the precipitousness of the decline varied. Those people whose ctDNA levels dropped a hundredfold after the first round or three-hundredfold by the second round were much more likely to live 24 months or more without experiencing a recurrence of their disease than those whose ctDNA levels declined more slowly.

“We found that ctDNA levels serve as a very sensitive and specific biomarker of response to therapy within as few as 21 days,” Kurtz said. “Every year, about 30,000 people in the United States are diagnosed with diffuse large B cell lymphoma and, for the most part, they’re treated with six cycles of combination therapy. But we know that not all patients need six cycles. A large fraction could be cured with fewer cycles — maybe even just two. If we can identify those people who are responding extremely well, we could spare them additional treatments. Conversely, we could intensify the therapy or seek other options for those who are not responding as well as we would

have hoped.”

The researchers are encouraged that they saw a similar correlation between changes in ctDNA levels and outcomes in patients from each of the six



Aaron Newman, PhD

participating medical centers, confirming the global usefulness of the analysis. They’re currently planning a clinical trial based on the results, and they’re eager to learn whether they can make similar predictions about the prognoses of patients other than those with diffuse large B cell lymphomas.

“These findings confirm the value of tracking cancer genetics in the blood in real time,” Alizadeh said.

“We are thinking about how to use the tools to best benefit patients, and are very excited to test this approach in other types of cancers.”

‘Chemo brain’ caused by malfunction in three types of brain cells

Three types of cells in the brain’s white matter show interwoven problems during the cognitive dysfunction that follows treatment with the cancer drug methotrexate, Stanford neuroscientists have found.

Three types of cells in the brain’s white matter show interwoven problems during the cognitive dysfunction that follows treatment with the cancer drug methotrexate, Stanford neuroscientists have found.

More than half of cancer survivors suffer from cognitive impairment from chemotherapy that lingers for months or years after the cancer is gone. In a new study explaining the cellular mechanisms behind this condition, scientists at the institute have demonstrated that a widely used chemotherapy drug, methotrexate, causes a complex set of problems in three major cell types within the brain’s white matter. The study also identifies a potential remedy. A drug now in clinical trials for other indications reversed symptoms of “chemo brain,” as the condition is known, in a mouse model, the researchers found. Chemo brain is becoming more common as cancer therapies increasingly allow patients to live many years beyond their diagnoses. There are 15.5 million cancer survivors alive today in the United States, a figure expected to reach 20 million by 2026, according to the National Cancer Institute. But the cognitive side effects of cancer treatment can be debilitating and prolonged: Adults may be unable to return to work, and children often struggle in school. “It’s wonderful that they’re alive, but their quality of life is really suffering,” said the study’s lead author, Erin Gibson, PhD, a research scientist at Stanford. “If we can do anything to improve that, there is a huge population that could benefit.”

Scientists have long known that drugs like methotrexate impair all of the body’s rapidly dividing cells, but how such drugs affect the function of brain cells has been poorly understood.

“Cognitive dysfunction after cancer therapy is a real and recognized syndrome,” said institute member Michelle Monje, MD, PhD, associate professor of neurology and neurological sciences and the study’s senior author. “In addition to existing symptomatic therapies — which many patients don’t know about — we are now homing in on potential interventions



Michelle Monje, PhD

to promote normalization of the disorders induced by cancer drugs. There’s real hope that we can intervene, induce regeneration and prevent damage in the brain.” Chemo brain is especially severe in childhood cancer patients, Monje added, and children have the most to gain from better remedies. In addition to neurons, which transmit nerve impulses, the brain’s white matter contains other cells that help the neurons function. The research focused on three types of those cells: oligodendrocytes, which produce and maintain myelin, the fatty insulating sheath around nerve fibers; astrocytes, which link neurons to their blood supply, promote proper connections between neurons and maintain the neurons’ environment; and microglia, immune cells that can engulf and destroy foreign invaders in the brain, as well as sculpt neural circuitry.

Comparing postmortem frontal lobe brain tissue from children who had and had not received chemotherapy, the researchers showed that there were far fewer oligodendrocyte lineage cells in the brains of the chemotherapy-treated children. If we understand the cellular and molecular mechanisms that contribute to cognitive dysfunction after cancer therapy, that will help us develop strategies for effective treatment. To figure out what was happening to these cells, the researchers injected young mice with methotrexate at

“Cognitive dysfunction after cancer therapy is a real and recognized syndrome.”

levels designed to replicate human exposures during cancer treatment. The mice received three doses at weekly intervals. Four weeks later, the researchers compared the mice’s brains to those of mice that had not received the drug.

Methotrexate chemotherapy was found to damage the brain’s populations of oligodendrocyte precursor cells. Normally, these cells can quickly divide to replace any that are lost, but after methotrexate was administered, this self-renewal process did not happen correctly. More precursor cells than normal were starting down the path of maturation to oligodendrocytes, but they were getting stuck in an intermediate, immature state. The same problem was seen in mice brains six months after methotrexate was administered.

Transmission electron microscopy of the mouse brains after methotrexate administration revealed deficiencies in the thickness of the myelin insulation around nerve fibers, similar to changes in the brains of humans who have received chemotherapy. Mice exposed to methotrexate also exhibited behavioral problems after four weeks that were similar to humans with chemo brain, including motor impairment (slower movement of their forepaws), signs of anxiety on an “open field” test used to assess how threatened the animal feels in an unsheltered environment, and impaired attention and short-term memory function, evidenced by the inability to discern between novel and familiar objects — a symptom that persisted for six months after methotrexate was given.

The researchers injected oligodendrocyte precursor cells from healthy animals into the brains of animals that had received methotrexate to see if the cells’ maturation problems were caused by some aspect of the brain environment after chemotherapy. The precursor cells still began maturing at higher-than-normal rates but did not get stuck partway through the maturation process, indicating that the brain environment was partly responsible for the cells’ abnormal maturation.

Further study showed that microglia, the brain’s immune cells, were persistently activated after methotrexate exposure for at least six months. The activated microglia caused problems for astrocytes, the cells that help neurons get nutrients and function properly. Administering a drug that selectively depleted microglia to mice that had been treated with methotrexate reversed many of the cognitive symptoms of chemo brain and reversed the abnormalities in maturation of oligodendrocyte precursor cells, activation of astrocytes and myelin thickness.

“The biology of this disease really underscores how important intercellular crosstalk is,” Monje said. “Every major neural cell type is affected in this pathophysiology.” She suspects this type of complex dysfunction may also underlie other cognitive disorders. “I think that is probably more the rule than the exception,” she said.

More research is needed to understand exactly how the different cell types are signaling to each other, as well as when and how medications could be best deployed against chemo brain.

“If we understand the cellular and molecular mechanisms that contribute to cognitive dysfunction after cancer therapy, that will help us develop strategies for effective treatment,” Monje said. “It’s an exciting moment.”

Researchers develop urine test for bladder cancer

The researchers found that by testing for fragments of cancer DNA in urine, they could find the cancer in early stages of development, when it's easier to treat.

Researchers at the institute have developed a highly sensitive urine test for diagnosing and monitoring bladder cancer.

The test involves looking for fragments of cancer DNA in urine samples. "This study describes a new diagnostic approach to bladder cancer focused on analysis of urine samples," said institute member Maximilian Diehn, MD, PhD, associate professor of radiation oncology. "Urine is in direct contact with bladder tumors, which shed some of their DNA into it."

