2017 ANNUAL REPORT

STANFORD INSTITUTE FOR STEM CELL BIOLOGY AND REGENERATIVE MEDICINE

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This annual report is produced by the staff of the Institute for Stem Cell Biology and Regenerative Medicine, with some material from the School of Medicine Office of Communications and Public Affairs.

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Message from the Director

The mission of the Institute for Stem Cell Biology and Regenerative Medicine is to make discoveries, to translate those discoveries, and to train the next generation of scientists in stem cell biology and regenerative medicine. This year, in pursuit of all these goals, the institute carried out two searches for new assistant professors, while related departments carried out three searches...

for stem cell faculty. Candidates came from all geographic regions and were trained in many independent institutions. Candidates who had trained wholly or in part at Stanford had to be judged by faculty who had not collaborated with them during their training. Despite these restrictions, when the top five researchers emerged from hundreds of candidates, the most significant portion of these five had received their training at Stanford, speaking to the strength of our program and the competitive abilities of those trained in it. In the past year, many of our trainees have seeded the faculties at Harvard, Duke, Yale, UCSF, UCSD, UCLA and other high-caliber institutions. Here is an introduction to the five new Stanford stem cell faculty and their already remarkable accomplishments.

In 2017, **Sidd Jaiswal** became an assistant professor in the Stanford School of Medicine, Department of Pathology. He received his college and medical training at Stanford, working in the Weissman lab. As a medical student in the MD-PhD program, he was a co-discoverer of the 'don't eat me' molecule CD47 on both circulating blood-forming stem cells and all leukemias.
He then carried out independent research while completing pathology training at the Massachusetts General Hospital at Harvard.
While at Harvard, he independently analyzed the phenomenon of blood-forming stem cell competition.
He looked at blood samples from a large patient cohort that had been studied over the years, and found that some people had massively expanded populations of particular blood-forming stem cells. In these people, out of the 15 million blood forming stem cells in the body, about a million were clones of just one or two stem cells. This abnormal situation is the result of clonal expansion, where a single mutated stem cell multiplies and outcompetes other stem cells.
This result was shocking in a few regards. Firstly, it was surprising that there are many individuals who, as they age, have 'mutant' clones of blood stem cells that outcompete their normal stem cells. Secondly, these were mutations that were previously also found by Majeti, Quake, and myself to be the first steps in the progression to leukemia, but the people in this cohort had not (yet) progressed to leukemia. They lacked the final mutations that switch a normal blood stem cell to a leukemia stem cell.
Jaiswal went on to show another shocking result: The cohort of patients with overly competitive blood-forming stem cells also had highly significant increases in inflammation of the blood vessels, which can lead to heart attacks and strokes. This resonates with a study published last year by another young Stanford medical scientist, Nick Leeper, who in collaboration with the Weissman lab, showed that smooth muscle cells in those with heart disease or stroke had increased expression of the "don't eat me" molecule CD47. Previously Sidd, Ravi Majeti, and I had found CD47 to be over-expressed in acute myelogenous

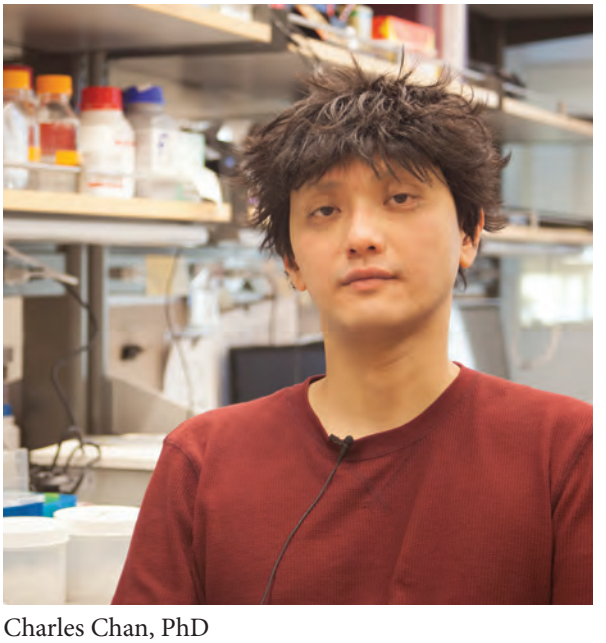

Siddhartha Jaiswal, MD, PhD

leukemia cells, and all other cancers. CD47 upregulation inhibits scavenger cells in the body called macrophages from eating the cancer cells. It has been shown in preclinical research and in clinical trials that blocking the "don't eat me" signal can lead to many cases of cancer regression. This reveals a picture that the increase in expression of the "don't eat me signal" allows both cancer cells and diseased blood vessel cells to survive instead of being eaten. Sidd's findings add another layer—the blood vessel cells may not be eaten if the blood forming system has these massively expanded mutant blood stem cells, and he and Nick will try to understand how a change in blood stem cells could lead to emergence of blood vessel cells that proliferate enough to damage or close off critical coronary or neck blood vessels.

Kyle Loh, PhD

A major issue in stem cell biology is understanding how the earliest cells in an embryo begin the process of going from a fertilized egg to become organ or tissue stem cells committed to making heart, blood, or brain, etc. **Kyle Loh**, another new faculty member with a secondary appointment in developmental biology, has become a world leader in deciphering the positive and negative signals that occur in the developing embryo to make the body's tissues and organs. This research has provided a road map for developing tissue stem cells and their progeny, as well as producing tissues as diverse as liver, heart, bone, cartilage, and of course blood-forming stem cells themselves.

He has found a simple yet elegant way to guide these early embryonic cells so that they give rise to daughter cells that make tissue stem cells for specific tissues and organs. Many labs at the institute have previously identified and isolated tissue-forming stem cells, but they cannot expand these cells in culture so that they can be transplanted in any effective way. Kyle has found how these tissue stem cells originate from human embryonic stem cell lines so that specific stem cell types can be expanded massively in tissue culture dishes. It follows that the future donors of tissue stem cells may be cell lines rather than living (or recently deceased) people.


Charles Chan, PhD

Charles Chan is a new assistant professor of surgery in the institute. He (working with the Weissman and Longaker labs) played a key role in the discovery of the skeletal stem cell that can give rise to bone and cartilage in both mouse and humans. He discovered that this skeletal stem cell also gives rise to cells that control and guide blood-forming stem cells and their progeny in the bone marrow. Skeletal stem

cells eventually should be able to help heal broken bones, replace worn-out cartilage, and renew the bone marrow helper cells that blood stem cells need for their survival and differentiation. Additionally, he and Longaker have found the factors that steer skeletal stem cells to become either bone or cartilage, and designed systems to release those factors into specific areas of healing bones or joints.


Agnieszka Czechowicz, MD, PhD

Agnieszka Czechowicz returns to Stanford from Boston Children's Hospital/Dana Farber Cancer Institute following her clinical training in general pediatrics and pediatric hematology, oncology, and bone marrow transplantation. She joins the pediatric hematopoietic stem cell transplantation group led by Maria Grazia Roncarolo. As an MD/PhD medical student in my lab, Czechowicz was the first author on 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. (The extension of those studies were the main topic in the fall, 2017 institute newsletter.) 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. Accumulated mutations block blood formation or lead almost universally to the development of childhood acute myelogenous leukemias. The chemotherapy or irradiation usually used to prepare for transplantation of blood stem cells comes with a high morbidity and mortality, especially for children with Fanconi anemia. Antibody conditioning instead of chemo or radiotherapy will hopefully give them a chance to live longer lives. Czechowicz brings novel methods of antibody conditioning that she developed at Harvard to provide more "shots on goal" for the types of antibody conditioning that will emerge, mostly here at Stanford. She is also interested in exploring other causes of bone marrow failure syndrome, and broadly in understanding the interactions between blood-forming stem cells and their microenvironment, to be able to develop novel cures for a wide variety of blood and immune diseases.

Aaron Newman is a new assistant professor billeted in the institute. He trained initially in computer science, but has since worked closely with institute researchers Ash Alizadeh and Max Diehn to develop methods for detecting evidence of cancer in the blood by looking for bits of cancer DNA that are released into blood plasma when cancer cells die (and when they are not removed by macrophages). Newman has extended the analysis of cancer and

tissue mutations by developing "CIBERSORT," a method to analyze gene expression in individual cells using statistical techniques rather than by physically sorting the cells. The application of CIBERSORT to analyze previously stored histopathological samples (from patients whose diagnostic records we have) should reveal new patterns of disease development not only in cancers but also other disorders.

These fabulous five new faculty members—Jaiswal, Loh, Chan, Czechowicz, and Newman—demonstrate the strength of scientific training in stem cell biology and regenerative medicine at Stanford, while also providing the promise that the triumphs of the past will continue on into the future. We expect great things of these new faculty members, as well as of the many graduate students and postdocs who are creating the future of medicine in institute labs.

Aaron Newman, PhD

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 for blood stem cell transplantion with much lower toxicity than caused by the standard chemotherapy or radiation.

Using antibody in treatment of 'bubble boy disease' shows early promise, institute researchers say

In a clinical trial, participants were given an antibody to CD117, a cell surface marker, in an effort to wipe out their defective blood stem cells without high-risk chemotherapy or radiation

Judith Shizuru, MD, PhD

Researchers at the institute said they are encouraged by early results from a clinical trial in which participants are being given an antibody-based treatment rather than chemotherapy or radiation to prepare patients for a blood stem cell transplant.

The trial is the first time that the approach has been tested in humans, said trial leader Judith Shizuru, MD, PhD.

The phase-1 trial involves participants who have a condition known as severe combined immunodeficiency (SCID). Also known as "bubble boy disease," SCID is a genetic disorder that disturbs the normal development of immune cells, leaving people with the condition vulnerable to infections that most people ward off easily.

SCID patients can be given infusions of stem and progenitor blood-forming cells to boost their immune response, but that effect can wear off over time if significant numbers of the healthy stem cells can't replace the diseased stem cells. The only cure for SCID involves a blood stem cell transplant, in which the patient's defective stem cells are wiped out with chemotherapy or radiation so that large numbers of normal blood stem cells from a donor can take their place.

The problem with chemotherapy or radiation is that they can be very damaging. "Physicians often choose not to give chemotherapy or radiation to young children with SCID because there are lifelong effects: neurological impairment, growth delays, infertility, risk of cancer, etc.," Shizuru said.

