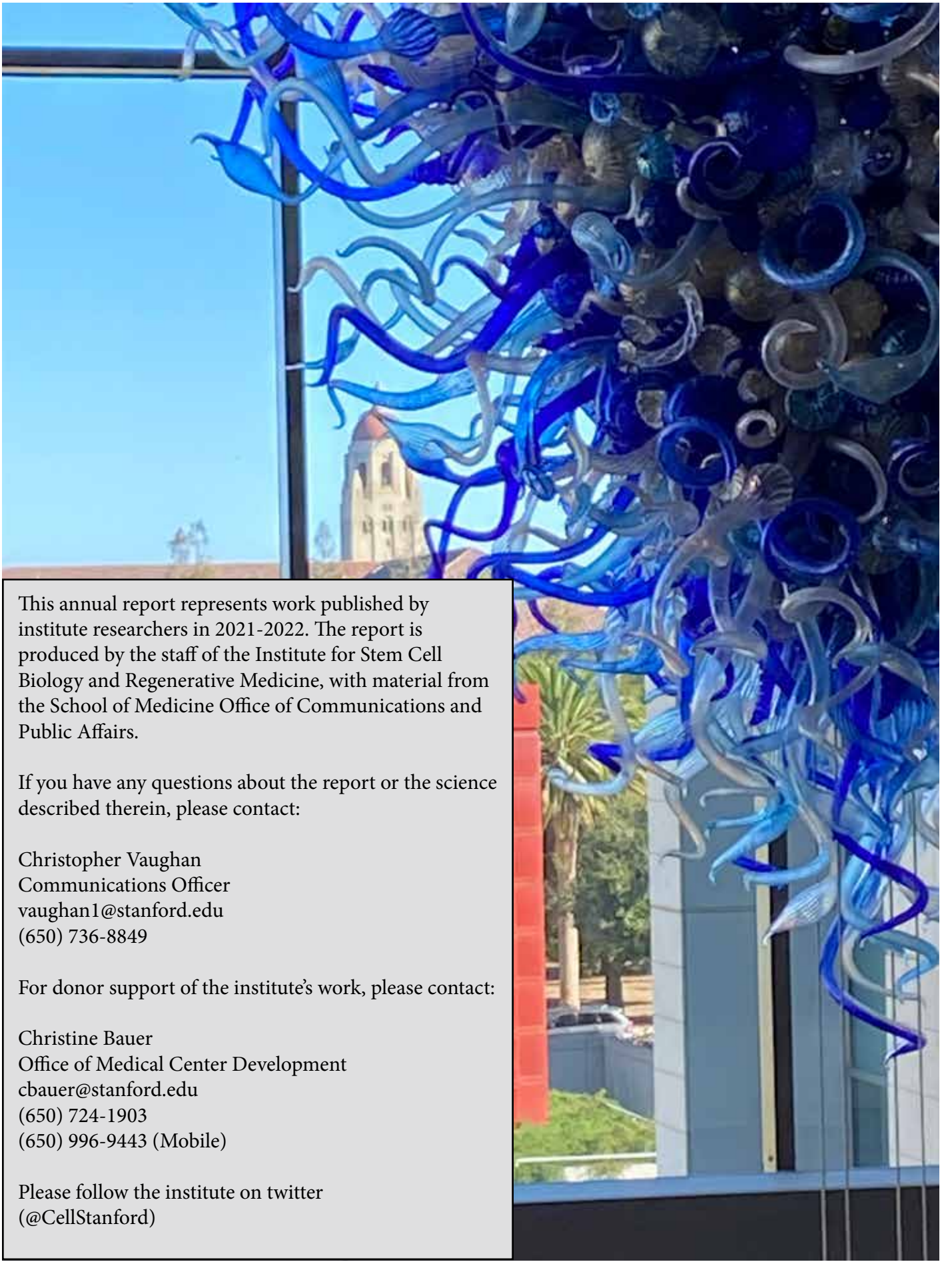
2022 ANNUAL REPORT

STANFORD INSTITUTE FOR STEM CELL BIOLOGY AND REGENERATIVE MEDICINE



This annual report represents work published by institute researchers in 2021-2022. 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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STANFORD MEDICINE | Institute for Stem Cell Biology and Regenerative Medicine

2022 ANNUAL REPORT

Message from the Director

Dear Friends of the Stanford Institute for Stem Cell Biology and Regenerative Medicine,

It is my pleasure to greet you as the new Director of the ISCBRM, and it is my honor and privilege to take the lead from my mentor, colleague, and our founding director Irv Weissman. The ISCBRM has seen many amazing successes in our first 20 years including major advances in stem cell biology basic research, clinical trials helping patients based on our discoveries, and training the next generation of leaders in stem cell biology. Our vision for the next term at the institute is to build on this amazing foundation to continue pushing the boundaries of stem cell science and translate these discoveries to patients. We look to expand our faculty with a commitment to diversity, equity, and inclusion, and to continue advancing in cutting edge areas. We also look to work with the broad scope of departments and institutes across Stanford Medicine and Stanford University to enhance our impact. And finally, importantly, we are excited and very grateful to continue working alongside our philanthropic partners, without whose visionary generosity we would be unable to pursue some of the most promising ideas our faculty might investigate. We thank you for your support and look forward to your continued partnership with the ISCBRM.

Best Regards,

Ravi Majeti MD, PhD
RZ Cao Professor of Medicine, Hematology
Director, Institute for Stem Cell Biology and Regenerative Medicine
Stanford University School of Medicine

Ravi Majeti becomes director of the institute, taking over from founding director Irv Weissman

In September 2022, after a long search, Ravi Majeti, MD, PhD, became the new director of the Stanford Institute for Stem Cell Biology and Regenerative Medicine. Majeti took over from Irv Weissman, MD, who stepped down as founding director of the institute after about two decades. Majeti has long been a member of the institute and stepped down from his position as chief of Stanford's Division of Hematology to become the institute director.