The findings were published in the journal *Cancer Discovery*. Diehn shares senior authorship with Ash Alizadeh, MD, PhD, associate professor of medicine. Postdoctoral scholars Jonathan Dudley, MD, and Joseph Schroers-Martin, MD, are the lead authors.

Sixth most common cancer

Bladder cancer is the sixth most common cancer. More than 80,000 people are diagnosed with it every year in the United States. Currently, the most accurate method of diagnosing bladder cancer is through cystoscopy, an invasive method to visualize the bladder and take tissue samples. Another method is to look for cancer cells in the urine via a cytology test. Although noninvasive, this approach has suboptimal sensitivity, Diehn said.

The research builds on earlier studies co-authored by Diehn and Alizadeh in which they showed that they could detect certain cancers by looking for DNA fragments of tumors circulating in the bloodstream using a method called CAPP-Seq, an abbreviation for cancer personalized profiling by deep sequencing. In the new study, the researchers modified molecular and bioinformatics aspects of this technique to apply to bladder cancer DNA fragments found in urine. They analyzed a total of 67 healthy adults and 118 patients with early stage bladder cancer who either had urine collected prior to treatment or during surveillance.

The researchers found that by testing for bladder cancer in urine, they could detect cancer in the early stages of development, when it can be treated more easily. Their approach correctly identified the presence of bladder cancer in 83 percent of patients

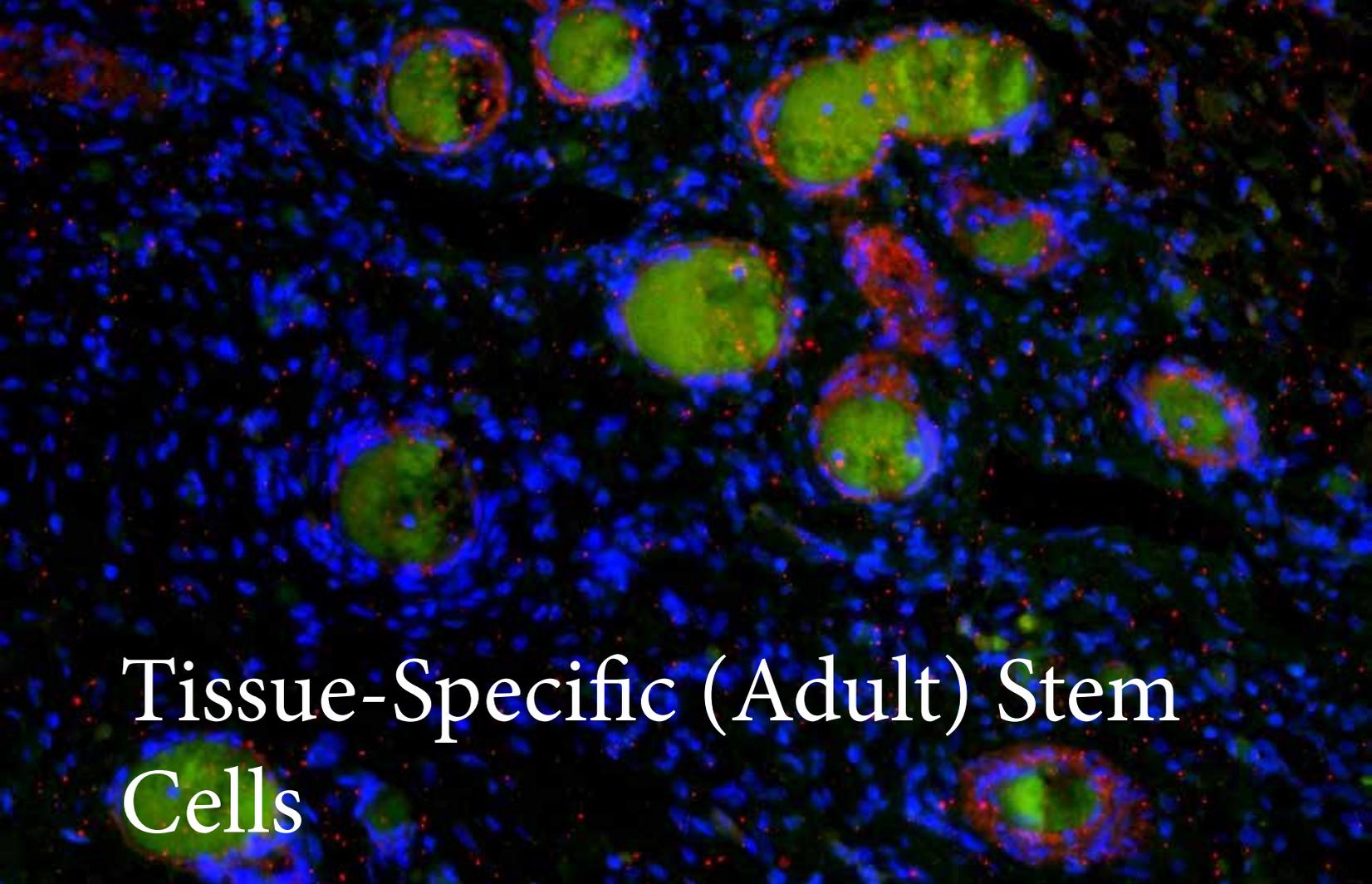
with early stage bladder cancer, compared with only 14 percent for the clinically available urine cytology test.

One of the greatest benefits of the new approach may be its ability to detect the recurrence of bladder



Maximilian Diehn, MD, PhD

cancer after someone has been treated for the disease. "In our test samples, we were able to detect bladder cancer recurrence an average of 2.7 months earlier than could be done with cystoscopy," Alizadeh said. With the new approach, they detected almost all cases of recurrent bladder cancer, nearly double the sensitivity of cystoscopy and cytology. The researchers believe that the method of looking for cancer DNA in body fluids other than blood could be more widely applied. "It may eventually be useful for testing saliva for oral cancer, cerebrospinal fluid for neurological cancers or sputum for lung cancer," Diehn said.

A fluorescence microscopy image showing a dense population of cells. The cells are stained with a blue dye, likely DAPI, which highlights the nuclei. Several cells are also stained with a green dye, which likely targets a specific protein or marker associated with stem cells. The background is dark, making the stained cells stand out.

Tissue-Specific (Adult) Stem Cells

Embryonic stem cells are able to become any kind of cell in the body. As an organism grows, however, stem cells become more specialized. At that point they become what is often called “adult” stem cells, able to become only **specific kinds of tissue**.

For most of our lives, every organ and tissue in the body is regenerated by these tissue-specific stem cells. Learning how these tissue-specific stem cells operate will help us bolster our **natural regenerative abilities**.

Skeletal stem cells regress when tasked with extensive regeneration

New research shows that skeletal stem cells in mice assume a more primitive developmental state in response to extensive regeneration needs and environmental cues.

Adult mouse skeletal stem cells in the jaw revert to a more developmentally flexible state when called upon to regenerate large portions of bone and tissue, according to a study by researchers at the Institute for Stem Cell Biology and Regenerative Medicine. The finding is the first to show that mammalian adult stem cells can march backward along the developmental timeline in a process called de-differentiation to become more primitive in response to environmental signals. In particular, the cells appeared to regress to a cell type that normally occurs within weeks of conception in humans and that give rise to the bones, cartilage and connective tissue of the head and face.