The beginnings of this therapy came in 2007 when graduate student Agnieskwa Czechowicz, with Daniel Kraft and Deepta Bhattacharya, all in Irving Weissman's lab, sought to replace radiation or chemotherapy with antibodies that could deplete blood-forming stem cells, which were first discovered by Weissman in 1988. They found that, of all the antibodies to different molecules on the stem cell surface, only an antibody to the molecule

c-kit (also known as CD117), shown by Weissman and colleagues to be essential to keep these stem cells alive, depleted the stem cells from SCID mice. After that depletion, pure blood stem cells could be transplanted to replace the defective blood system in these mice for life.

Weissman and Shizuru later interacted with a company that had made large quantities of the antibody to human CD117, and obtained the antibody and the right to do collaborative experiments. Later, Weissman, Shizuru, Maria Grazia Roncarolo, MD, and Czechowicz co-wrote an application to the California Institute of Regenerative Medicine, the agency established by passage of Proposition 71, to use this antibody in preclinical testing and in clinical trials funded by the agency.

Early data from the clinical trial show that the antibody's activity in humans is similar to what was observed in mouse studies. Specifically, the antibody appears to be effective in the depletion of genetically defective stem cells.

Nine and six months after the treatment, respectively, the two participants in the current clinical trial have shown evidence that the donor stem cells have taken

Maria Grazia Roncarolo, MD

root and are producing immune cells, Shizuru said.

After these few but significant successes, the researchers hope to continue their clinical trial in infants who have SCID. The technique could prove highly beneficial in infants, who are known to suffer more highly from the side effects of chemotherapy or radiation.

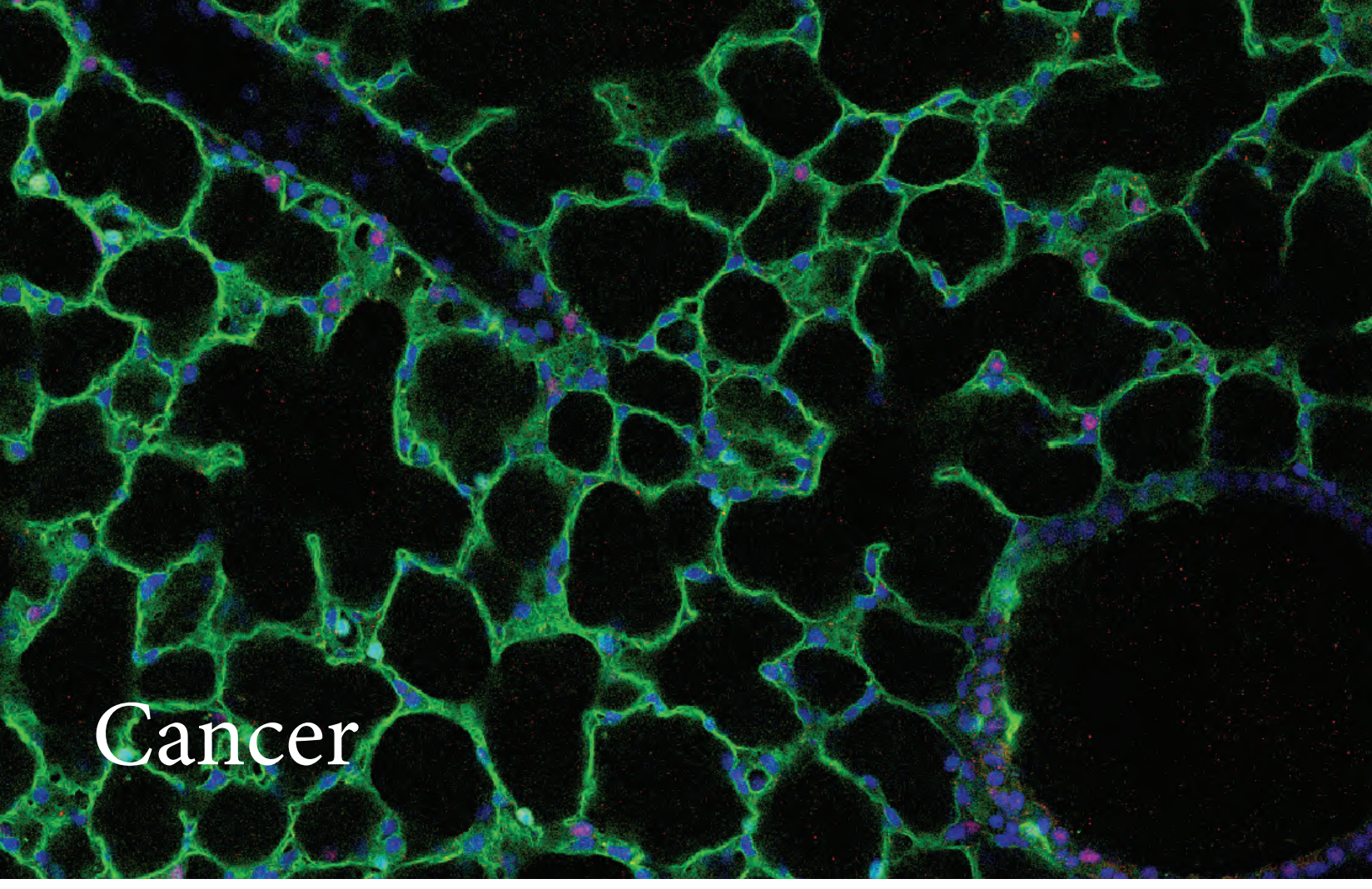
The researchers also have plans to use highly purified blood-forming stem cells (also first isolated by Weissman and colleagues) in the actual transplantation. Such highly purified blood stem cells have no immune cells from the donor that could attack the patients' tissues, a serious complication that goes by the name of graft-versus-host disease. Roncarolo and David DiGiusto, PhD, at Stanford's Laboratory of Cell and Gene Medicine, have produced the protocols to isolate these cells in the rigorous way required by the FDA for any biological substance used in a human clinical trial.

> "These clinical trials ... represent another example of how Proposition 71 funding is beginning to pay off in human therapies"

The first cases also suggest that the antibody-based conditioning may be useful in other diseases. Shizuru, Weissman and colleagues have shown in animal models that autoimmune diseases like juvenile diabetes and lupus should be curable through blood stem cell transplantation. They are not now treated this way because the dangers of the current methods of preparing for transplant, using chemotherapy or radiation, can outweigh the benefits. The collaborative efforts of the Weissman and Shizuru laboratories have found and developed antibody cocktails that not only eliminate diseased blood stem cells, but also immune cells in the patient that would reject the incoming stem cells.

These clinical trials, dating back to research that was first published 30 years ago, represent not only proof of principle that the mouse studies can lead to otherwise difficult to treat or incurable human diseases, but also represent another example of how Proposition 71 funding is beginning to pay off in human therapies.

The trial is being supported by a grant from the California Institute for Regenerative Medicine. Lucile Packard Children's Hospital Stanford and Stanford's departments of Medicine and of Pediatrics also support the work.



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.

Second 'don't eat me' signal found on cancer cells

CD47 is an important inhibitor of cancer-killing immune cells called macrophages. Now Stanford researchers have identified another, similar way to activate macrophages to destroy cancer cells.

Irv Weissman, MD

A second biological pathway that signals immune cells not to engulf and kill cancer cells has been identified by researchers at the Stanford University School of Medicine.

An antibody that blocks the "don't eat me" signal has already shown promise as a cancer treatment in animal models and is currently in clinical trials. Combining that antibody, known as anti-CD47, with another that blocks this newly discovered pathway could further enhance the ability of the immune system to eradicate many types of cancers, the researchers believe.

"The development of cancer cells triggers the generation of SOS molecules that invite attack by the body's scavenger cells, called macrophages," said institute director Irving Weissman, MD. "However, aggressive cancers protect themselves with a 'don't eat me' signal in the form of CD47 on their surfaces. Now we've identified a second 'don't eat me' signal

> "These findings help us understand the many ways cancer cells can evade macrophages, and how we might block these escape pathways"

and its complementary receptor on macrophages. We've also shown that we can overcome this signal with specific antibodies and restore the ability of macrophages to kill the cancer cells."

A paper describing the findings was published online in Nature Immunology. Weissman shares senior authorship of the study with former postdoctoral scholar Roy Maute, PhD, who is now head of biology at Ab Initio Biotherapeutics Inc. Graduate student Amira Barkal shares lead authorship with former graduate student Kipp Weiskopf, MD, PhD, who is now a resident at Brigham and Women's Hospital.

"Simultaneously blocking both these pathways in mice resulted in the infiltration of the tumor with many types of immune cells and significantly promoted tumor clearance, resulting in smaller tumors overall," Barkal said. "We are excited about the possibility of a double- or perhaps even triple-pronged therapy in humans in which we combine multiple blockades to cancer growth."

Macrophages are large white blood cells found in nearly all the body's tissues. As part of what's known as the innate immune system, they engulf and kill foreign invaders like bacteria or viruses. They also destroy dead and dying cells and, in some cases, cancer cells whose internal development cues have gone haywire.

The link between cancer and the "don't eat me" signal was identified in Weissman's laboratory in 2009. His team found that nearly all cancer cells express high levels of a CD47 on their surfaces. They showed that CD47 binds to a protein called SIRPalpha on the surface of macrophages, inhibiting their ability to kill the cancer cells.

Animal studies showed that treatment with an anti-CD47 antibody vastly improved the ability of macrophages to kill cancer cells and even led to some cures in mouse models of cancer. Phase-1 clinical trials are currently underway at Stanford and in the United Kingdom to test the safety and efficacy of the treatment in humans with a variety of blood and solid tumors.

The newly discovered mechanism used by cancer cells to evade macrophages capitalizes on a protein structure on the cancer cells' surface, called the

Amira Barkal

major histocompatibility complex class 1, or MHC class 1. Human tumors that have high levels of MHC class 1 on their surfaces are more resistant to anti-CD47 treatment than are those with lower levels of the complex, the researchers found.

MHC class 1 is an important component of adaptive immunity, the second major arm of the immune system, which relies on immune cells called T cells and B cells to nimbly and specifically respond to foreign invaders and cell damage. Most cells of the body express MHC class 1 on their surfaces as a way to indiscriminately display bits of many proteins found within the cell — a kind of random sampling of a cell's innards that provides a window into its health and function. If the protein bits, called peptides, displayed by the MHC are abnormal, a T cell destroys the cell. Although the relationship between MHC class 1 and T cells has been well-established, it's been unclear whether and how the complex interacts with macrophages.