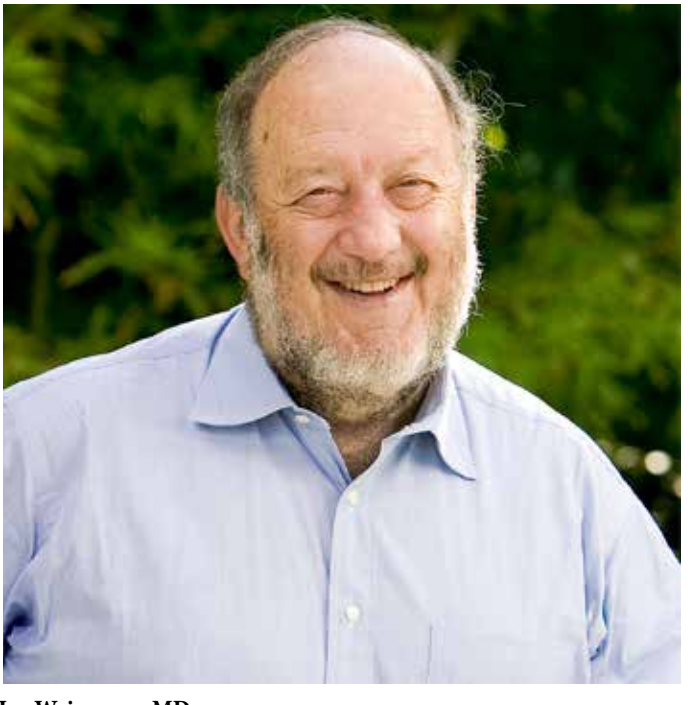
Ravi Majeti, MD, PhD

"In his time at Stanford Medicine, Prof. Majeti has contributed to many breakthrough research projects," Minor said. "Scientifically, he has been a leader in the characterization of leukemia stem cells in acute myeloid leukemia (AML) and has been a pioneer in the identification and investigation of pre-leukemic hematopoietic stem cells in this disease." Majeti also served as co-principal investigator on a multiple year effort to discover, develop and bring to clinical trials the CD-47 monoclonal antibody, which targets AML and other cancers. "Beyond his research, Dr. Majeti has proven himself a strategic and collaborative leader," Minor added.

Weissman has served as director of the institute since its founding in 2002. Weissman is the Virginia and D. K. Ludwig Professor of Clinical Investigation in Cancer Research. Weissman will remain as an active faculty member and will continue to do research. Weissman has said that he will be stepping away from being director of the institute in order to devote himself more fully to conducting a definitive clinical trial for a breast cancer therapy that showed great promise in a small clinical trial over two decades ago.

"My first objective is to bring cancer-free autologous HSC transplants to patients with metastatic disease following essentially lethal high dose chemotherapy, as we did 25 years ago," Weissman said.

Majeti said he is "honored and excited" to have the opportunity to lead the institute in the coming years. "The stem cell field is poised to make major advances in biology and medicine, and our Institute and investigators will be major contributors," Majeti said. "It is an exciting time for the field and for Stanford."



Irv Weissman, MD

In Memoriam: Philanthropist and stem cell science supporter Lorry Lokey (1927 - 2022)

Irv Weissman and Lorry Lokey at the dedication of the Lorry I. Lokey Stem Cell Research Building in 2010

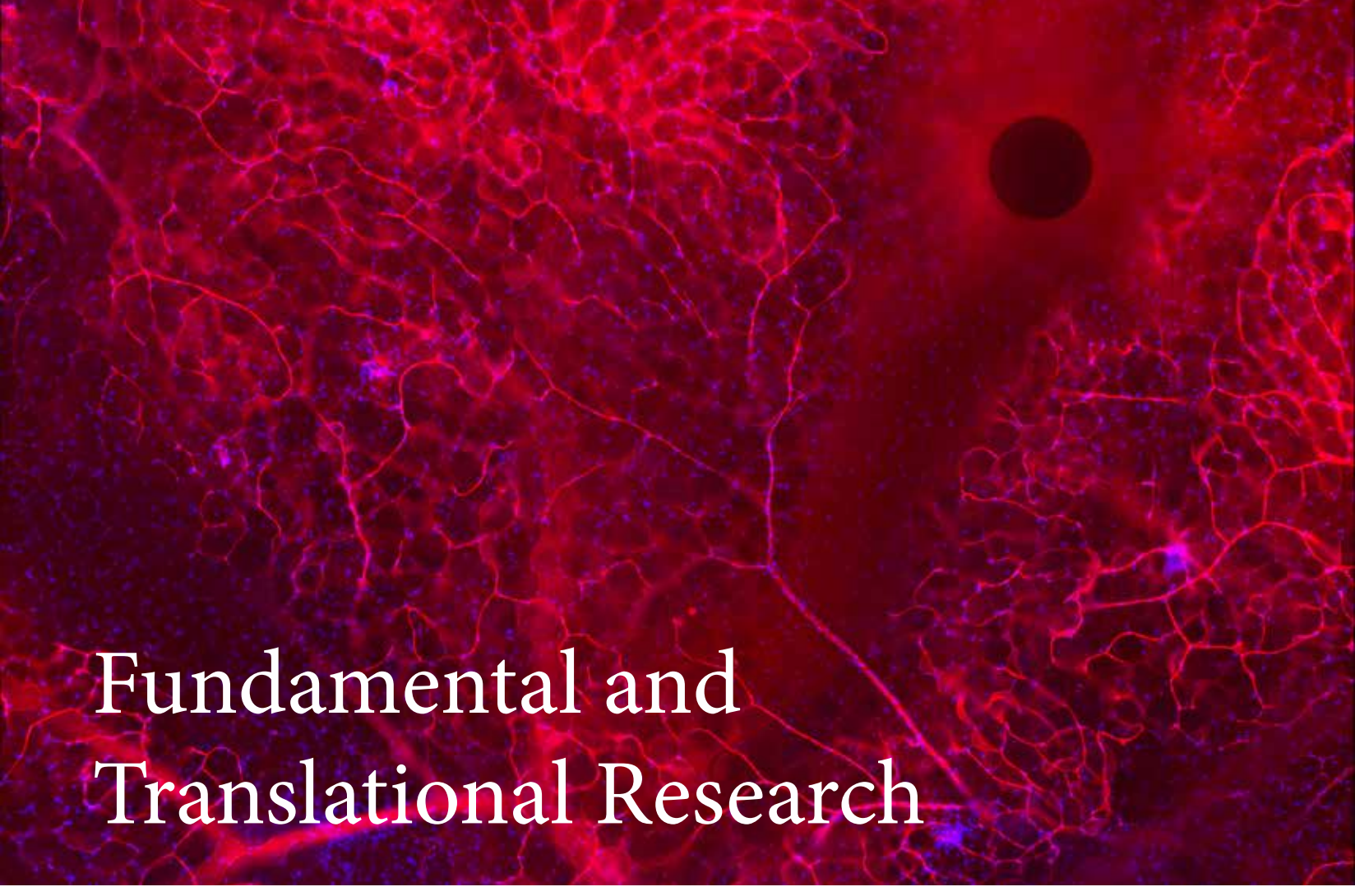
Lorry I. Lokey, a Stanford graduate and philanthropist who supported stem cell research and other causes, died October 1st. Lokey built a business empire and then proceeded to give nearly all of his money away, steering much of it to Stanford. Lokey was the largest private donor supporting the construction of the building called SIM1, which was named the Lorry I. Lokey Building for Stem Cell Research in his honor.