The results suggest the possibility of using naturally occurring adult stem cells, which are usually restricted to generate only a limited panel of closely related progeny, to carry out more extensive regeneration projects throughout the body — much in the way that salamanders or newts can replace entire limbs or tails.

“It’s pretty remarkable that this would happen in an adult animal,” said institute Co-Director Michael Longaker, MD, professor of plastic and reconstructive surgery. “It changes the way we look at skeletal development and regeneration.”



Ava Carter, PhD

A paper describing the research was published in the journal *Nature*. Longaker, the Deane P. and Louise Mitchell Professor in the School of Medicine, shares senior authorship with Howard Chang, MD, PhD, professor of dermatology and of genetics and director of

Stanford’s Center for Personal Dynamic Regulomes. Graduate students Ava Carter and Ryan Ransom are the lead authors. Institute member and assistant professor of surgery Charles K.F. Chan, PhD, and other Stanford scientists also contributed to the



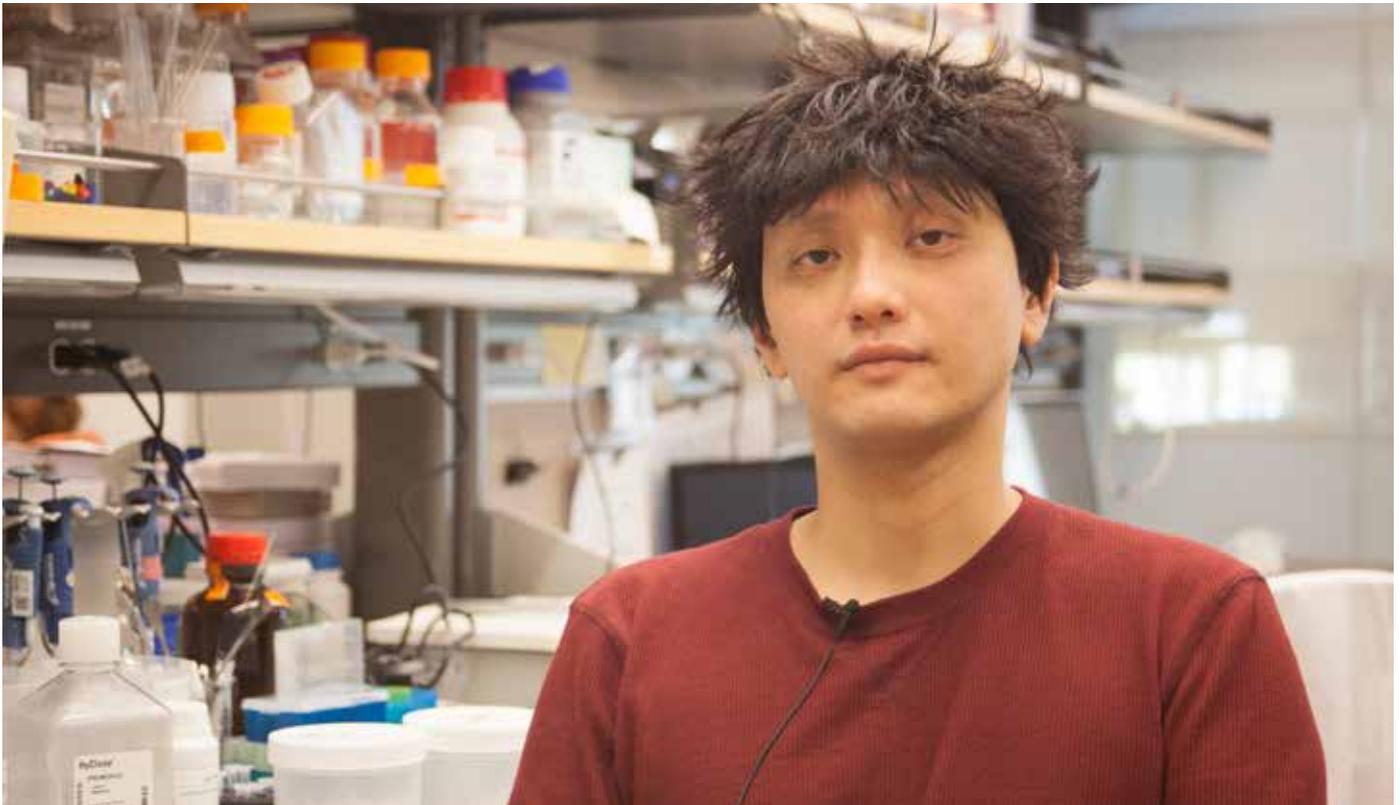
Michael Longaker, MD

research.

The researchers were studying a common surgical technique called distraction osteogenesis, which is often used in newborns or infants to lengthen abnormally stunted bones in the lower jaw. These malformations occur in conditions such as Pierre-Robin sequence, Treacher Collins syndrome and craniofacial microsomia.

During distraction osteogenesis, the bone is surgically fractured, and an adjustable device is inserted to gradually increase the distance between the ends of the bone over the course of weeks. This encourages new bone growth to fill in the gap and create what resembles a normally developed mandible.

“This is a very special system of regeneration that echoes what normally happens in development,” said Chang, who is the Virginia and D.K. Ludwig Professor of Cancer Genomics and a Howard Hughes Medical Institute investigator. “If you cut the bone, and stretch it, you get more bone. But this regeneration requires mechanical force. We wanted



Charles Chan, PhD

to know how skeletal stem cells respond to this kind of environmental signal.”

Carter and Ransom used a technique developed in the Chang laboratory called ATAC-seq to identify gene switches that are turned on in mouse skeletal stem cells in response to the mechanical force of distraction. They found that the cells began to express genes normally found in cranial neural

This is an opportunity to change how we think about the development of not just the skeleton, but also other tissues and organs.

crest cells — cells that arise in humans about five to six weeks after conception and that form the bones, cartilage and connective tissue of the head and face. At the same time, the cells tamped down the expression of genes involved in normal fracture repair.

“This was really a surprise,” Longaker said. “These cells appear to revert back to a cell type responsible

for forming the jaw during early development. That’s why the regenerated mandible looks like one formed in early embryogenesis.”

Further research identified the focal adhesion kinase molecular pathway as a key player in the ability of the skeletal stem cells to detect and respond to mechanical force. Inhibiting this pathway abolished the ability of the cells to make new bone during distraction osteogenesis.

The finding has provocative clinical implications, the researchers believe.

“Now that we’ve identified one of the molecular pathways responsible for this developmental shift, it may be possible to target the proteins in that pathway to achieve a similar outcome without the requirement for physical force,” Carter said.

“We’re beginning to understand in detail how skeletal stem cells are likely to respond to environmental cues in humans,” Longaker said. “This is an opportunity to change how we think about the development of not just the skeleton, but also other tissues and organs. Can we go back in time after an organ is formed to trigger more extensive regeneration? This at least opens the door to that possibility.”

Honeybee protein keeps stem cells youthful

An active protein component of royal jelly helps honeybees create new queens. Stanford researchers have identified a similar protein in mammals, which keeps cultured embryonic stem cells pluripotent.

A mammalian protein similar in structure to the active component of honeybee royal jelly — the queen-making goop that helps worker bees raise a new egg-laying diva for the hive — functions as kind of a fountain of youth for mouse embryonic stem cells, according to Stanford stem cell researchers. The protein causes the cells to remain pluripotent, meaning they can become any cell in the body, under conditions that would normally trigger them to develop into specialized cells.