Barkal and her colleagues found that a protein called LILRB1 on the surface of macrophages binds to a portion of MHC class 1 on cancer cells that is widely shared across individuals. This binding inhibits the ability of macrophages to engulf and kill the cancer cells, both when growing in a laboratory dish and in mice with human tumors, the researchers found. Inhibiting both the CD47-mediated pathway and the LILRB1 pathway significantly slowed tumor growth in mice.

Understanding the balance between adaptive and innate immunity is important in cancer immunotherapy. For example, it's not uncommon for human cancer cells to reduce the levels of MHC class 1 on their surfaces to escape destruction by T cells. People with these types of tumors may be poor candidates for cancer immunotherapies meant to stimulate T cell activity against the cancer. But these cells may then be particularly vulnerable to anti-CD47 treatment, the researchers believe. Conversely, cancer cells with robust MHC class 1 on their surfaces may be less susceptible to anti-CD47.

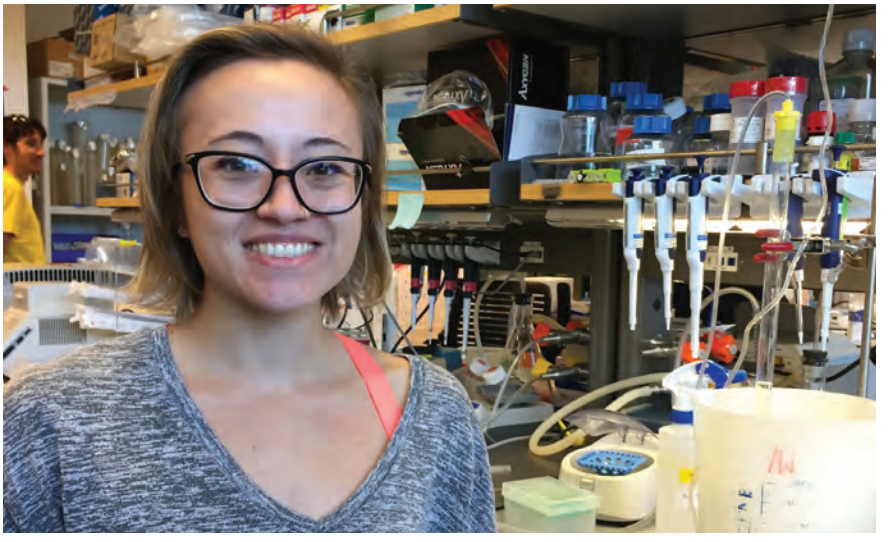
"In some cancers, MHC class 1 expression, for a variety of reasons, is not reduced," Weissman said, "and this helps the cancer cells escape from macrophages. These findings help us understand the many ways cancer cells can evade macrophages, and how we might block these escape pathways."

"The fact that there are at least two redundant mechanisms to modulate macrophage activity is a testament to how critically important it is to tightly control our immune responses," Barkal said. "It's possible that future studies will identify even more of these pathways, which will give us additional targets for cancer immunotherapy."

Other Stanford authors are technician Kevin Kao; former graduate student Sydney Gordon, PhD; postdoctoral scholar Benyamin Rosental, PhD; graduate students Ying Yiu, Benson George, Jonathan Tsai and James Chen; research associate Maxim Markovic; former medical fellow Nan Ring, MD; former research assistants Kelly McKenna and Po Yi Ho; and former undergraduate student Robin Cheng.

Cancer therapy works in unexpected way

Blocking the PD-1 pathway initiates a two-pronged attack by both T cells and macrophages



Sydney Gordon, PhD

Antibodies to the proteins PD-1 and PD-L1 have been shown to fight cancer by unleashing the body's T cells, a type of immune cell. Now, researchers at the Stanford University School of Medicine have shown that the therapy also fights cancer in a completely different way, by prompting immune cells called macrophages to engulf and devour cancer cells.

The finding may have important implications for improving and expanding the use of this cancer treatment, the researchers said.

The work, which was done in mice, was published in Nature. The senior author is institute director Irving Weissman, MD. The lead author is former graduate student Sydney Gordon, PhD.

PD-1 is a cell receptor that plays an important role in protecting the body from an overactive immune system. T cells, which are immune cells that learn to detect and destroy damaged or diseased cells, can at times mistakenly attack healthy cells, producing autoimmune disorders like lupus or multiple sclerosis. PD-1 is what's called an "immune checkpoint," a protein receptor that tamps down highly active T cells so that they are less likely to attack healthy tissue.

About 10 years ago, researchers discovered that cancer learns to use this immune safeguard for their own purposes. Cancer cells crank up the production of PD-L1 proteins, which are detected by the PD-1 receptor, inhibiting T cells from attacking the tumors. In effect, the proteins are a "don't kill me" signal to the immune system. Cancer patients are now being treated with antibodies that block the PD-1 receptor or latch onto its binding partner, PD-L1, to turn off this "don't kill me" signal and enable the T cells' attack.

"Using antibodies to PD-1 or PD-L1 is one of the major advances in cancer immunotherapy," said Weissman, who is also director of the Ludwig Center for Cancer Stem Cell Research and Medicine at Stanford. "While most investigators accept the idea that anti-PD-1 and PD-L1 antibodies work by taking the brakes off of the T-cell attack on cancer cells, we have shown that there is a second mechanism that is also involved."

What Weissman and his colleagues discovered is that PD-1 activation also inhibits the anti-cancer activity of other immune cells called macrophages. "Macrophages that infiltrate tumors are induced to create the PD-1 receptor on their surface, and when PD-1 or PD-L1 is blocked with antibodies, it prompts those macrophage cells to attack the cancer," Gordon said.

As it stands, it's unclear to what degree macrophages are responsible for the therapeutic success of the anti-PD-1 and anti-PD-L1 antibodies.

"This could lead to novel therapies that are aimed at promoting either the T-cell component of the attack on cancer, or promoting the macrophage component," Gordon said.

Other Stanford co-authors of the study are associate professor of pathology Andrew Connolly, MD, PhD; visiting scholar Gregor Hutter, MD, PhD; instructor Rahul Sinha, PhD; postdoctoral scholars Roy Maute, PhD, Daniel Corey, MD, and Melissa McCracken, PhD; graduate students Benjamin Dulken, Benson George and Jonathan Tsai; and former graduate student Aaron Ring, MD, PhD.

Ravi Majeti, MD, PhD

Technique pinpoints cancer genes' partners in crime

Targeting backup biological pathways often used by cancers can lead to more efficient drug development and less-toxic therapies. Stanford researchers have developed a new way to identify these pathways

Cancers often rely on pairs of complementary genes to keep their cells plugging along as they spin increasingly out of the bounds of normal cellular control. If one partner is mutated, the other springs to the rescue; if both are compromised, the cell dies. Genes that work in this way are called synthetic lethals, and cancer researchers' ears perk up when one member of the pair is a known cancer-associated mutation. Blocking its partner could be an attractive therapeutic target that would specifically kill cancer cells while sparing normal cells without the mutation. But until now it's been difficult to identify these partners in crime.

Now another dynamic duo—institute researcher Ravi Majeti, MD, PhD, and Stanford professor of computer science David Dill, PhD—have partnered up to devise a new computer algorithm that churns through piles of existing data to find and target these genetic understudies in primary human tumors. Doing so is likely to lead to new, less-toxic treatments for many cancers, they believe. Dill and Majeti are collaborating with oncologists at Stanford and at M.D. Anderson Cancer Center in Texas to use the algorithm, which they've called MiSL, to find new, mutation-specific therapies for patients with a variety of cancers.

> "We've found that even though many known cancer-associated mutations are difficult to target clinically, their synthetic lethal partners may be much more druggable."

"Using data from real human tumors gives us important, fundamental advantages over using cancer cell lines, which often don't display the same mutation profiles," said Majeti. "We've found that even though many known cancer-associated mutations are difficult to target clinically, their synthetic lethal partners may be much more druggable."

The researchers tackled 12 different types of cancers and over 3,000 cancer-associated mutations to identify thousands of new genetic partnerships that

could be amenable to drug treatment. In particular, they found that 17 of the 89 potential synthetic lethal partners for a well-known, leukemia-associated mutation are likely to be susceptible to drugs that are either already clinically available or are under development.

Majeti and Dill share senior authorship of the study, which was published in Nature Communications. Research associate Subarna Sinha, PhD, and postdoctoral scholar Daniel Thomas, PhD, share lead authorship.

The researchers capitalized on the fact that cancer cells are often a genomic hot mess. As they proliferate out of control, they play fast and loose with the normal rules for DNA duplication and cellular division. It's not uncommon for genes to be summarily deleted from the genome or, conversely, to be "amplified" so that they occur two, three or more times in the cells' DNA.

In this study, the researchers taught the computer a simple "if this, then that" concept to help them identify pairs of genes whose expression levels were co-dependent — a hallmark of synthetic lethals.

"We were looking for situations in which, if gene A is mutated, gene Y is amplified to compensate for the loss of function of gene A," said Dill, who is the Donald E. Knuth Professor in the School of Engineering. "Conversely, gene Y is only ever deleted in cells in which gene A is not mutated." In other words, these genetic partners have each others' backs.

"We found these strong relationships much more often than we had expected, even among seemingly unrelated genes," said Dill.

The researchers analyzed more than 3,000 known cancer-associated genes and identified more than 140,000 potential synthetic lethal partners through a study of the DNA sequences of the cells. They winnowed this number down by limiting the prospects to only those that displayed a true difference in gene expression levels of the partner based on whether the first gene was mutated. In most cases, this narrowed the contenders down to 50 or fewer for each mutation.

They found that MiSL pinpointed some synthetic lethals that had previously been identified by other means — confirming that their approach was working. But they also identified some new relationships, including one between a mutation in a gene called IDH1 that's associated with the development of leukemia and another gene called ACACA. They validated this synthetic lethal partnership by a variety of tests in laboratory grown cells and human tumor tissue.

David Dill, PhD

"We have just scratched the surface of what we think we can learn with MiSL," said Majeti. "It's an incredibly powerful way to analyze large amounts of data to quickly identify relationships of potential interest, and it's likely to make drug development much more efficient and quick."

"It's an incredibly powerful way to analyze large amounts of data to quickly identify relationships of potential interest. It's likely to make drug development much more efficient and quick."