"With the passing of Lorry Lokey, Stanford University, and especially the Institute for Stem Cell Biology and Regenerative Medicine have lost the energy, the vision, and the continuous commitment he brought to realizing stem cell research and clinical translation here," said Irv Weissman, founding director of the ISCBRM. The construction of the Lorry I. Lokey Building for Stem Cell Research, and our biomedical stem cell discoveries and their clinical translations have attested to Lorry's vision. The lives saved now and in the future stand as perhaps his most enduring gift to people with what had been incurable diseases."

Lokey majored in journalism at Stanford and was editor-in-chief of the Stanford Daily. After graduating in 1949, he worked for the wire service United Press and in public relations for Shell Oil, among other jobs. In 1961, he realized that there might be a market for a subscription service that distributed business press releases over teletype machines, much as wire services like United Press distributed news stories. Lokey founded Business Wire, which went on to become a huge success. It was sold in 2006 for $500 million.

In 2012, Lokey joined Bill Gates, Warren Buffet and many of the other world's wealthiest people in promising to give away at least half their wealth. Before he died, Lokey gave away nearly all his wealth—over $800 million. Stanford was always high on Lokey's list when donating money. He served as the lead donor for the Lorry I. Lokey Laboratory for the Life Sciences, the Lorry I. Lokey Stanford Daily Building, as well as the Lorry Lokey SIM1 Building for Stem Cell Research.

"I do not really view the giving program as giving or spending or paying back," Lokey told the Stanford News Service during one campus visit. "When you get down to it, the action is one of reinvestment. Or, as farmers put it, plowing riches back into the soil in order to continue the run of good harvests."



Fundamental and Translational Research

Clues from Down syndrome hint at new Alzheimers finding

For a long time, common diseases (think Alzheimer's, cancer and other ailments) were thought to arise mostly from molecular or genetic mishaps. But scientists are finding that there seems to be increased involvement of an unexpected culprit: stem cells.

Nine years ago, institute member Michael Clarke, MD, made a connection between stem cells and Down syndrome: He showed in a mouse model of Down syndrome that many signature elements of the disorder -- including physical deformities and cognitive impairment -- were caused by reduced stem cell activity.

Clarke has now discovered that a molecule associated with Down syndrome may also be partly responsible for the pathologies of Alzheimer's disease.

Zeroing in on USP16

Down syndrome and Alzheimer's disease seem worlds apart on the disease spectrum. People with Down syndrome are born with the condition, which arises when embryos have three copies of chromosome 21 instead of two, and causes physical malformations and cognitive deficits. Alzheimer's, on the other hand, usually strikes late in life, is not caused by a particular genetic defect, and is primarily associated with memory problems.

During previous research, Clarke, associate director of the Institute for Stem Cell Biology and Regenerative Medicine, worked with mice that were genetically engineered to carry an extra copy of a variety of genes that researchers believe are tied to human Down syndrome.

But Clarke and his colleagues found that many of the signs of Down syndrome in these mice were associated with an extra copy of only one of those genes. This gene produces a protein called USP16, which regulates stem cell activity. Down syndrome-afflicted mice, it turned out, were making too much USP16, which suppresses levels of stem cell activity during development.

Besides physical changes and cognitive impairment, Down syndrome is also is associated with such disorders as cardiovascular disease and Alzheimer's disease. "If people with Down syndrome live into their 20s and 30s, they almost always start to develop Alzheimer's disease," said Clarke, the Karel H. and Avice N. Beekhuis professor of Cancer Biology. "We wondered if USP16 might also be playing a role in the development of Alzheimer's disease." Could too much USP16 be suppressing the activity of neural stem cells in both Down syndrome and Alzheimer's disease?

Michael Clarke, MD

In research published in March in eLife, the group showed that, at least in a mouse model of Alzheimer's, neural stem cells' reduced production led to a reduction of neural precursor cells, which develop into many kinds of brain cells and support repair and regeneration in the brain.

They also showed that lowering the level of the USP16 protein in Alzheimer's mice, increased levels of neural precursor cell production and, perhaps most importantly, improved cognitive performance on mazes and object recognition tests.

"USP16 seems to be one piece of the puzzle, a targetable component that has not been explored before," said Felicia Reinitz, MD, PhD, a former graduate student in the Clarke lab and first author of the eLife-paper. The researchers acknowledge that there is a lot more work to be done, but hope that this information might help them devise therapies to treat the worst aspects of Alzheimer's disease.

When it comes to healing without scarring, it pays to be small

Have you ever seen a salamander with a scar? No? Neither have scientists studying why some animals -- humans included -- scar and some don't.