The unexpected finding is likely to fan the flames of a millennia-old debate as to the regenerative power of royal jelly. More importantly, the discovery reveals new pathways to pluripotency and suggests novel ways to keep stem cells in a state of suspended animation until needed for future therapies.

“In folklore, royal jelly is kind of like a super-medicine, particularly in Asia and Europe,” said assistant professor of dermatology Kevin Wang, MD, PhD, “but the DNA sequence of royalactin, the active component in the jelly, is unique to honeybees. Now, we’ve identified a structurally similar mammalian protein that can maintain stem cell pluripotency.” Wang is the senior author of the study, which was published in *Nature Communications*. Associate professor of surgery Derrick Wan, MD, is the lead author. Assistant professor of obstetrics and gynecology Vittorio Sebastiano, PhD and professor



Vittorio Sebastiano, PhD

of developmental biology Roel Nusse, PhD, both Institute members, contributed to the study. Wang and his colleagues focused on a protein — appropriately called royalactin — that previously had been suggested to be the active ingredient in royal jelly. They applied royalactin to mouse embryonic stem cells to study the cells’ response.

“For royal jelly to have an effect on queen development, it has to work on early progenitor cells in the bee larvae,” Wang said. “So we decided to see what effect it had, if any, on embryonic stem cells.”

To their surprise, Wang and colleagues found that the addition of royalactin stopped the embryonic stem cells from differentiating, even in the absence of the inhibitors. Yet the cells’ response was confusing because mammals don’t make royalactin.

Wang found a mammalian protein called NHLRC3 that was predicted to form a structure similar to royalactin and that was produced early in embryonic development in all animals from eels to humans. Furthermore, they discovered that NHLRC3, like royalactin, was able to maintain pluripotency in mouse embryonic cells, and that it caused a similar gene-expression pattern in them as in those cells exposed to royalactin. They renamed the protein Regina, which is Latin for queen.

The researchers next plan to investigate whether Regina has any therapeutic value in wound healing or cell regeneration in adult animals. They also hope their finding will help researchers discover more or better ways to keep embryonic stem cells pluripotent when grown in the laboratory.



Roel Nusse, PhD

Study identifies stem cell that gives rise to new bone, cartilage in humans

Identification of the human skeletal stem cell by institute scientists could pave the way for regenerative treatments for bone fractures, arthritis and joint injuries.

A decade-long effort led by institute scientists has been rewarded with the identification of the human skeletal stem cell.

The cell, which can be isolated from human bone or generated from specialized cells in fat, gives rise to progenitor cells that can make new bone, the spongy stroma of the bone's interior and the cartilage that helps our knees and other joints function smoothly and painlessly.

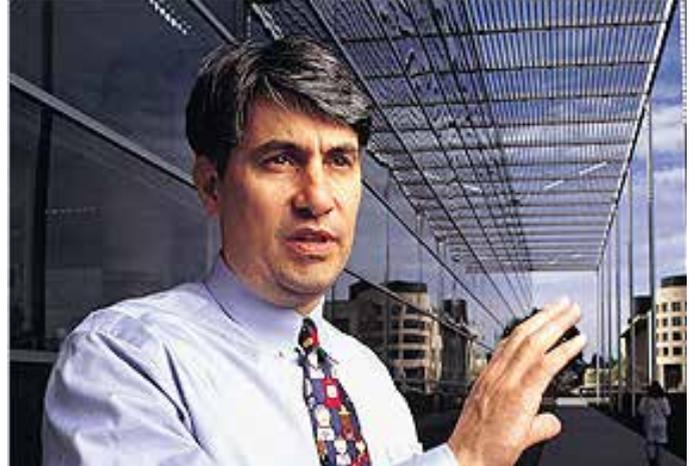
The discovery allowed the researchers to create a kind of family tree of stem cells important to the development and maintenance of the human skeleton. It could also pave the way for treatments that regenerate bone and cartilage in people.

"Every day, children and adults need normal bone, cartilage and stromal tissue," said institute Co-Director Michael Longaker, MD, professor of plastic and reconstructive surgery. "There are 75 million Americans with arthritis, for example. Imagine if we could turn readily available fat cells from liposuction into stem cells that could be injected into their joints to make new cartilage, or if we could stimulate the formation of new bone to repair fractures in older people."

A paper describing the finding was published in 2018 in the journal *Cell*. Longaker is the senior author. The lead authors are institute member Charles K.F. Chan, PhD, assistant professor of surgery; medical student Gunsagar Gulati, MD; Rahul Sinha, PhD, instructor of stem cell biology and regenerative medicine; and research assistant Justin Vincent Tompkins.

The skeletal stem cells are distinct from another cell type called the mesenchymal stem cell, which can generate skeletal tissues, fat and muscle.

Mesenchymal stem cells, which can be isolated from blood, bone marrow or fat, are considered by some clinicians to function as all-purpose stem cells. They have been tested, with limited success, in clinical trials and as unproven experimental treatments



Michael Longaker, MD

for their ability to regenerate a variety of tissues. Recently, three elderly patients in Florida were blinded or lost most of their sight after mesenchymal stem cells from fat were injected into their eyes as an experimental treatment for macular degeneration. "Mesenchymal stem cells are loosely characterized and likely to include many populations of cells, each of which may respond differently and unpredictably to differentiation signals," Chan said. "In contrast, the skeletal stem cell we've identified possesses all of the hallmark qualities of true, multipotential, self-renewing, tissue-specific stem cells. They are restricted in terms of their fate potential to just skeletal tissues, which is likely to make them much more clinically useful."

Skeletal regeneration is an important capability for any bony animal evolving in a rough-and-tumble world where only the most fit, or the fastest-healing, are likely to survive very long into adulthood. Some vertebrates, such as newts, are able to regenerate entire limbs if necessary, but the healing ability of other animals, such as mice and humans, is more modest. Although humans can usually heal a bone fracture fairly well, they begin to lose some of that ability with age. And they are completely unable to regenerate the cartilage that wears away with age or repetitive use. Researchers have wondered whether the skeletal stem cell could be used clinically to help replace damaged or missing bone or cartilage, but it's been very difficult to identify.

Unlike embryonic stem cells, which are present only in the earliest stages of development, adult stem cells are thought to be found in all major tissue types, where they bide their time until needed to repair damage or trauma. Each adult stem cell is lineage-restricted — that is, it makes progenitor cells that give rise only to the types of cells that naturally occur in that tissue. For our skeleton, that means cells that make bone, cartilage and stroma.

Chan, Longaker and their colleagues had hoped to use what they learned from identifying the mouse skeletal stem cell to quickly isolate its human counterpart. But the quest turned out to be more difficult than they had anticipated. Most cell isolation efforts focus on using a technology called fluorescence activated cell sorting to separate cells based on the expression of proteins on their surface. Often, similar cell types from different species share some key cell surface markers.

But the human skeletal stem cell turned out to share few markers with its mouse counterpart. Instead, the researchers had to compare the gene expression profiles of the mouse skeletal stem cell with those of several human cell types found at the growing ends of developing human bone. Doing so, they were able to identify a cell population that made many of the same proteins as the mouse skeletal stem cell. They then worked backward to identify markers on the surface of the human cells that could be used to isolate and study them as a pure population.