Interestingly, the researchers found that some synthetic lethal pairs predicted by MiSL were found in multiple human cancers. In particular, the genes tended to be involved in pathways of broad biological significance, including the Krebs cycle, which releases energy stored in carbohydrates, fats and proteins; the DNA repair machinery used by cells to correct genetic mistakes; and the Wnt signaling pathway, which has been shown to be critical in normal development and many human cancers.

Other Stanford co-authors are hematologist Steven Chan, MD; research assistant Damoun Torabi; postdoctoral scholar Andreas Reinisch, MD, PhD; former CIRM Bridges intern David Cruz; resident Andy Chan, MD; and assistant professor of radiation oncology Erinn Rankin, PhD.

Cancer uses inflammatory pathways to protect itself

In recent years, scientists in the laboratory of institute director Irving Weissman, MD, discovered that cancer cells cover themselves in copies of the CD47 "don't eat me" protein to protect themselves from being engulfed and devoured by immune cells called macrophages. But it was never clear how cancer cells increased the production of CD47.

Now, Weissman and his colleagues have discovered that cancer cells accomplish this trick by recruiting molecular pathways usually used for inflammatory processes. One particular pathway involves a protein called tumor necrosis factor (TNF-alpha), which is produced in response to infection or trauma. It attracts and activates macrophage cells, which destroy sick or damaged cells. Ironically, that same genetic machinery is being used by cancer cells to protect themselves from those macrophages. The researchers published a paper describing the research in the journal Nature Communications.

"Usually TNF-alpha is involved in attracting immune cells to tissues that need to be healed," said Paola Betancur, PhD, a postdoctoral researcher in the Weissman lab and first author on the paper. "But in this case, the tumor is using the same inflammatory mechanisms to protect itself by increasing CD47 production."

The researchers found that TNF-alpha stimulates transcription by binding to a genomic region associated with the CD47 gene known as a "super-enhancer." Tumor cells can evolve super-enhancers by leaving more of their DNA free for multiple transcription factors to bind, resulting in a kind of critical mass that markedly increases the production of the associated protein.

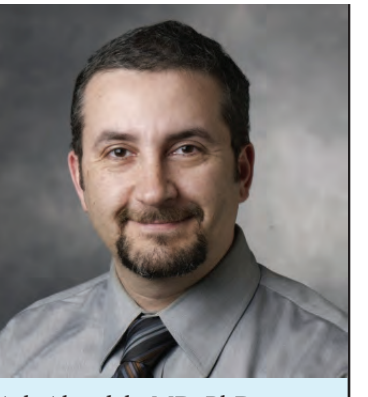
"By mapping enhancers, we showed that each cancer can lock in the permanent increase in CD47 production by turning enhancers into super-enhancers," said Weissman, who is also director of the Stanford Ludwig Center for Cancer Stem Cell Research and Medicine.

The team then tested possible anti-cancer therapies. After implanting human breast cancer samples into mice, they treated the breast tumors with antibodies to TNF-alpha and/or to CD47, with the goal of triggering an immune attack. Since both these mechanisms can decrease the amount of CD47 "don't eat me" signal that the macrophages see, using both antibodies together should boost the anti-cancer activity of the immune system, which is exactly what the scientists saw. The team now plans to test if this strategy works with other kinds of cancers.

Ash Alizadeh awarded Parker Institute grant

The Parker Institute for Cancer Immunotherapy at Stanford has awarded its first round of bench-to-bedside grants to four research teams at the School of Medicine.

These grants are designed for faculty with early-stage projects in cancer immunotherapy that might not be funded through traditional sources. Each team, consisting of both basic science and clinical investigators, will receive $200,000 over two years. Institute member Ash Alizadeh, MD, PhD, assistant professor of medicine, and Russ Altman, MD, PhD, professor of bioengineering, of genetics and of medicine, are studying cancer antigens — molecules that induce an immune response and that are key to controlling or curing cancer. They are developing a method for predicting which tumor antigens are most likely to be useful in this process. Their team includes graduate student Binbin Chen and research scientist Chih Long Liu, PhD.



Ash Alizadeh, MD, PhD

Antibody fights pediatric brain tumors in early tests

Five types of pediatric brain cancer were safely and effectively treated in mice by an antibody that causes immune cells to engulf and eat tumors without hurting healthy brain cells, according to a new study by institute researchers. The immune therapy studied consists of antibodies against a cellular "don't eat me" signal called CD47. Developed in the Weissman lab, anti-CD47 antibodies are already being tested in early clinical trials in adults who have tumors outside the central nervous system, but they have never been tried against pediatric brain tumors until now.

Anti-CD47 antibodies are already being tested in early clinical trials in adults who have tumors outside the central nervous system, but they have never been tried against pediatric brain tumors until now

The new study pitted anti-CD47 antibodies against human cancer cells that had been grown in a dish and implanted in mice. The tests targeted five aggressive pediatric brain tumors: Group 3 medulloblastoma, atypical teratoid rhabdoid tumor, primitive neuroectodermal tumor, pediatric glioblastoma and diffuse intrinsic pontine glioma.

"For many of these tumors, there's just no treatment," said institute member Samuel Cheshier, MD, PhD, former Stanford assistant professor of neurosurgery. "Diagnosis is synonymous with a death sentence."

The study was published in Science Translational Medicine. Cheshier shares senior authorship of the paper with institute director Irv Weissman, MD, the Virginia and D.K. Ludwig Professor for Clinical Investigation in Cancer Research and professor of pathology and of developmental biology. The lead authors are postdoctoral scholar Sharareh Gholamin, MD, and senior research scientist Siddhartha Mitra, PhD.

Many childhood brain tumors are inoperable. Some also lack effective chemotherapy drugs, or require radiation and chemotherapy so toxic to the developing brain that they cause devastating long-term side effects. In contrast with the toxic profile of existing treatments, the preclinical trials conducted by Cheshier's team indicate that anti-CD47 antibodies specifically target cancer cells while leaving healthy brain cells alone.

"The most exciting aspect of our findings is that no matter what kind of brain tumor we tested it against, this treatment worked really well in the animal models," said Cheshier, who is now an associate professor of neurosurgery at the University of Utah School of Medicine. In mice that had been implanted with both normal human brain cells and human brain cancer cells, "there was no toxicity to normal human cells but very, very active tumor-killing in vivo," he said.

Given the encouraging results of the new study and the ongoing research on anti-CD47 antibodies in adults, the antibodies are expected to reach clinical trials in children with brain cancer in one to two years, he added.

Irv Weissman, MD

The anti-CD47 antibodies did not completely eliminate all tumors, suggesting that the antibodies may not be able to completely penetrate large tumors, the researchers noted.

To maximize their effects, the antibodies will likely need to be combined with other forms of cancer treatment, a concept the researchers plan to investigate further, Cheshier said. In the future, patients may receive combinations of immune therapies and lower doses of standard cancer treatments, he said, adding, "The question is: Can we wisely combine immune therapies and other approaches to make cancer treatment more efficacious and less toxic?"

Researchers turn leukemia cells back into iPS cells

The question sounds more like sociology than biology: What would happen if you could take a cell gone bad — a cancer cell — bring it back to its infancy, before it turned to the dark side, and let it grow up again? Would it become cancerous again? What if you raised it in a different environment? Stanford Professor Ravi Majeti, MD, PhD, and his colleagues posed this simple question about a leukemia cell. And the answer they got gave them a new set of tools for studying leukemia and designing better therapies against it.

Ten years ago, Japanese scientist Shinya Yamanaka discovered that researchers could chemically push the reset button on mature cells, turning them into something very close to embryonic stem cells, which he called induced pluripotent stem (iPS) cells. If genetic instructions are like the recipes in a cookbook, epigenetic markers are like the notations and sticky notes that accumulate over time in that cookbook as the chef gains experience, annotating which recipes should be modified, ignored or are favorites. Mature cells of different types may have the same DNA (the same recipes) as embryonic cells, but how they look and behave is different because the epigenetic annotations are different. Yamanka's process for creating iPS cells is like taking out all the notes in a cookbook—all the modifications and annotations are wiped out and the cell reverts to its naïve, embryonic state. These iPS cells could then be grown back up into mature cells of various types

Over the last decade, researchers around the world had successfully turned mature cells of various types into iPS cells, but no one had yet succeeded in creating iPS cells from leukemia. When Majeti and former post-doc Mark Chao, MD, PhD, figured out how to create an iPS cell out of a leukemia cell they were curious to see what would happen. After all, although the epigenetic annotations had been wiped out, the cells still had the genetic mutations that the leukemia cells contained. When they grew the leukemia iPS cells up into other kinds of cells like heart cells or neurons, the cells behaved completely normally. But when the cells grew into blood cells, they once again became cancerous.

"This was super surprising to us," says Majeti, who is a member of the Stanford Ludwig Center in addition to being a member of the Institute for Stem Cell Biology and Regenerative Medicine. "What this tells us is that context matters. Those leukemic gene mutations only cause cancer when they exist in the context of a blood cell."