Researchers at the institute have found that a phenomenon tied to animal size helps determine whether animals heal without scarring after injury -- in this case, burns. What›s more, the team has also found a way to manipulate this feature to allow burns to heal without leaving a permanent mark.

Burns not only cause disfiguring scars that mark a person for life, but the inflexible, plastic-like tissue found in scars can also interfere with physical motion and hinder normal physiological needs, such as sweating. Two researchers in the department of surgery have long focused on the process of scar formation, trying to understand why scars form and how they can be prevented.

Previous research by professor of surgery Michael Longaker, MD, has centered on how the human fetus can heal without scarring in the womb and why humans lose that ability once born. Geoffry Gurtner, MD, a professor of surgery and collaborator of Longaker's who has long been interested in the healing of burns, also noted that some animals, such as salamanders, can heal without scarring and can regenerate tissues their whole lives.

Michael Longaker, MD

Longaker and Gurtner shared senior authorship of a paper published in Science in April on how skin can heal without scarring after incisions, and on the more recent study of burn scarring, which published in September in Nature Communications.

So what is the difference between animals that can regenerate tissue without scarring, and those that scar? The answer, it turned out, stemmed from something few if any scientists had previously focused on: animal size and the physical forces on their cells.

"When animals get big, the forces on their bones and between cells are much, much greater; the cells and tissues change to withstand what are called allometric scaling forces," Gurtner said. Allometric scaling is a way to describe how various properties change, often drastically, as a result of changes in body size.

In some small animals -- think zebra fish and salamanders -- you don't need strong bonds between cells to help them glom onto one another, but when animals grow as large as elephants or dinosaurs, the bonds between cells must be much stronger. Without such strong bonds, even tissues of medium-sized animals, like cats or dogs, would never hold together on the animals' skeleton, the researchers said. It would be like trying to pile pudding on a coat hanger.

"All the animals capable of scarless regeneration have very small bones and their tissues are almost gelatinous," Gurtner said. "We wondered if these allometric scaling forces might be part of the reason that zebrafish can regenerate tissues, but humans cannot. Allometric scaling forces are known to change bone size and muscle strength, but no one had looked into how they affect tissue regeneration."

Scar-free healing

To investigate the role of these forces in scarring,

the researchers disrupted sensors that all cells have to detect mechanical stresses in their environment. The scientists blocked a molecule called focal adhesion kinase, the most evolutionarily conserved component of this sensing system, and observed how it affected the tissue healing process in pigs, which have the most similar skin to humans, through a very small lesion on anesthetized skin.

"To our surprise, simply blocking this one component allowed burn injuries that normally result in scars to heal with completely normal skin architecture and morphology, just like a salamander would," Gurtner said.

The researchers then cultured human cells engineered to mimic human skin, and precisely manipulated mechanical stresses to imitate the changes that would be observed with changes in body size. "We found that blocking these same biologic sensors allowed mechanically stressed human cells to revert back to a non-stressed state, similar to that in a smaller-sized animal," said Kellen Chen, PhD, a postdoctoral scholar in Dr. Gurtner's lab and one of the lead authors on the paper.

The finding has strong clinical implications for the treatment of burn injuries for humans and animals, especially because "there are zero Food and Drug Administration approved treatments that reduce scar formation," said Gurtner. "Patients with severe injuries or burns can suffer a lifetime of pain and disfigurement from scarring. The potential to heal burn injuries with regeneration, rather than scarring, would dramatically change the lives of these patients."

The researchers are now pursuing clinical trials to reduce scarring in burn patients or also use techniques described in the previously published research to reduce scarring after accidents or surgery. They are also hopeful that looking at these same mechanisms might be useful in helping other organs, such as the lungs, liver and heart, to heal without scarring after injury or disease.

CD47 reveals a possible cause of autism

Researchers at the Institute for Stem Cell Biology and Regenerative Medicine have linked an immune molecule to brain growth aberrations associated with autism. Their research points the way to potential treatments for this kind of brain alteration.

"It's very exciting to able to find underlying cellular mechanisms that may be at play in at least these forms of autism, and to start looking at ways we might be able to intervene therapeutically," said assistant professor Sundari Chetty, PhD. Chetty did her research in association with professor Irv Weissman, MD, director of the Institute for Stem Cell Biology and Regenerative Medicine. The research was recently published in the Proceedings of the National Academy of Sciences (PNAS)

The most severe forms of autism spectrum disorder often occur in people with larger than average heads, and many of these people also have deletions in a region of chromosome 16 known as 16p11.2, Chetty said. To understand what might be happening on a cellular level in people with alterations of the 16p11.2 gene locus, Chetty and her colleagues studied cell samples from autism patients with this genetic alteration. The cells were turned into iPS cells—chemically reset so that they can grow into the brain cells they wanted to study.

Normally brain development is a process of rapid cell multiplication and growth, combined with a process of pruning away cells that are not serving a functional purpose. "We wanted to test the hypothesis that the pruning process is aberrant in people with this chromosomal change, leading to brain overgrowth as cells that should be pruned away are not," Chetty said.

In the lab, the researchers grew brain progenitor cells from patient-derived iPS cells to see how they behaved and began to focus on the action of a protein called CD47. CD47 is well documented to act as a "don't eat me" signal that impedes the activity of immune cells whose job it is to devour and destroy cells in the body that are defective or infected with pathogens. Nearly all cancers overexpress CD47 to

protect themselves against the immune system, and a drug that blocks this "don't eat me" signal is currently in clinical trials as an anti-cancer therapy.

"We found that brain progenitor cells with 16p11.2 deletion syndrome overexpressed CD47, leading us to believe that cells that should be eliminated during brain development are not," Chetty said. They also found that cells with 16p11.2 deletion show increased cell surface levels of a protein called calreticulin, an "eat me" marker that signals the immune system that something is wrong with the cells and should be eliminated. "So we think these cells without the 16p11.2 locus are marked for elimination and would be pruned away if it weren't for the increased presence of the CD47 "don't eat me" signals that counteract that message," Chetty said.