“This was quite a bioinformatics challenge, and it required a big team of interdisciplinary researchers, but eventually Chuck and his colleagues were able to identify a series of markers that we felt had great potential,” Longaker said. “Then they had to prove two things: Can these cells self-renew, or make more of themselves indefinitely, and can they make the three main lineages that comprise the human skeleton?”

The researchers showed that the human skeletal stem cell they identified is both self-renewing and capable of making bone, cartilage and stroma progenitors. It is found at the end of developing bone, as well as in increased numbers near the site of healing fractures. Not only can it be isolated from fracture sites, it can also be generated by reprogramming human fat cells or iPS cells to assume a skeletal fate.

Intriguingly, the skeletal stem cell also provided a nurturing environment for the growth of human hematopoietic stem cells — or the cells in our bone

marrow that give rise to our blood and immune system — without the need for additional growth factors found in serum.

“Blood-forming stem cells love the interior of spongy bone,” Chan said. “It’s the perfect niche for them. We found that the stromal population that arises from the skeletal stem cell can keep hematopoietic stem cells alive for two weeks without serum.”

By studying the differentiation potential of the human skeletal stem cell, the researchers were able to construct a family tree of stem cells to serve as a foundation for further studies into potential clinical applications. Understanding the similarities and differences between the mouse and human skeletal stem cell may also unravel mysteries about skeletal formation and intrinsic properties that differentiate mouse and human skeletons.

The skeletal stem cell we’ve identified possesses all of the hallmark qualities of true, multipotential, self-renewing, tissue-specific stem cells.

“Now we can begin to understand why human bone is denser than that of mice, or why human bones grow to be so much larger,” Longaker said.

In particular, the researchers found that the human skeletal stem cell expresses genes active in the Wnt signaling pathway known to modulate bone formation, whereas the mouse skeletal stem cell does not.

The ultimate goal of the researchers, however, is to find a way to use the human skeletal stem cell in the clinic. Longaker envisions a future in which arthroscopy — a minimally invasive procedure in which a tiny camera or surgical instruments, or both, are inserted into a joint to visualize and treat damaged cartilage — could include the injection of a skeletal stem cell specifically restricted to generate new cartilage, for example.

“I would hope that, within the next decade or so, this cell source will be a game-changer in the field of arthroscopic and regenerative medicine,” Longaker said. “The United States has a rapidly aging population that undergoes almost 2 million joint replacements each year. If we can use this stem cell for relatively noninvasive therapies, it could be a dream come true.”

diseases and then to use those models to identify disease-specific pathways that can be targeted.”

Protein promotes small artery growth to damaged heart tissue in mice

Stanford scientists have discovered a molecule that promotes the growth of collateral arteries in mice. The finding could lead to therapies that help heal heart tissues damaged by disease or heart attack.

A collaboration between scientists at two different Stanford institutes has revealed a protein that promotes the growth of small arteries leading into oxygen-starved heart tissues in mice.

Stem Cell Institute member Kristy Red-Horse, PhD, associate professor of biology, and Cardiovascular Institute member Joseph Woo, MD, professor of cardiothoracic surgery, think the growth of these new arteries may help heal damage caused by heart disease or heart attack, or even help prevent that damage.

In clinical practice, Woo has observed that patients with blockages in major arteries feeding the heart often have confoundingly different outcomes. “Some patients have a blockage in one coronary artery and die; other patients have multiple blockages in multiple areas but can run marathons,” said Woo. The difference, Woo said, may be that this second group of patients has collateral arteries, tiny arteries that bypass blockages in hearts’ major arteries and feed areas of the heart starved of oxygen. “They are like the side streets that let you get around a traffic jam on the freeway,” Woo said. Such collateral arteries could help people with atherosclerosis or people recovering from a heart attack, except that collateral arteries are only seen in a minority of patients.

Now Red-Horse, Woo and their colleagues have discovered how these collateral arteries are formed and a signaling molecule that promotes their growth in adult mice, offering hope that collateral arteries may be coaxed to grow in human patients.

The researchers began by looking at newborn mice. “Neonatal mice have a robust ability to heal injured heart tissue, but they no longer have that ability in adulthood,” Red-Horse said. “Understanding why could identify ways of reigniting regeneration in adults.”

They documented that the young mice’s healing was

due in part to the growth of new collateral arteries into the injured area. Through advanced imaging that let them look at the intact newborn hearts at the cellular level, the researchers showed that this happened because arterial endothelial cells exited the artery, migrated along existing capillaries that extended into injured heart tissue and reassembled to form collateral arteries.

Then the researchers investigated how the cells knew to do this. The molecule CXCL12 is known to be an important signal during embryonic development of arterial cells, and has been shown to improve cardiac recovery and function after heart attacks. The scientists wondered if this molecule had a beneficial effect by promoting collateral artery growth in injured heart tissue. They found that CXCL12 was mostly restricted to arterial endothelial cells in uninjured neonatal mouse hearts. In newborn mice with heart injuries, it shows up in the capillaries of the injured area. The researchers found evidence that low oxygen levels in the injured area turned on genes that create CXCL12, signaling the areas to which arterial endothelial cells should migrate.

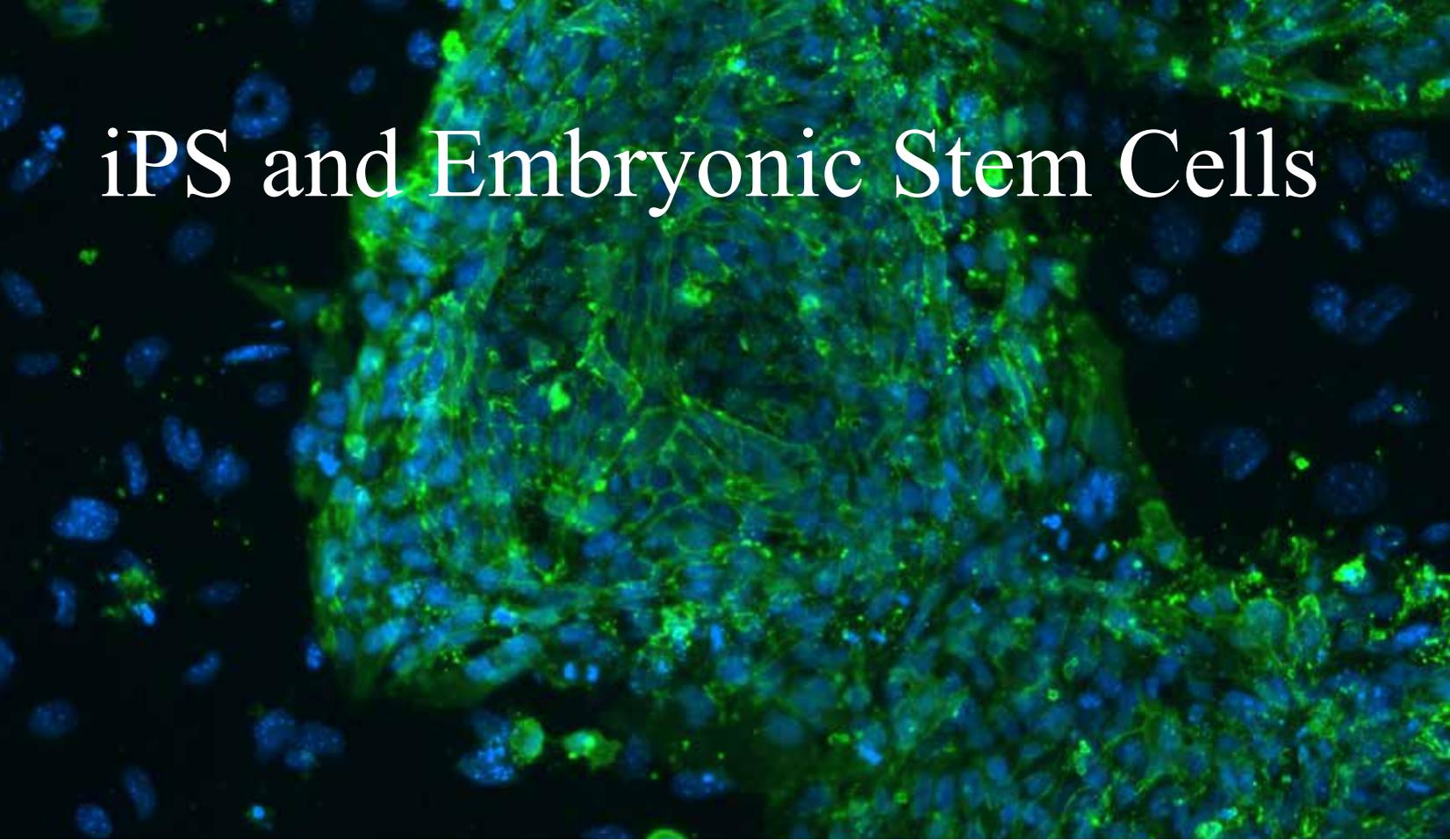
Next, they investigated whether CXCL12 could help adult heart tissue grow collateral arteries.