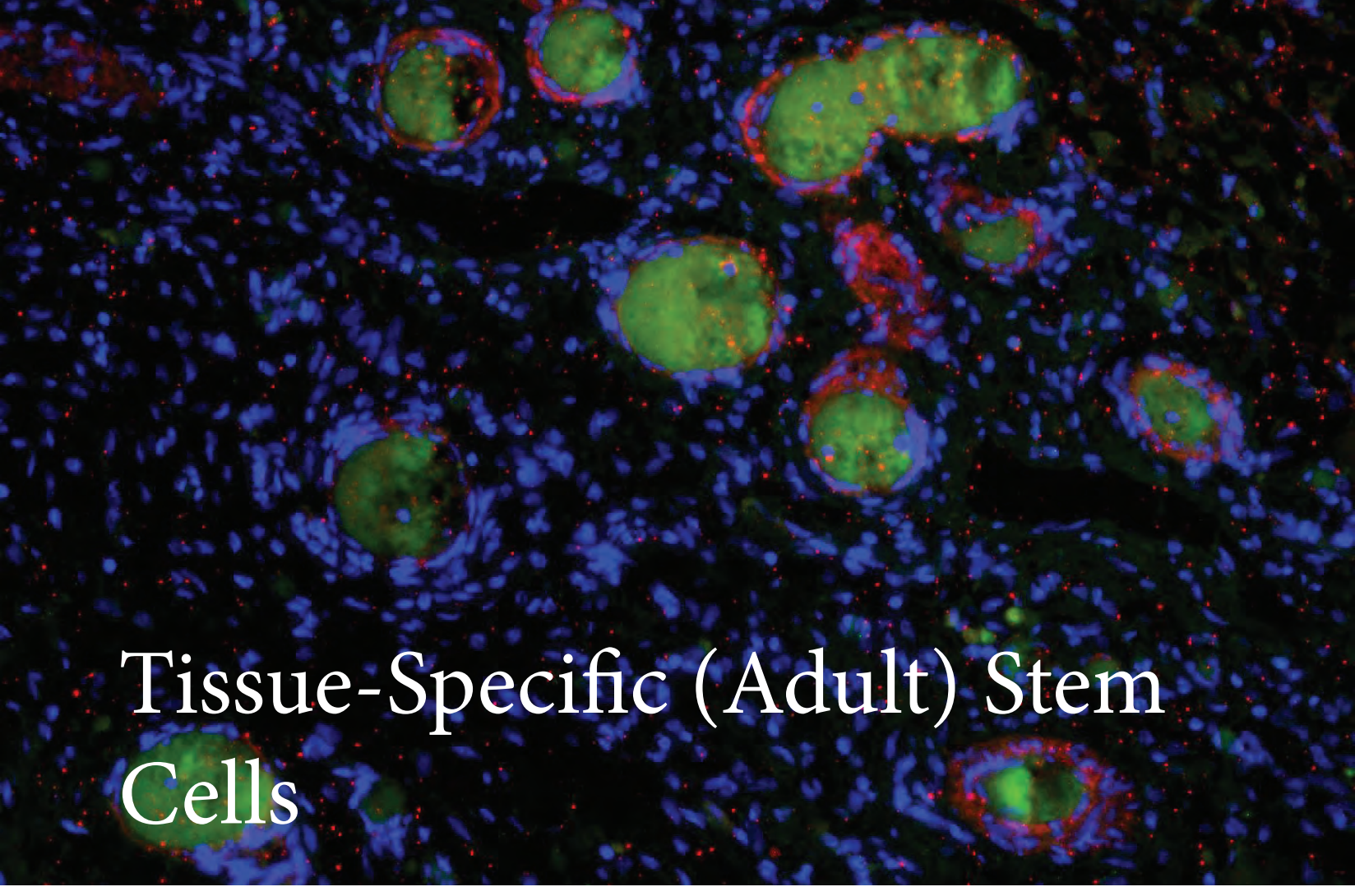
Ravi Majeti

The ability to make lots of leukemic cells from a single iPS cell also provides a number of important tools. "We can now grow up a lot of leukemia cells," Majeti says. "Getting a large number of cancer cells to study can be a limiting issue since patient cells are difficult to grow."

Creating iPS cells from a single leukemia cell can also help researchers study the natural variations that occur among leukemia cells. "Leukemia cell populations actually contain a number of subclones that have different genetic mutations," Chao says. "Before this there was no good way to separate out and expand the different subclones, since some can be very rare. But now we can use this technology to create and study leukemias from each subclone within a patient's cancer."

This can be useful if physicians want to know how to treat a particular leukemia. If a patient has leukemia, pure populations of various subclones can be grown and tested to see how well they react to various chemotherapy agents. If a leukemia reacts well to chemotherapy but one subclone is less affected, that may set the patient up for relapse. In fact, using this technique, Majeti and Chao were able to verify that this is what happened in one particular patient. By analyzing blood samples stored over the course of the patient's treatment, they found that one subclone was more resistant to chemotherapy, and that this subclone ended up being the source of the patient's eventual relapse.

This technology now offers a platform to more accurately investigate how the genetics of individual cells contribute to leukemic disease. Majeti and Chao hope that these findings will lead to a better understanding of how leukemia evolves and to the development of better personalized therapies.



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.

Nerve cells actively repress alternative cell fates

A regulatory protein actively blocks the expression of non-neuronal genes in nerve cells. The finding suggests there are many master regulators to help cell types maintain their identities.

A neural cell maintains its identity by actively suppressing the expression of genes associated with non-neuronal cell types, including skin, heart, lung, cartilage and liver, according to an institute study led by Marius Wernig, MD, PhD.

It does so with a powerful repressor protein. "When this protein is missing, neural cells get a little confused," said Wernig. "They become less efficient at transmitting nerve signals and begin to express genes associated with other cell fates."

The study marks the first identification of a near-global repressor that works to block many cell fates but one. It also suggests the possibility of a network of as-yet-unidentified master regulators specific to each cell type in the body.

"The concept of an inverse master regulator, one that represses many different developmental programs rather than activating a single program, is a unique way to control neuronal cell identity, and a completely new paradigm as to how cells maintain their cell fate throughout an organism's lifetime," Wernig said.

Marius Wernig, MD, PhD

Because the protein, Myt1l, has been found to be mutated in people with autism, schizophrenia and major depression, the discovered mode of action may provide new opportunities for therapeutic intervention for these conditions, the researchers said.

Wernig is the senior author of the study, published in Nature. Postdoctoral scholars Moritz Mall, PhD, and Michael Kareta, PhD, are the lead authors.

Myt1l is not the only protein known to repress certain cell fates. But most other known repressors block only one type of developmental program, rather than many. For example, a well-known repressor called REST is known to block the neuronal pathway, but no others.

"Until now, researchers have focused only on identifying these types of single-lineage repressors," said Wernig. "The concept of an 'everything but' repressor is entirely new."

In 2010, Wernig showed that it is possible to convert skin cells into functional neurons over the course of three weeks by exposing them to a combination of just three proteins that are typically expressed in neurons. This "direct reprogramming" bypassed a step called induced pluripotency that many scientists had thought was necessary to transform one cell type into another.

One of the proteins necessary to accomplish the transformation of skin to neurons was Myt1l.

But until this study the researchers were unaware precisely how it functioned.

"Usually we think in terms about what regulatory programs need to be activated to direct a cell to a specific developmental state," said Wernig. "So we were surprised when we took a closer look and saw that Myt1l was actually suppressing the expression of

many genes."
These genes, the researchers found, encoded proteins important for the development of lung, heart, liver, cartilage and other types of non-neuronal tissue. Furthermore, two of the proteins, Notch and Wnt, are known to actively block neurogenesis in the developing brain.

> The discovered mode of action may provide new opportunities for therapeutic intervention, the researchers say

Blocking Myt1l expression in the brains of embryonic mice reduced the number of mature neurons that developed in the animals. Furthermore, knocking down Myt1l expression in mature neurons caused them to express lower-than-normal levels of neural-specific genes and to fire less readily in response to an electrical pulse.
Wernig and his colleagues contrasted the effect of Myt1l with that of another protein called Ascl1, which is required to directly reprogram skin fibroblasts into neurons. Ascl1 is known to specifically induce the expression of neuronal genes in the fibroblasts.
"Together, these proteins work as a perfect team to funnel a developing cell, or a cell that is being reprogrammed, into the desired cell fate," said Wernig. "It's a beautiful scenario that both blocks the fibroblast program and promotes the neuronal program. My gut feeling would be that there are many more master repressors like Myt1l to be found for specific cell types, each of which would block all but one cell fate."


Moritz Mall, PhD

Other Stanford co-authors of the paper are postdoctoral scholars Soham Chanda, PhD, Bo Zhou, PhD, Xuecai Ge, PhD, and Philip Brennecke, PhD; graduate students Cheen Ang, Thomas Vierbuchen and Daniel Fuentes; research assistant Sarah Grieder; undergraduate student Brandon Walker; professor of genetics Lars Steinmetz, PhD; and professor of molecular and cellular biology Thomas Sudhof, MD.

Vittorio Sebastiano receives $100,000 research grant


Vittorio Sebastiano, PhD

Institue researcherVittorio Sebastiano, PhD has received a $100,000 Research Grant from the American Federation for Aging Research (AFAR). Founded in 1981, AFAR is the premier not-for-profit organization supporting biomedical research to advance healthy aging and address age-related diseases.
Dr. Sebastiano's AFAR-supported research will study transient reprogramming for efficient cell-autonomous reversal of age-associated phenotypes.
On receiving an AFAR Research Grant for Junior Faculty, Dr. Sebastiano notes: "AFAR puts particular attention to the thriving of young investigators that have "out-of-the-box" ideas but with potential implications on the field of aging. I feel honored that my proposal was considered one of the most promising by the panel of investigators that reviewed the proposals. At this stage of my career, it is critical to obtain the resources and the recognition to build a strong and impactful research program."
The AFAR Research Grant for Junior Faculty provides an early career investigator with up to $100,000 for a one- to two-year award to support research focused on aging processes and age-related diseases. Moreover, this grant provides flexible support at a critical juncture in their career development when research funding is most difficult to obtain.

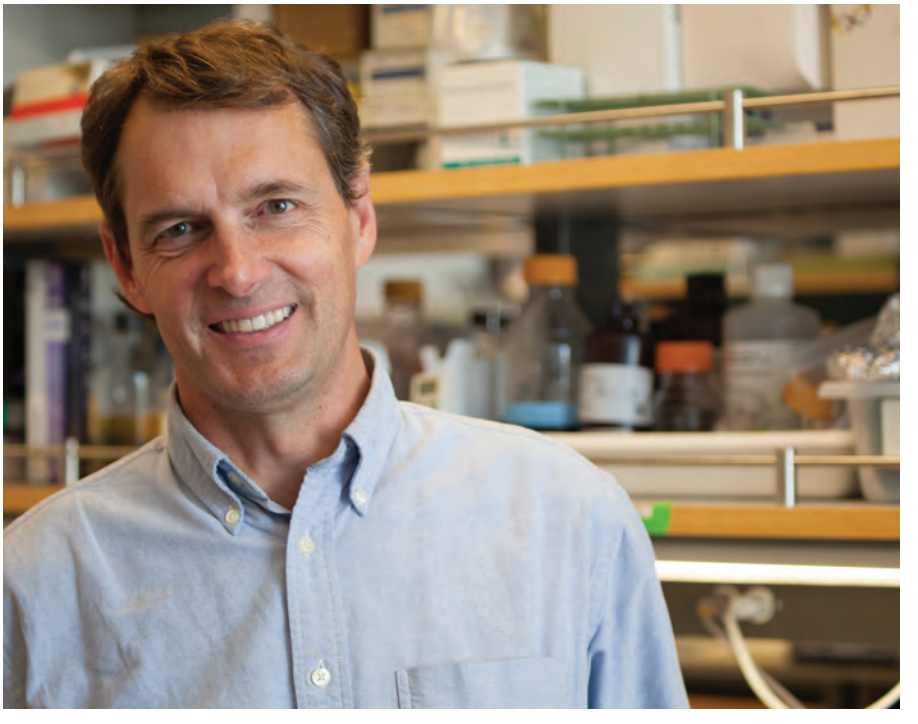
Porteus awarded grant for work on possible treatment for sickle cell anemia

The grant will be used to fund work that needs to be done before asking the Food and Drug Administration to sign off on the potential treatment as an investigational new drug.