The researchers next tested that idea by growing the brain progenitor cells in the presence of macrophages, the immune cells that devour and destroy defective cells. They observed that the cells with 16p11.2 deletion syndrome were not only making more CD47, the cells were also much less likely to be eaten by macrophages than control cells, as they hypothesized.

"These results raised the possibility that the normal state of affairs could be restored if the CD47 "don't eat me" signal was blocked," Chetty said. "Luckily we have antibodies that can do this." They performed the same experiments with the 16p11.2-deleted brain progenitor cells and macrophages, but this time also added an antibody that blocked the CD47 signal. "When we blocked the CD47 signal, it resulted in a more normal pattern of elimination of the 16p11.2-deleted cells," she said.

Lastly, the researchers wondered if blocking the CD47 signal would also restore normal brain cell elimination outside of a laboratory dish. They implanted 16p11.2-deleted brain progenitor cells in young mice, and also administered cd47-blocking antibody to some of them. Chetty and her colleagues found that CD47-blocking antibodies did indeed result in increased elimination of the 16p11.2-deficient brain cells and a more normal pattern of brain growth.

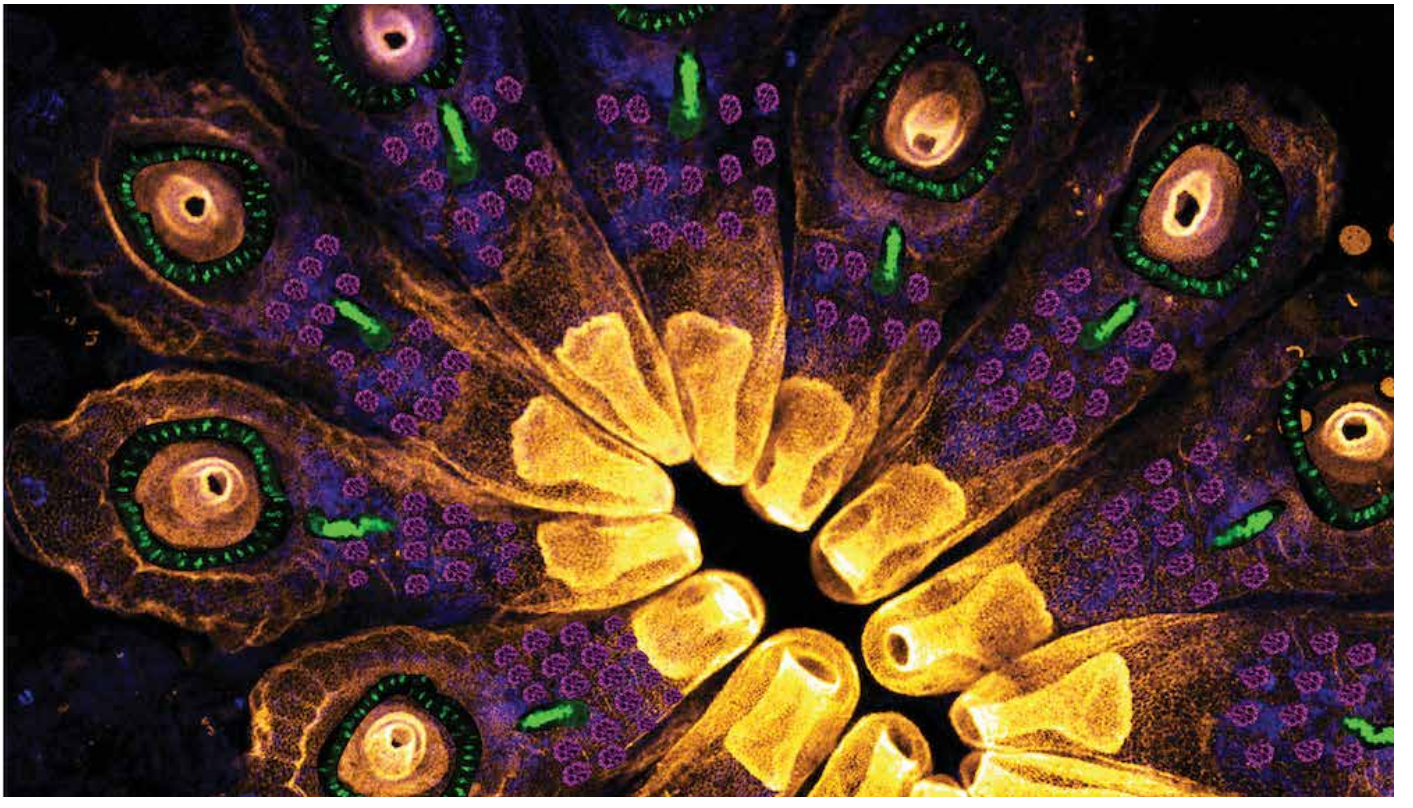
"This raises the possibility that, at least for people with this particular syndrome, we might develop a way to intervene as the brain is developing so that proper pruning of brain cells takes place," Chetty said.

Chetty and her colleagues note that improper pruning of the developing brain is also implicated in other psychiatric disorders like bipolar disorder, and it may be worth investigating whether CD47 or other immune regulating molecules are involved in those syndromes.

Sundari Chetty, MD

For Weissman, the research represents the fruits of scientific ideas he brought forth decades ago. "Nearly 20 years ago I suggested that the reprogramming of adult cells to bring them back to an embryonic-stage stem cells could be used to understand human genetic diseases," Weissman said. "Specifically, this technique can be used to understand which variant genes lead to which kind of disease, and how variant genes cause the disease at a cellular and mechanistic level." It was about ten years ago that Weissman and his colleagues discovered CD47, and intervening years they and others have documented how dangerous cells use it to protect themselves from the immune system, spawning a wide variety of diseases.

"Thanks to the brilliant work of Chetty and our collaborators, it is gratifying to me that this example provides evidence that combining these two scientific approaches can open the door to understanding diseases that cannot be studied in living humans," Weissman said.



A colony of Botryllus organisms (Photo by Chiara Anselmi)

Humans' closest marine invertebrate relative gives insights into neurodegenerative disorders

A tiny marine creature with a strange lifestyle may end up giving us valuable insights into human neurodegenerative disorders like Alzheimer's disease, say scientists at Stanford School of Medicine.