“Our studies showed that adult hearts do not form collateral arteries in the way newborns do after injury,” Red-Horse said. After inducing heart attacks in adult mice, they injected CXCL12 into the injured areas. Sure enough, 15 days after the injuries, there were numerous new collateral arteries formed by the detaching and migrating artery cells. Almost none were present in control mice.

Red-Horse and Woo think the complete story is not this simple. “We speculate that there is a whole suite of proteins that support cell migration out of arteries and promotes cell proliferation among the injured cells,” Red-Horse said. Nonetheless, they hope that this discovery can become the basis for a new therapy.

“The question now is whether this mechanism we have discovered can be manipulated therapeutically to generate collateral arteries in human patients,” Woo said

iPS and Embryonic Stem Cells

A fluorescence microscopy image showing a dense cluster of cells. The cells are stained with a blue dye, likely DAPI, which highlights the nuclei. Interspersed among the blue-stained cells are numerous bright green spots and some green filamentous structures, indicating the presence of a specific marker or protein expressed by some of the cells.

Embryonic stem cells naturally develop into every kind of cell in our body. Institute researchers are exploring the mysteries of how this occurs naturally, and how errors occur during the development process. Part of this exploration involves research on how genetic instructions are carried out.

Researchers at the institute are also creating embryonic-like cells, called **induced pluripotent stem (iPS)** cells. They are now investigating the use of iPS cells therapeutically and for creating disease-in-a-dish models of clinical disorders.

Human blood cells transformed into functional neurons

Fresh or frozen human blood samples can be directly transformed into patient-specific neurons to study disorders such as schizophrenia and autism, say institute researchers.

Human immune cells in blood can be converted directly into functional neurons in the laboratory in about three weeks with the addition of just four proteins, institute researchers have found.

The dramatic transformation does not require the cells to first enter a state called pluripotency but instead occurs through a more direct process called transdifferentiation.

The conversion occurs with relatively high efficiency — generating as many as 50,000 neurons from 1 milliliter of blood — and it can be achieved with fresh or previously frozen and stored blood samples, which vastly enhances opportunities for the study of neurological disorders such as schizophrenia and autism.

“Blood is one of the easiest biological samples to obtain,” said Marius Wernig, MD, associate professor of pathology and a member of Stanford’s Institute for Stem Cell Biology and Regenerative Medicine. “Nearly every patient who walks into a hospital leaves a blood sample, and often these samples are frozen and stored for future study. This technique is a breakthrough that opens the possibility to learn about complex disease processes by studying large numbers of patients.”

A paper describing the findings was published in the Proceedings of the National Academy of Sciences. Wernig is the senior author. Former postdoctoral scholar Koji Tanabe, PhD, and graduate student Cheen Ang are the lead authors.

The transdifferentiation technique was first developed in Wernig’s laboratory in 2010 when he and his colleagues showed that they could convert mouse skin cells into mouse neurons without first inducing the cells to become pluripotent — a developmentally flexible stage from which the cells can become nearly any type of tissue. They went on to show the technique could also be used on human skin and liver cells.

But these approaches have been dogged by challenges, particularly for researchers wishing to study genetically complex mental disorders, such as autism or schizophrenia, for which many hundreds of individual, patient-specific samples are needed in order to suss out the relative contributions of dozens or more disease-associated mutations.

Although it’s possible to directly convert skin cells to neurons, the biopsied skin cells first have to be grown in the laboratory for a period of time until their numbers increase — a process likely to introduce genetic mutations not found in the person from whom the cells were obtained.

The researchers wondered if there was an easier, more efficient way to generate patient-specific neurons.

In this study, Wernig and his colleague focused on highly specialized immune cells called T cells that circulate in the blood. T cells protect us from disease by recognizing and killing infected or cancerous cells. In contrast, neurons are long and skinny cells capable of conducting electrical impulses along their length and passing them from cell to cell. But despite the cells’ vastly different shapes, locations and biological missions, the researchers found it unexpectedly easy to complete their quest.

“It’s kind of shocking how simple it is to convert T cells into functional neurons in just a few days,” Wernig said. “T cells are very specialized immune cells with a simple round shape, so the rapid transformation is somewhat mind-boggling.”

The resulting human neurons aren’t perfect. They lack the ability to form mature synapses, or connections, with one another. But they are able to carry out the main fundamental functions of neurons, and Wernig and his colleague are hopeful they will be able to further optimize the technique in the future. In the meantime, they’ve started to collect blood samples from children with autism.

“We now have a way to directly study the neuronal function of, in principle, hundreds of people with schizophrenia and autism,” Wernig said. “For decades we’ve had very few clues about the origins of these disorders or how to treat them. Now we can start to answer so many questions.”

Institute Leadership



Irving Weissman, MD

Institute Director

Director of the Ludwig Center for Cancer Stem Cell Research

Virginia and D.K. Ludwig Professor for Clinical Investigation and Cancer Research

Irving Weissman has directed the institute since its founding, providing the vision and leadership to build one of the nation's top stem cell programs. In 1988, Dr. Weissman became the first researcher to isolate in pure form any stem cell in any species when he found hematopoietic (blood-forming) stem cell in mice. He subsequently found the human hematopoietic stem cell, the human neuronal stem cell, and the human leukemia stem cell. His work has opened up an entirely new area of scientific research with enormous potential for life-saving therapies.