The California Institute for Regenerative Medicine has awarded a researcher at the School of Medicine a grant of $5.2 million to lay the groundwork for a clinical trial of a possible treatment for sickle cell disease.

Institute researcher Matthew Porteus, MD, PhD, associate professor of pediatrics, has shown that he can take human blood stem cells with the gene defect that causes sickle cell disease and use gene-editing tools to repair the faulty gene. He also showed that he could successfully transplant those repaired blood stem cells into mice.

"We are extremely excited that, with CIRM support, we may be able to use gene correction to treat this terrible disease," Porteus said.



Matt Porteus, MD, PhD

Sickle cell disease damages tissues, causes pain and suffering and can even be life-threatening. It is caused by a single mutation in a gene that is the blueprint for one of the proteins in hemoglobin, the molecule that carries oxygen in red blood cells. Under certain conditions, red blood cells with the sickle cell defect will change from a soft, rounded form to a rigid, sickle shape. This change makes red blood cells clump together, clogging arteries and causing organ damage. There is currently no cure for the disease, and medical treatments are mostly restricted to efforts to limit the damage it can cause.

Porteus and his colleagues are preparing to conduct a clinical trial of the technique in patients with the disease. In such a trial, clinicians would draw participants' blood, separate out their stem cells and then use a gene-editing tool called CRISPR to fix the sickle cell defect. After this, patients would be given a chemotherapy regimen that would kill off some of the patient's defective stem cells, creating places in the bone marrow where the corrected blood stem cells could take up residence when they are given back to the patient. If the treatment worked, the repaired stem cells could possibly create enough normal red blood cells for the patient to be symptom-free for life.

The interdisciplinary team at Stanford, which includes people from the new Stem Cell and Gene Therapy Clinical Trials Office and the Laboratory of Cell and Gene Medicine, is excited to be part of what may be the first instance in which a stem cell correction strategy will be given to participants in a clinical trial, Porteus said.

The grant from CIRM will be used to do the work necessary before asking the Food and Drug Administration to give the treatment the status of an investigational new drug (IND), Porteus said. Getting this status is one of the last regulatory hurdles before a clinical trial can be put together.

Fibrosis reversed when 'don't eat me' signal blocked

A common signaling pathway unites diverse fibrotic diseases in humans, Gerlinde Wernig has found. CD47 reverses fibrosis in mice.

Irv Weissman, MD, Gerlinde Wernig, MD and their colleagues have identified a pathway that, when mutated, drives fibrosis in many organs of the body. The pathway turns out to underly what seem to be disparate conditions, including scleroderma, idiopathic pulmonary fibrosis, liver cirrhosis, kidney fibrosis and more, the researchers found. These diseases are often incurable and life-threatening. Importantly, the researchers were able to reverse lung fibrosis in mice by administering an antibody called anti-CD47 now being tested as an anti-cancer treatment.

"The variety of diseases caused by overproduction of fibroblasts has made finding a common root cause very challenging, in part because there has been no good animal model of these conditions," said institute director Weissman. "Now we've shown that activating a single signaling pathway in mice causes fibrosis in nearly all tissues. Blocking the CD-47 signal, which protects cancer cells from the immune system, can also ameliorate these fibrotic diseases even in the most extreme cases."

The researchers hope their findings will lead to the development of a reliable treatment of many types of fibrotic diseases. They are also planning to investigate whether the anti-CD47 antibody could be an effective treatment for people with fibrosis.

Fibrosis occurs when the body's normal response to injury goes astray. An overenthusiastic or inappropriately timed proliferation of cells called fibroblasts, which make up the connective tissue surrounding and supporting all of our organs, can lead to many devastating diseases. Until now, it's not been clear whether these diseases share a common biological pathway.

Gerlinde Wernig, MD

The researchers were building upon previous work by Wernig on a condition called myelofibrosis, or fibrosis of the bone marrow. In a mouse model she developed, she had found that fibroblasts were producing unusually high levels of an important signaling molecule called c-Jun. C-Jun is a transcription factor that drives the production of many proteins involved in critical cellular processes. It's been implicated in many types of human cancer.

In the current study, Wernig investigated c-Jun expression levels in 454 biopsied tissue samples from patients with a variety of fibrotic diseases. She found that, in every case, the fibroblasts from the patients with fibrosis expressed higher levels of c-Jun than did control fibroblasts collected from people with nonfibrotic conditions.

"We found that c-Jun is not just over-expressed, but it's also highly activated," Wernig said. "We

$2.2 million CIRM grant for liver cell generation

The California Institute for Regenerative Medicine has awarded Irv Weissman and colleagues a $2.2 million grant to research the production of liver stem cells from pluripotent stem cells. The research is based in large part on the work of Lay Teng Ang, PhD.

Liver failure is one of the 12 greatest causes of death in the United States. The only existing treatment is liver transplantation, but there is a huge shortage of available organs. Every day, 5 people die while waiting for a liver transplant.

The newly funded research is aimed at generating large numbers of liver cells from human pluripotent stem cells. The researchers will then evaluate how those stem cell-derived human liver cells incorporate themselves into a damaged liver in a mouse model.

The CIRM award provides an initial $1.4 million grant, with the rest of the award only becoming available if the researchers reach certain milestones.

wondered if its activity is necessary to maintain the disease."

Blocking the expression of c-Jun in laboratory-grown lung fibroblasts collected from people with idiopathic pulmonary fibrosis substantially decreased the proliferation of these cells, but not of lung fibroblasts collected from people without fibrosis, Wernig said. Furthermore,mice genetically engineered to overexpress c-Jun in all their body's tissues developed fibrosis in nearly every organ, including lung, liver, skin and bone marrow. Finally, she also found an intriguing link to past work from the Weissman lab.

"We found that c-Jun overexpression and over-activation is a unifying mechanism in many types of fibrosis," Wernig said. "But an even more exciting part of the story is the fact that we observed that the diseased, c-Jun-expressing fibroblasts are surrounded by immune cells called macrophages. This is reminiscent of what's often seen in human cancers."

Over the past eight years, researchers in Weissman's laboratory have shown that many human cancers evade the immune system by expressing high levels of a protein called CD47 on their surfaces. Blocking this protein with an anti-CD47 antibody restores the ability of the macrophages to gobble up the cancer and has proven to be a promising treatment in animal models of the disease. Anti-CD47 antibody is currently undergoing a phase-1 clinical trial in humans with advanced solid tumors.

"Like in cancer, these fibroblasts are proliferating excessively beyond what should be their natural limit," Weissman said. "We therefore wondered whether they are also expressing the 'don't eat me' signal on their surfaces to protect them from the immune system."

When Wernig treated mice with c-Jun-induced lung fibrosis with daily injections of anti-CD47 antibody, the animals exhibited significantly better lung function, lived longer than their peers and cleared the fibrosis.

The researchers plan to investigate whether any patients in the phase-1 trial of the anti-CD47 antibody also suffered from any fibrotic conditions. If so, they are eager to learn whether they experienced any relief as a result of participating in the trial.

"We have hit upon something unique in this study," Wernig said. "We identified a highly activated pathway that causes fibrosis in many tissues in mice, and we showed that treating the animals with an anti-CD47 antibody reverses the fibrosis. We're hopeful that this could be a potential treatment for people with many types of fibrotic conditions."

Wernig also tested inhibitors of other genes activated by c-Jun in the abnormal fibroblastic cells, and inhibitors of two pathways also reduced the fibrotic lesions.

"This study shows once again how basic science investigations in one field can lead to advances in what appeared to be unrelated diseases," Weissman said. "Here, our studies of human cancer have led to the discovery of the mechanisms of how other 'dangerous' cells in fibrosis escape removal by the body's scavenger cells. It shows how important it is to develop appropriate animal models of human diseases and then to use those models to identify disease-specific pathways that can be targeted."

Matt Porteus, others get $3 million grant to study Huntington's disease

Matt Porteus, MD, PhD

Scientists at Stanford and at the Gladstone Institute have received a $3 million gift from Taube Philanthropies to fund Huntington's disease research. The donation will support the first efforts to use gene editing and stem cell therapies to ameliorate Huntington's disease, a progressive, inherited neurodegenerative disease that lacks approved drugs to slow its progress and for which there is no cure.

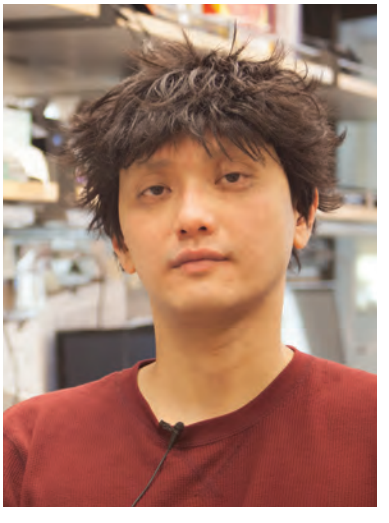
The research team is composed of Matthew Porteus, MD, PhD, who will lead the work; Frank Longo, MD, PhD, professor of neurology at Stanford; and Steve Finkbeiner, MD, PhD, of the Gladstone Institutes' Taube-Koret Center for Neurodegenerative Disease Research.

Diabetes impairs fracture repair by inhibiting bone stem cell activity in mice

Institute researchers found that activating bone stem cells helps repair fractures in diabetic mice. Applying a protein to the fracture site increased the expression of key signaling proteins and enhanced healing in the animals

Bone fractures in diabetic mice heal better in the presence of a protein that stimulates the activity of skeletal stem cells, according to a study by researchers at the Institute for Stem Cell Biology and Regenerative Medicine.

The protein counteracts a decrease in stem cell activity that the researchers observed both in mouse models of diabetes and in bone samples from diabetic patients who had undergone joint replacements. The researchers hope the discovery will lead to ways to help people with diabetes heal more efficiently from broken bones.