Botryllus schlosseri, also called the star tunicate, is most visible as a tiny flower-shaped organism that attaches to rocks along the coast. It is also humans' closest evolutionary relative among invertebrates in the seas. It starts life swimming in the ocean as a tiny tadpole-like creature with two brains, but then at some point it drifts down from the surface to settle into a stationary life on a rock, joining a colony of invertebrate organisms.

As it adapts to a sedentary life on the rock, like a human couch-potato, the tunicate loses brain power—one of the two brains, now unneeded for navigating the seas, is dissolved. But the way the brain degenerates and disappears has important parallels to the way the brain degenerates in human neural disorders, says Irv Weissman, MD, director of the Institute for Stem Cell Biology and Regenerative Medicine.

In a paper published this week in the Proceedings of the National Academy of Sciences (PNAS), Weissman and his colleagues show that many of the genes associated with neurodegeneration in Botryllus have analogues to the genes associated with neurodegeneration in humans. What's more, the researchers say, genetic changes that build up over decades in the Botryllus colonies affect neurodegeneration in many of the same ways as age-relationed genetic changes affect elderly humans.

"We think that Botryllus is the beginning of the vertebrate line," Weissman said. "And although the path that led to humans split far back in time, the essential stuff might stay the same." Weissman, who is the Virginia and D.K. Ludwig Professor in Pathology, is the co-senior author on the PNAS paper, along with Stanford Senior Scientist Ayelet Voskoboynik, PhD who is leading studies on this marine organism at Stanford's Hopkins Marine Station in Pacific Grove. Postdoctoral scholar Chiara Anselmi, PhD, is first

author on the paper.

Botryllus offers a lot of advantages as a model organism for studying neurodegeneration, the researchers say. Every week, each Botryllus organisms in a colony reproduces a-sexually, producing 2-4 buds that become new organisms. Each bud complete its development within two weeks, lives as an adult for one week, and then deteriorates and dies on the the last day of the third week.

"At the beginning we thought the number of neurons would be stable" during the adult stage during the third week, Anselmi said. "But the number is not stable, there is a specific pattern of neural degeneration." What's more, the process of neural degeneration is very similar to that in humans. "Out of about 1,000 genes that are involved in neural degeneration, we found that 428 are homologous genes shared by humans and Botryllus." So being able to study the process of neural degeneration in Botryllus may tell us a lot about neural degeneration in humans.

Things really get interesting when they looked at neural degeneration in the aged Botryllus colonies. One challenge when using mice to study humans neural degeneration is that the degenerative process is thought to be driven in part by changes that accumulate in neural stem cells over decades. But mice reach old age and die in about 3 years, long before they acquire the kinds of stem cell alterations that human have in old age. The Botryllus colonies at the Stanford Hopkins Marine Station in Pacific Grove have been around for over 20 years, however. Since the organisms in the colony reproduce asexually through stem cell mediated organogenesis, their stem cells are the only cells in the colonies that maintained throughout the years and most likely accumulate defects over time in the same way that ours will.

Elderly humans get neurodegenerative diseases more often than young people, and human neural stem cells are less active than they are when young. A similar pattern is seen in aged Botryllus colonies, which regenerate smaller brains than young colonies. "Something happens to the stem cells in the colony along the way, and after 20 years, they can't regenerate the way they did when they were young," Voskoboynik said. "There is a reduction in neurons of almost 30 percent in individuals brains in the aged colony, and even at the peak of neural generation it doesn't reach the peak of young colonies." Future studies on the differences between young and old neural stem cells aged in this relatively simple model organism will shed light on their role in neurodegenerative diseases in elderly humans.

"It is amazing that in an invertebrate you can see the same changes in genes from young to old that you see in aging humans," Voskoboynik said.

Furthermore, the individuals in those aging colonies show definite parallels to people with Alzheimer's disease, the neurodegenerative disease that usually strikes only in the last decades of life. One of the hallmarks of Alzheimer's disease is the accumulation amyloid plaques, which are created when amyloid precursor proteins (APP) glom together. "When individuals in the aged colonies go through that asexual cycle, not only do they make far fewer neurons, but those neurons have a lot of APP," Weissman said.

> Being able to study neural degeneration in Botryllus may tell us a lot about neural degeneration in humans.

Since no one yet knows the cause of Alzheimer's disease or the significance of amyloid in the neurons, the researchers are hopeful that Botryllus might be a powerful platform for studying the disease. "We can easily create 250 offspring every week and study various aspects of their neural development and degeneration," Weissman says. "We can put anti-sense signals in the water and block specific pathways that might lead to amyloid accumulation or other aspects of Alzheimer's neurodegeneration."

"Even though we are way behind the fly and mouse people in terms of understanding neural stem cells and the formation of brains in Botryllus, we believe this is an ideal animal to look through the window of so many biological processes that they share with humans," Weissman said.

Institute researchers create a detailed biological profile of the lives of a strange sea creature

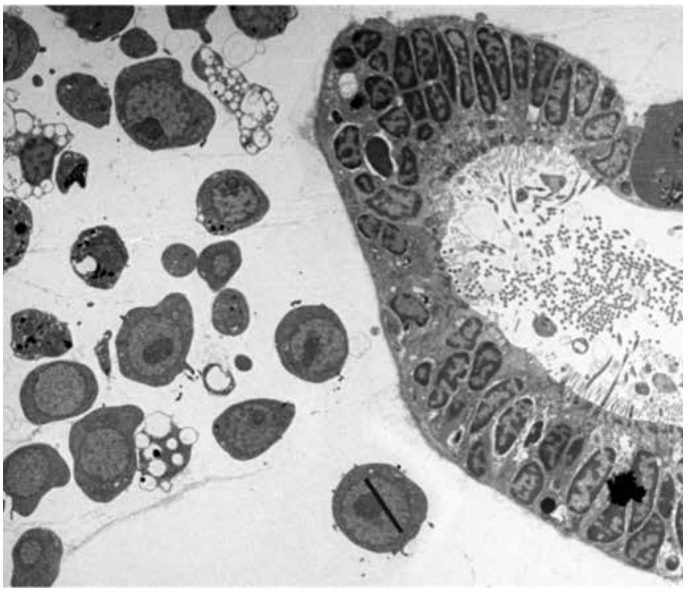
Stanford's Hopkins Marine Station

Researchers at the Institute and Stanford's Bio-X have compiled a thorough genetic and microscopic atlas of the developmental history of the unusual sea creature Botryllus schlosseri, which reproduces sexually during one part of its life, to make the chordate form in its life history, and asexually as an invertebrate during another part of its life. The researchers documented similarities and differences between the developmental programs guiding each form of reproduction and made surprising findings that may inform our understanding of how reproduction and regeneration occurs in all animals.