Michael T. Longaker, MD, MBA, FACS

Co-Director

Director, Program in Regenerative Medicine

Deane P. and Louise Mitchell Professor

Michael Longaker has broad experience in pediatric plastic surgery, developmental biology, epithelial biology, tissue repair, and tissue engineering. He has extensive research experience in the cellular and molecular biology of extracellular matrix, with specific applications to the differences between fetal and post-natal wound healing, the biology of keloids and hypertrophic scars, and the cellular and molecular events that surround distraction osteogenesis with respect to craniofacial development. Most recently, his research has focused on multipotent mesenchymal cells derived from adipose tissue and their applications for tissue repair, replacement, and regeneration.



Maria-Grazia Roncarolo, MD

Co-Director

George D. Smith Professor in Stem Cell and Regenerative Medicine

Maria Grazia Roncarolo is a world leader in stem cell and gene therapies. She is the former scientific director of the San Raffaele Scientific Institute in Milan, Italy, where she showed that gene therapy could be used effectively in treating formerly untreatable diseases. Dr. Roncarolo was recruited to lead the institute's efforts to translate basic scientific discoveries in the field of regenerative medicine into novel patient therapies, including treatments based on stem cells and gene therapy.



Michael F. Clarke, MD

Associate Director

Karel H. and Avice N. Beekhuis Professor in Cancer Biology

In addition to his clinical duties in cancer treatment, Michael Clarke maintains a laboratory focused on stem cells and the role they play in cancer. Dr. Clarke's research is aimed at the identification and characterization of cancer stem cells, and at increasing our knowledge of the factors that control self-renewal in normal stem cells and their malignant counterparts. Dr. Clarke was the first researcher to find cancer stem cells in a solid tumor (breast cancer) and discovered that the inhibition of programmed cell death is essential for the growth of breast cancers.

Agnieszka Czechowicz joins the institute

Agnieszka Czechowicz was an MD/PhD student in the Weissman lab before doing her clinical training at Boston Children's Hospital/Dana Farber Cancer Institute. She returned to Stanford to join the pediatric hematopoietic stem cell transplantation group. In the Weissman lab, Czechowicz was the first author in the first paper that led to using antibodies instead of irradiation or chemotherapy to clear space in the blood stem cell niche in preparation for blood stem cell transplantation. Czechowicz is especially interested in this form of conditioning for children whose defects in blood formation cause a lethal anemia (Fanconi anemia). These children suffer from defects in the repair of mutations in their DNA, which almost always lead to childhood cancers. Antibody conditioning instead of chemo or radiotherapy will hopefully give them a chance to live longer lives.



Agnieszka Czechowicz, MD, PhD

Kristy Red-Horse joins the institute

Kristy Red-Horse, PhD, studies coronary artery development and arterial cell differentiation. Red-Horse's studies of the basic biology of the stem cells that differentiate into cardiovascular tissue, and of the signals that govern these stem cells, has the potential to lead to therapies that help people avoid damage from heart attacks and recover quickly after such attacks.



Kristy Red-Horse, PhD

Siddhartha Jaiswal joins the institute

Siddhartha Jaiswal, MD, PhD is another Weissman lab alumni who left to do work in Boston and returned to become an assistant professor at Stanford. While in the Weissman lab, Jaiswal was the first to document that the CD47 protein protects cancer cells from macrophage. Now, his research is focused on blood stem cell mutations that are prevalent in the aging population and that cause clonal hematopoiesis, a disorder that increases the risk of blood cancer, cardiovascular disease, and overall mortality. Understanding the biology of these mutations and how they contribute to the development of cancer and other age-related diseases is the current focus of work in his lab.



Siddhartha Jaiswal, MD, PhD

Anthony Oro joins the institute

In 2018, Anthony E. Oro, MD, PhD, became a member of the institute. He is the Eugene and Gloria Bauer Professor of Dermatology, Associate Director of the Center for Definitive and Curative Medicine, and the co-director of the Child Health Research Institute. Oro is co-founder of the Program in Epithelial Biology, and is also an active member of the Children's Health Research Institute, Bio-X, and the Program in Cancer Biology. His research interests encompass cancer genomics and tumor evolution, stem cell biology and hair/skin development and regeneration, and definitive molecular and cellular therapeutics. His clinical interests include hair biology, non-melanoma skin cancer, and stem cell-based therapies for genetic skin diseases. Oro has been working with institute member Marius Wernig to create new skin stem cells from iPS cells with genetic corrections to fix the skin disease epidermolysis bullosa. He also studies how molecules guide the differentiation and development of stem cells.



Anthony Oro, MD,

Kyle Loh joins the institute

On February 1, 2018, Kyle Loh, PhD became an assistant professor at Stanford and the 7th faculty member billeted in the institute. His home department is Developmental Biology. Loh's work concerns a major challenge of regenerative medicine and stem cell therapy: how can you create batches of stem cells that are purely that type of cell so that you can transplant them into patients. Typically, when embryonic or induced pluripotent stem cells divide and specialize (differentiate) they can become many different kinds of cells. Some of these cells should not be transplanted because they may lead to teratomas (non-cancerous tumors) or other problems. Current methods to direct cell development are not able to solve this problem. Loh's method is to look for the positive and negative signals that seem to be needed to guide cell development at every decision point. He finds that cells not only need a signal that tells cells "turn into this kind of cell," but also a signal that tells cells "do not turn into this other kind of cell." By supplying both positive and negative signals at key times in the growth of cell cultures, Loh find that he can develop batches of cells that are purely one type of cell. These pure cell populations can be the building blocks for future regenerative therapies.

Loh received his BA from Rutgers University and his PhD from Stanford University (with Irv Weissman). He then continued as a Siebel Investigator and later, as an Assistant Professor, at Stanford. He is a scientific advisor to the Americans for Cures foundation, whose goal is to communicate stem cell research to the U.S. public-at-large. The work of Loh and his colleagues has been recognized by the NIH Director's Early Independence Award, the Harold Weintraub Graduate Award, the Hertz Foundation Thesis Prize and the A*STAR Investigatorship.



Kyle Loh, PhD

SCBRM Graduate Program

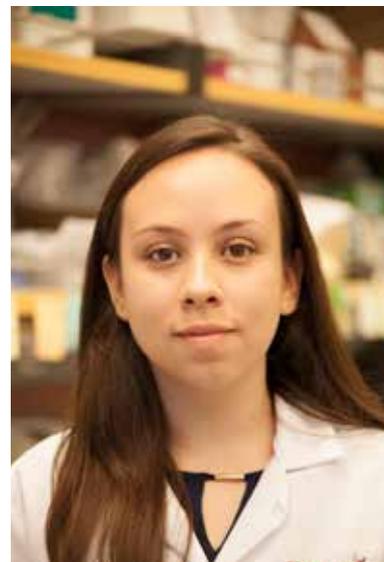
The 2018 entering class of SCBRM grad students

Karen Gonzalez

BS Molecular, Cell and Developmental Biology

University of California, Los Angeles

I am proud to have completed my Bachelor of Science degree at UCLA, where I had the opportunity to develop my research interests in Dr. April Pyle's laboratory. My scientific interests lie in understanding how tissue-specific stem cell populations are established and contribute to tissue patterning during human embryonic development, so that we can develop reliable in vitro models for different tissues and therapies for a spectrum of conditions. Beyond the lab, I am passionate about mentorship and increasing diversity in STEM. As a first-generation Latina pursuing a career in science, I aim to be an accessible role model, especially for students from underrepresented backgrounds.