Charles Chan, PhD

"We've uncovered the reason why some patients with diabetes don't heal well from fractures, and we've come up with a solution that can be locally applied during surgery to repair the break," said institute co-director Michael Longaker, MD. "Diabetes is rampant worldwide, and any improvement in the ability of affected people to heal from fractures could have an enormously positive effect on their quality of life."

Longaker shares senior authorship of the study with Charles Chan, PhD, an instructor at the institute. Postdoctoral scholar Ruth Tevlin, MD, is the lead author.

Diabetes mellitus is a metabolic disease characterized by the inability to either produce or to respond appropriately to insulin. It affects hundreds of millions of people worldwide and is increasing in prevalence. In addition to causing dangerous swings in blood sugar levels after meals, the condition leads to many other debilitating symptoms, including an impaired ability to heal soft tissue injuries and skeletal fractures. The precise molecular reason behind this impaired bone healing has been unknown, however.

Longaker, Chan and Tevlin built on previous research in which they and colleagues in the laboratory of institute director Irv Weissman, MD, identified and described a population of cells in the bones of mice that serve as skeletal stem cells, or SSCs. These adult stem cells can become all components of the skeletal system, including bone, cartilage and a part of the bone marrow known as the stroma. They subsequently showed that fracture healing in mice was severely impaired when these stem cells were depleted. That finding got them thinking.

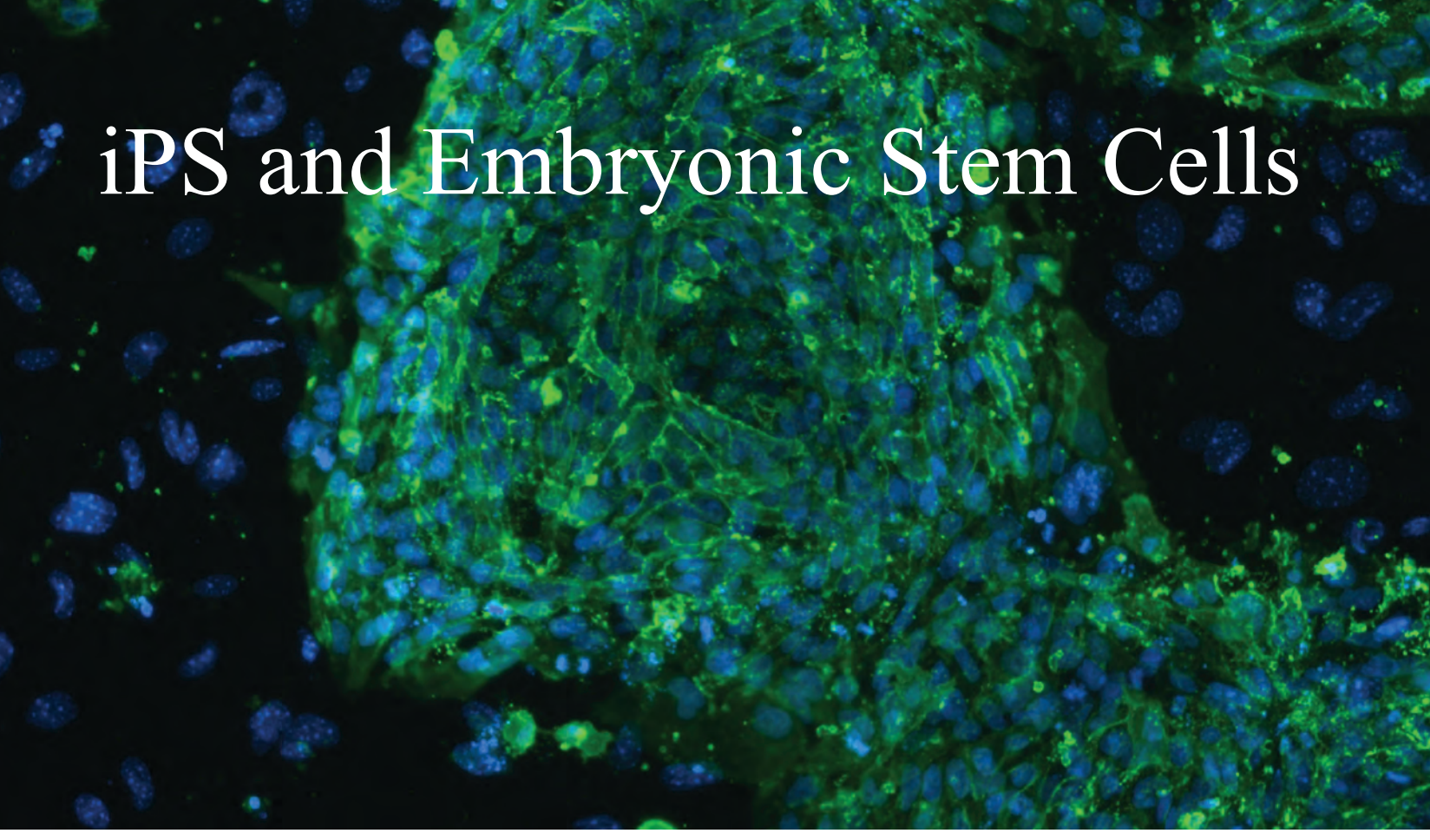
"We wanted to apply what we knew about skeletal stem cells to the problem of impaired bone healing in people with diabetes," said Chan. "Does the disease affect fracture healing by somehow modulating the activity of these stem cells?"

The researchers first used a mouse model of Type-2 diabetes. Later they replicated their findings in human bone.

"What we saw in human samples completely echoed what we saw in the mice," said Chan. "The bones from the diabetic patients displayed significantly reduced expression of important signaling proteins."

Longaker, Chan and Tevlin believe the inhibition of the hedgehog signaling pathway arises from diabetes-associated inflammation that causes high levels of a molecule called tumor necrosis factor alpha. TNF-alpha levels are known to be elevated in patients with diabetes, and the researchers observed a corresponding increase in their mouse models of the disease. They also showed that these increased levels of TNF-alpha inhibited the expression of some hedgehog family members.

"We anticipate that hedgehog-mediated molecular therapies that directly target stem cells in human patients could be therapeutic."



iPS and Embryonic Stem 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.

Dynamic DNA dance identified with CRISPR labeling

DNA twitches during transcription to bring distant regions in contact and enhance gene expression, according to Stanford researchers who devised a new way to label individual, nonrepetitive DNA sequences

DNA flails about during transcription like a strand of spaghetti being sucked through pursed lips, institute researchers have found. Like the resulting out-of-control flying globs of sauce, the surprising discovery flies in the face of conventional wisdom, which says that static loops of DNA are required to bring together distant gene regions in order to enhance and promote gene expression.

A new DNA labeling technique, which can precisely tag any individual stretch of DNA with fluorescent molecules to track their three-dimensional locations and movements, revealed this genetic dance. The technique, which the researchers have termed CARGO, for chimeric array of gRNA oligo, is a variation of the CRISPR/Cas9 gene-editing tool, and it promises to revolutionize the study of genome dynamics.

"We've found that, as the polymerase plows across the DNA, it provides a source of molecular agitation that increases mobility within a local chromosome domain and can repeatedly bring distant regions of the genome together," said Joanna Wysocka, PhD, professor of developmental biology and of chemical and systems biology. "This was entirely unexpected and surprising, and directly counters the prevailing beliefs about transcription. It's just one example of what we and others can now learn by using CARGO to label specific DNA regions."

CRISPR is most commonly used to seek out and replace specific DNA sequences in the genome with other DNA sequences. To do so, an enzyme called Cas9 uses a short RNA sequence to guide the DNA sequences to the correct spot in the genome.

A variation of the technique that was developed by other researchers instead uses guide RNAs and the CRISPR system with a catalytically inactive form of Cas9 to recognize and label specific stretches of DNA with fluorescent molecules. But that works best on highly repetitive regions where a single guide RNA can marshal the critical mass of fluorescent tags necessary to generate enough light to be seen through a microscope.

Joanna Wysocka, PhD

Wysocka, graduate student Bo Gu and another co-author of the study, Tomasz Swigut, PhD, devised a way to introduce an array of many different guide RNAs into a cell to precisely recognize nonrepetitive, unique stretches of DNA and label them with multiple fluorescent tags so they can be easily visualized under a microscope.

"All the most interesting stuff in the genome is present as single copies," Wysocka said. "People have been trying unsuccessfully to label single regions, or loci, for some time. But CARGO solves the delivery problem. Now we can label any region, or locus, that we want by using many different guide RNAs to blanket the DNA so we can see it clearly." She and Gu emphasize that the CARGO technique will be useful to researchers pursuing many different questions about the genome or gene expression.

Already it's opened their eyes about the process of transcription, which is often stimulated when distant enhancer regions are brought into close proximity with other DNA regions called promoters.

"We found that any locus we looked at moved about four times faster in its active state, when nearby genes are being transcribed into RNA," Wysocka said. "We propose that this enhanced movement, or diffusion, is likely to bring distant regions of the DNA together and further promote transcription."

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.

Aaron Newman becomes ISCBRM faculty member

Aaron Newman, PhD

In 2017, Aaron Newman, PhD, officially became an assistant professor at Stanford. Newman is the 6th faculty member who is billetted in the institute. His home department is Biomedical Data Science. Newman was hired after a long and extensive international search for someone who could invent and deploy high-powered data analysis tools to make new discoveries in stem cell and cancer biology.

Newman was an undergraduate and graduate student at UC Santa Barbara, earning his PhD in the Biomolecular Science and Engineering Program in 2010. He then came to Stanford as a postdoctoral scholar, developing genomic methods to advance the diagnoisis and treatment of cancer. In 2015, he became an instructor at Stanford.

"We are in an era of unprecedented volumes of genomic data, and by using novel computational techniques, we can paint a highly detailed picture of cells and complex tissues," Newman says. He has invented computational tools that allow researchers and clinicians to find extremely rare bits of cancer DNA in the bloodstream, which allows better treatment and diagnosis of cancer. He has also developed methods that allow researchers to study the cellular heterogeneity of tumors and other tissues, documenting their development and evolution. "I believe computational tools can significantly accelerate the discovery and analysis of clinically relevant cell subsets," Newman says.

Kyle Loh joins the institute as a faculty member

Kyle Loh, PhD

On February 1, 2018, Kyle Loh, PhD became an assistant professor at Stanford and the 7th faculty member billetted in the institute. His home department is Developmental Biology.