"One can compare the reproduction and development of Botryllus after fertilization as parallel to our embryonic and fetal development, while its asexual reproduction and development is akin to the maintenance of our own tissues and organs throughout life with tissue-specific stem cells," said Irv Weissman, MD, director of the Stanford Institute for Stem Cell Biology and Regenerative Medicine.

Botryllus is a creature with a very interesting life cycle. The sea-dwelling organism is the closest living invertebrate relative to humans, but it lives a very different life from us. Botryllus goes through two distinct phases—In one phase it reproduces sexually to create a tadpole-like creature with two brains which direct the muscles in the tail to swim directionally. This creature swims around until it finds a nice bit of underwater real estate to latch onto, settling down and absorbing one of its brains, its eye and its muscular tail, to begin an even stranger stage of life. In this phase, post-settlement, it begins reproducing asexually, budding off new individuals from the initial Botryllus. This budding process utilizes both tissue-specific stem cells as well as the germ stem cells.

All members live together communally in a colony that appear to be one organism. The tadpole-like creatures that swim out to find a new home mysteriously sense whether a compatiblility gene is shared or different, and co-settle if they share the gene. The individuals who live together and share a compatibility gene join together blood vessels that extend into the adjacent sibling. The individuals who live together and share the same variant of the compatibility gene called BHF, join together blood vessels that extend into the adjacent sibling. If they don't share this gene, the adjacent colony rejects the interloper and scars off the place where it tried to enter, blocking future immigrants. Between the colonies that share



Botryllus endostyle

blood circulation and exchange cells, germline stem cells can colonize and take over the reproductive organs of other individuals in the colony.

Institute researchers in the Weissman laboratory have long studied this strange sea creature because it offers the chance to answer fundamental questions about how stem cells are programmed to behave during development, how cells can identify whether other cells belong in the group or not and how cells attack invaders. In a paper published this week in the journal Cell Reports, institute researchers reveal details of the molecular/genetic and stem cell programs that guide reproduction in both the sexual and asexual phases of Botryllus's life, along with the morphological changes that researchers can see under the microscope.

"Many organisms, such as corals and sponges, can reproduce both asexually and sexually, but no one has compared these processes in the same organism in detail before," said Ayelet Voskoboynik, PhD, a co-corresponding author on the paper.

The researchers took samples during different stages of development when Botryllus reproduces sexually through embryogenesis, and when it reproduces by budding a new individual off the old one, a process called blastogenesis. The co-lead author, Mark Kowarsky, a grad student in the laboratory of Professor Stephen Quake, DPhil, from Bio-X, used bioinformatic methods to precisely analyze which genes were being expressed at which stages of both processes.

"Despite only sharing 30 percent of the genes guiding the two developmental processes, we were surprised that organs develop in the same order and have the same timing for gene expression in specific tissues" said Mark .

"Convergent morphology need not imply convergent molecular mechanisms," added Chiara Anselmi, PhD, one of the paper's a co-lead authors.

Furthermore, many of the shared genes were for transcription factors, which turn on and off whole groups of genes. "What this means is that during embryogenesis and blastogenesis, the same transcription factors are promoting different sets of genes," Voskoboynik says.

Development of tissues in both embryogenesis and blastogenesis is governed by how those sets of genes are activated in tissue-specific stem cells, which may offer lessons for how gene programs in our own bodies change after we finish embryonic development and start using the same stem cells to regenerate and repair tissues in adulthood, the researchers say.

Overall, the detailed analysis of very different forms of development is now a platform on which other research can ask far-flung scientific questions, Weissman says. " For example, we have known since the 1970s that mate selection in vertebrates such as mice, dogs and humans has an olfactory component genetically linked to the MHC genes that define our immunological identity," he said. "Now in Botryllus we might define the cellular and molecular principles that this is based on."

Other Stanford scientists involved in the research are life science research professionals Karla Palmeri and Kathi Ishizuka.

Analyzing cells' behaviors in social networks provides powerful tools for biological analysis

Aaron Newman, PhD

A personal assistant, a tech CEO, an auto mechanic, and a stay-at-home dad might have very distinct personalities and behaviors in their day-to-day lives, but when they all get together at a weekly gathering of their French club, the way they behave and the way they interact with others might be completely different. A CEO who is gregarious and confident at her job might be unsure of her French and hang back. The stay-at-home dad, who spent a year in France in high school, might leave behind his usual introversion and take on a leadership role.

Cells are much the same way. One type of cell can behave very differently when its environment changes. The changes both influence, and are influenced by, the cells of various types that surround it. These changes in behavior and interaction have deep implications for the development of normal and diseased tissues.

Researchers at the Stanford Institute for Stem Cell Biology and Regenerative Medicine have devised a powerful method of combining computer analysis with other analytical methods to examine how cells behave and interact in various environments and have used the method create a new understanding of how cancer develops and can be treated.

"Now we can look at the building blocks of tissue, the way that the whole cellular ecosystem is structured, rather than just looking at the types of cells present," said Aaron Newman, PhD, and assistant professor of biomedical data science and a member of the Stanford Institute for Stem Cell Biology and Regenerative Medicine. "It's a much more powerful way of looking at tissue organization."