Katarina Klett

BS Chemical Engineering

University of Pittsburgh

The University of Pittsburgh campus is a unique blend of innovation, culture, and medicine. As an undergraduate student, I was captivated by the collaboration between experts in biological research and world-class physicians with the collective goal of better treating patients. I knew that I wanted to contribute to that united effort.

My first exposure to research was as a member of the Adipose Stem Cell Center, where I discovered how to leverage engineering principles to solve various clinical challenges, including improving drug delivery and optimizing decellularization techniques. That experience paved the way for me to travel to Stuttgart, Germany as a DAAD RISE student at the Fraunhofer Institute for Interfacial Engineering and Biotechnology. While in Germany, I worked with a team to design a bioreactor that allowed for real-time imaging of embryonic stem cells as they differentiated into cardiomyocytes. Following my experience in Germany, my research took me to Vail, Colorado, where I worked as a core team member of the Steadman Philippon Research Institutes Center for Regenerative Sports Medicine. There, I studied the impact of biomimetic microspheres on promoting cartilage tissue repair.



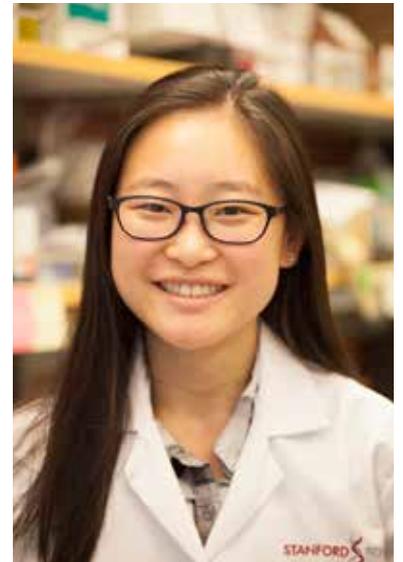
As a SCBRM graduate student, I plan to pair my understanding of basic biology with my background in engineering to take transformative steps in medicine. When not pipetting or changing cell media, you can find me exploring local eats or lost in nature.

Angela Liu

BA Molecular and Cell Biology

University of California, Berkeley

I was born in China, raised in southern California, and went to undergrad at UC Berkeley. My introduction to research was through the Essig Museum of Entomology at Berkeley, where I imaged countless Californian insect specimens. During undergrad, I also worked in Dr. Jennifer Lewis's lab studying plant-pathogen interactions in tomato. Subsequently, I spent 2 years in the lab of Dr. Bruce Conklin at The Gladstone Institutes developing a method to treat dominant negative genetic retinal disease. My current interests include technology development for regenerative medicine, crocheting, and nail art.

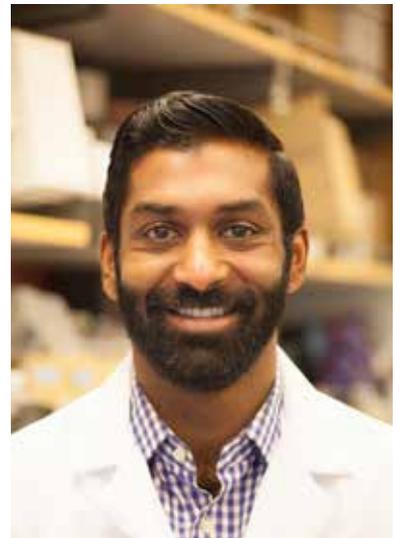


Sidd Menon

BS Biology

University of California, San Diego

I graduated from the University of California San Diego with a Bachelors of Science in Biology in 2008 and was a 4-year member of the men's varsity water polo team. From 2009-2014, I worked at Sacred Heart Preparatory in Atherton California as Science Faculty, teaching AP Biology, Chemistry and Physics in addition to coaching water polo and swimming. In June 2014 I joined the Laboratory of Dr. Michael Longaker as a Life Science Research Professional (LSRP) in the craniofacial group and now am continuing in the lab as a graduate student. My research focuses mainly on calvarial repair and development, specifically on the role of skeletal stem cells in calvarial suture development. When I am not in the lab you can find me in the mountains skiing, mountain biking, or camping.



Kenisha Puckett

MS Cell & Molecular Biology
San Francisco State University

I'm originally from Southern California and received my bachelors in Biological Sciences from the University of California, Riverside. Before starting grad school, I served as a STEM Educator at several colleges/universities to increase the number of highly-trained diverse individuals entering the STEM workforce. However, I missed being in the lab, so I continued my training through my master's degree in Cell and Molecular Biology with an emphasis in Stem Cell Biology at San Francisco State University. As a former California Institute for Regenerative Medicine (CIRM) fellow, I am no stranger to stem cells. My master's thesis project, housed in the UCSF Center for Reproductive Science, investigated the impact of the environmental toxins on human and placental development. Today, my research interests are to continue examining human development to improve Maternal-Fetal Medicine. If I am not annoying everyone with my talk of trophoblasts then you can find me engaged in some artistic venture. I enjoy spending my time as a freelance makeup artist, consultanting an interior design company and advisor for higher education programs supporting underrepresented populations in STEM.



Harsh Shah

MS Public Health
BS Biology
University of Alabama, Birmingham

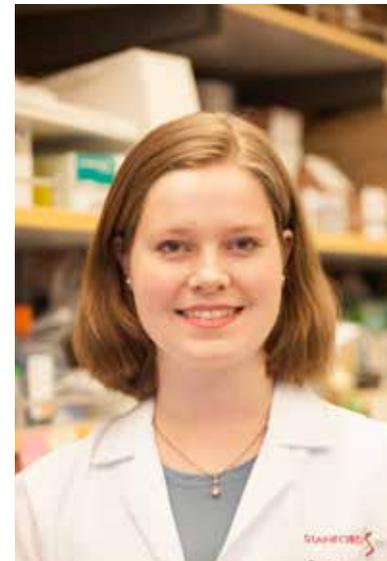
I am from Birmingham, Alabama, the land of Southern Charm, sweet tea, and where college football reigns supreme. I graduated from the University of Alabama at Birmingham with a Bachelors degree in Molecular Biology and a Masters of Public Health degree concentrating in Health Care Organization and Policy. I completed my undergraduate honors research thesis in Dr. Trygve Tollefsbol's laboratory focusing on alternative treatment strategies for triple negative breast cancer. After which, I traveled to Minnesota to attend medical school at Mayo Clinic Alix School of Medicine. In the midst of my medical education, I realized I missed basic science research; therefore, I applied to PhD programs and landed at Stanford University, away from the snow. I am pursuing to combine scientific research and clinical care in an effort to provide patients with the latest therapies. When I am not in the lab, you will find my in one of the many gyms at Stanford or choreographing for dance teams back in Birmingham.



Courtney Stockman

BS Biomedical Engineering
University of Cincinnati

I am from Cincinnati Ohio, the land of the flying pigs and #2 sunsets in the country (as of last time I checked). In Cincinnati we ask everyone where they went to high school, but I am going to mix it up and say where I went to undergrad, University of Cincinnati where I studied Biomedical Engineering. I became interested in Stem Cell Biology while participating in undergraduate research at our local Children's Hospital where I studied perinatal lung diseases. And to answer the question that just popped into your mind, Yes! That is as cool as it sounds. After 3.5 years in the lab, I moved out to Stanford where I hope to continue learning about mechanisms of disease that can lead to a therapy. When I am outside of lab you can find me drinking coffee, painting, eating a 4-way with beans (spaghetti+chili+cheese+beans) sometimes all at the same time.





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