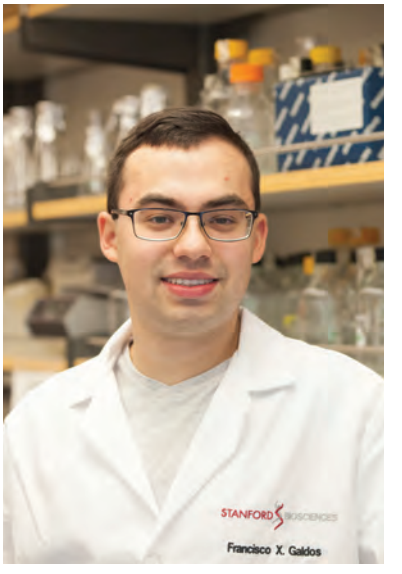
Loh's work concerns a major challenge of regenerative medicine and stem cell therapy: how can you create batches of stem cells that are purely one specific type of cell so that you can transplant them into patients. Typically, when embryonic or induced pluripotent stem cells divide and specialize (differentiate) they can become many different kinds of cells. Some of these cells should not be transplanted because they may lead to 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 finds 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.

SCBRM Graduate Program

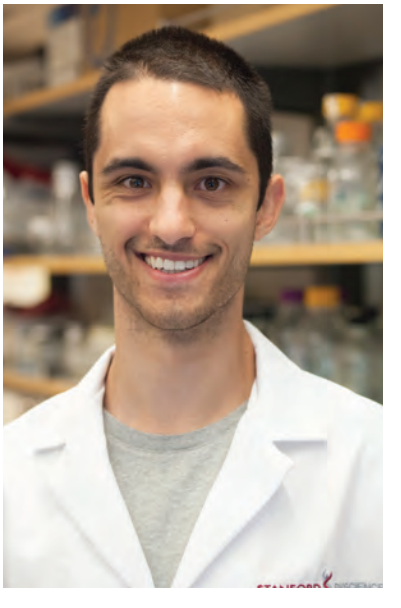
The 2017 entering class of SCBRM grad students

Francisco Galdos



I was born in El Paso, Texas and graduated from Michael E. DeBakey High School for Health Professions in Houston, Texas in 2011. I graduated magna cum laude in 2015 from Harvard University with a degree in Human Developmental and Regenerative Biology. I completed my undergraduate honors thesis in Dr. Richard Lee's laboratory focusing on identifying extracellular matrix components responsible for maintaining pancreatic beta cell function and maturity. Currently, I am an MD/PhD student in the Stanford Medical Scientist Training Program (MSTP) in Dr. Sean Wu's laboratory. I am passionate about pursuing a career researching and treating children with congenital heart disease and am currently working on a project to develop a genetic reporter system to study left ventricular development in a human induced pluripotent stem cell (hiPSC) model of hypoplastic left heart syndrome. I hope to combine the fields of stem cell biology and tissue engineering to provide novel ways to understand cardiac development in both normal and disease settings. I am very much in touch with my Colombian heritage and take yearly trips to Colombia. I enjoy salsa dancing, swimming, running, cycling, and love historical discussions.

Julien Roth

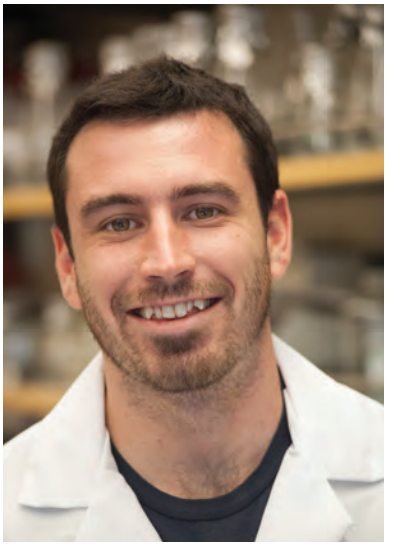


I graduated from UC San Diego in 2015 with degrees in Physiology and Neuroscience (BS) and Psychology (BS). Following my experiences working with patients with severe neurodevelopmental and neurodegenerative disorders, I became motivated to leverage the potential of stem cells to create novel platforms for understanding and treating neurological disorders. Before joining the Stem Cell Biology and Regenerative Medicine program at Stanford, I used human induced pluripotent stem cells to investigate the developmental trajectory of neuropsychiatric disorders like Schizophrenia (Gage Laboratory of Genetics at the Salk Institute) and Autism (Palmer Lab at Stanford University). Now, as a graduate student, I am eager to synergize my burgeoning interest in bioengineering and computational science with my background in stem cell neurobiology to explore complex questions and devise translational solutions. Outside the lab, I enjoy cooking (and eating) a wide variety of different cuisines, and am currently trying my hand at baking bread! Whenever time permits, I love being outside playing soccer, hiking/camping, or relaxing at the beach.

Malachia Hoover

I received my B.S. in Cell and Molecular Biology and Master's in Biology at California State University. During my undergraduate career at Cal State Northridge, I conducted research in Dr. Jonathan Kelber's Developmental Oncogene lab studying the mechanisms governing breast and pancreatic cancer. I continued in the Kelber lab for my master's thesis examining novel regulators of regeneration in the zebrafish caudal fin. At Stanford, I aim to continue my interest in stem cell biology as a potential therapy for degenerative diseases.

Carsten Chatsworth

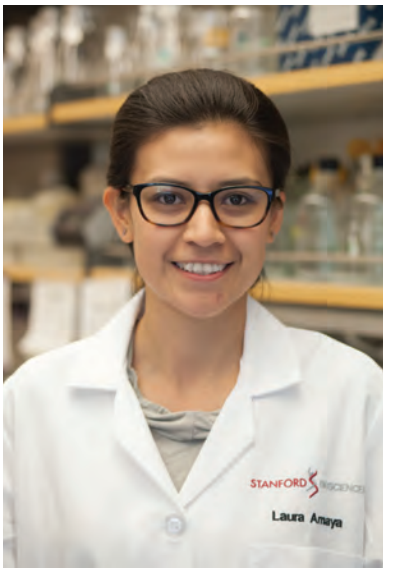


Growing up in Australia exposed me to a wide variety of wildlife. I knew from early on that I wanted to pursue a career in Biology. Moving to the United States at the end of High School, I earned my undergraduate degrea from Humboldt State, where I studied the role of paternal effect genes in C. elegans development. At the end of my undergraduate career I was fortunate to have the opportunity to come to Stanford as part of the CIRM Bridges program, where I worked on developing genome editing in hematopoietic stem cells for the treatment of genetic diseases in the lab of Dr. Matthew Porteus. After two years in the Porteus lab I joined Stanford's Stem and Regenerative Medicine PhD program, which I hope will serve as a platform in my attempts to become sole ruler of planet earth.

Carolyn Dundes

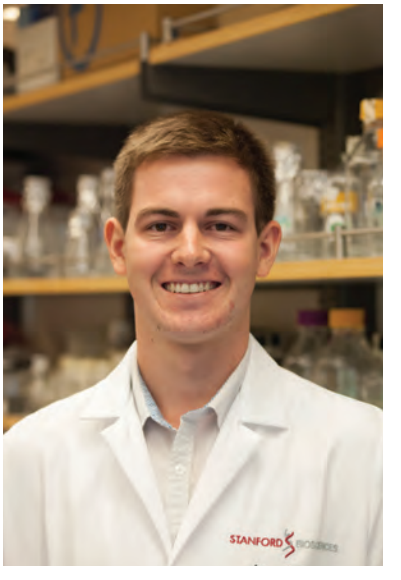
I received a B.A. in Biology from Wesleyan University in 2017. As an undergraduate, I worked with Dr. Laura Grabel on the differentiation of human embryonic stem cells into inhibitory interneurons. I also spent two summers studying Angelman Syndrome and Prader-Willi Syndrome using induced pluripotent stem cell models in the joint groups of Dr. Marc Lalande and Dr. Stormy Chamberlain at UConn Health. At Stanford, I aim to study developmental neurobiology through the lens of stem cell models and to apply this knowledge to novel therapies. When I'm not in lab, you can find me gleefully walking dogs, singing show tunes, or drinking lots of tea!

Laura Cristina Amaya Hernandez



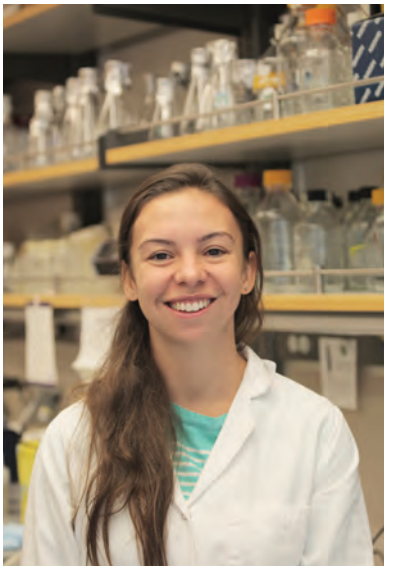
I completed my degree in Biotechnology Engineering in Mexico followed by a Master's in Biotechnology at the University of San Francisco. I interned at Distributed Bio under the supervision of Dr. Jacob Glanville, and in collaboration with Dr. Sarah Taylor from Clonetech Laboratories, Inc. I worked on characterizing the diversity of BCR and TCR repertoires among Hodgkins and Non-Hodgkin's Lymphomas. I also worked in the laboratory of Dr. Maria Grazia Roncarolo and Dr. Rosa Bacchetta at Stanford University investigating T cell development and plasticity through single-cell mass cytometry to better understand immune dysregulations. Outside the lab, I love Latin dance and I am mad about movies!

Jonas Fowler



I graduated from the University of Chicago in 2017 with a degree in biological sciences. My undergraduate research focused on the development of an optical clearing and multi-channel immunostaining technique for imaging thick human pancreatic samples for 3D analysis of endocrine islets and the role they play in diabetes progression. I am now interested in understanding the role of stem cells in the development and maintenance of tissues at different stages of life, from early embryonic development to adulthood. Outside of the lab, I enjoy being outdoors either swimming, hiking, climbing or snowboarding.

Renata Martin



I received my B.S. from Brown University, with a concentration in Biology. After graduating, I began doing research at Harvard in George Church's lab and worked on a project editing iPSCs and differentiating them into neurons to study Alzheimer's disease. Now I am in the Porteus Lab studying genome editing in embryonic stem cells using AAV6-mediated editing. Outside of science, I love to sing and play my electric guitar. I recently got this loop pedal and it is my favorite thing in the world!

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