Their method, called EcoTyper, combines new computer algorithms with those previously developed by the researchers to analyze cell types, single-cell RNA sequencing, cell sorting, and existing databases. One of the advantages of EcoTyper is that researchers can use vast reserves of stored tissue and huge public databases to run "virtual clinical trials" and analyze thousands of cancer cases on the computer in a highly cost-effective manner, Newman said.

Their work was published in the journal Cell. Newman, along with assistant professor of biomedical data science Andrew Gentles, PhD, are co-senior authors on the article, which showcase EcoTyper's capabilities with an analysis of the tissue architecture across different types of solid cancer tumors. In a companion research article also published September 30 in the journal Cancer Cell, Newman, along with professor Ash Alizadeh, MD PhD and colleagues, showcase EcoTyper's capabilities on lymphomas.

"Ecotyping" carcinoma, the most common cause of cancer-related death

While lung cancer might look very different from bladder cancer or other types of cancerous tumors under the microscope, the researchers found 10 clinically distinct subtypes of multicellular communities, dubbed "ecotypes", that existed across all different types of tumors. Furthermore, the presence or absence of certain ecotypes in a tumor was highly predictive for outcomes and often indicated what kinds of treatment would work best, even for very different types of cancer, the researchers say.

"We found one ecotype that was predictive of a good response to a particular immunotherapy," said Bogdan Luca, PhD, a postdoctoral researcher and co-first

author of the paper, who led the work with Chloé Steen, PhD. "In fact, it was even a better predictor than other candidate biomarkers that we tested, even ones that were specifically sought out to be predictive of response," Luca said. In addition, with the use of EcoTyper, the researchers were able to predict whether a premalignant lesion in the lungs would spontaneously regress or develop into lung cancer.

"EcoTyper can provide a platform for future therapies, because you have a better idea of the bad cells in a tumor you want to attack and spend less effort on the ones that are less bad," said Andrew Gentles, PhD, assistant professor of medicine. This focus on interacting cell populations in a tumor is different than current approaches, which usually target "driver mutations" or genes along a certain pathway. "Many cancer therapies are focused on a given cell type or gene, but there are always other cells contributing to the cancer or cells that don't have that gene mutation."

"EcoTyper can provide a platform for future therapies because you have a better idea of the bad cells in a tumor you want to attack and spend less effort on the ones that are less bad"

Ecotyping the most common blood cancer

In the research that was published in the Cancer Cell article, the researchers wanted to know if there really were two different subtypes of a certain kind of lymphoma, as has generally been accepted in the field. Using EcoTyper, they analyzed the microenvironment found amongst and around diffuse large B-cell lymphoma cells. When viewed in this way, the researchers were able to identify not two but nine different subtypes of this lymphoma.

Because the researchers were working from tissue samples from previous lymphoma cases, they also had a record of how these cancer cases turned out, and could correlate these statistics. "We found that not only were there many more subtypes of this B-cell lymphoma than previously recognized, but also were able to show that knowing which subtype people had gave us an improved ability to make predictions about how the cancer would likely progress," said Chloé Steen, PhD, a postdoctoral scholar and co-first author of the study.

One of the most striking features of this research is that the investigators were able to use their new understanding on a clinical trial that had seemed to be a failure.

Clinical trials for new therapies are vast undertakings, often costing a billion dollars or more to get move a drug from discovery to FDA approval. So, when a promising drug shows no statistically significant advantage in a clinical trial, it is not only a huge disappointment for doctors and patients, it is also a major financial hit for pharmaceutical companies.

Usually, clinical trials data is closely guarded by pharmaceutical companies, but in one case where a drug for lymphoma failed its clinical trial, the company felt that there was no economic value in that data anymore and put it on a public database. Newman, along with institute member Ash Alizadeh, MD, PhD, and their colleagues took that data and, in effect, reran the clinical trial, this time on the computer and including their new understanding of how many more types of this B-Cell lymphoma there were.

"What we saw was that there was in fact a specific lymphoma subtype that did respond to the therapy," Alizadeh said. "But in the original trial, they couldn't identify these other subtypes, and so this promising sign of efficacy was lost among the negative results for all the other lymphoma subtypes."

"Being able to find the right drug and craft effective cancer treatments based on the particular subtypes of cancer a patient is the epitome of precision health and personalized medicine," Alizadeh said. "Ecotyper helps us do that."

Stem cell molecule improves memory in mouse model of Alzheimer's disease

Researchers at the Stanford School of Medicine have shown that one of the earliest pathological changes in a mouse model of Alzheimer's disease is dysfunction in neural precursor cells (NPC), and that modifying NPC activity in these mice improved the memory problems that are a chief symptom of the Alzheimer's.

Currently, most proposed therapies for Alzheimer's disease target late-stage pathologies such as plaques and tangles, and none have proven very effective so far, said Michael Clarke, MD, the Karel H. and Avice N. Beekhuis Professor of Cancer Biology. "We wondered if targeting changes in neural precursor cells might be an earlier and more effective approach," Clarke said. Clarke is senior author on a paper documenting their results, published in the journal eLife. Former graduate student Felicia Reinitz, MD, PhD, is first author on the paper.

Clarke's research on the relationship between neural precursor cells and Alzheimer's disease grew out of research done many years ago on a seemingly very different disorder: Down's syndrome. Down's syndrome is caused by an aberrant replication of chromosome 21 in the developing embryo. People with Down's syndrome have 3 copies of chromosome 21 in their cells instead of two, and as a result, experience many developmental disorders such as brain defects and cardiovascular defects. In a dramatic finding, Clarke showed in 2013 that although there are thousands of genes on chromosome 21 that could possibly contribute to Down's syndrome, in fact many of the signs of disease were due to the overactivity of just one gene, which produced the protein USP16. They also showed that too much USP16 suppressed stem cell activity. Reducing levels of USP16 promoted stem cell activation.

Clarke and his colleagues then began to focus one notable characteristic of people with Down syndrome. "If people with Down syndrome live into their 20s and 30s, they almost always start to develop Alzheimer's disease," Clarke said. "We wondered if USP16 might also be playing a role in the development of Alzheimer's disease."

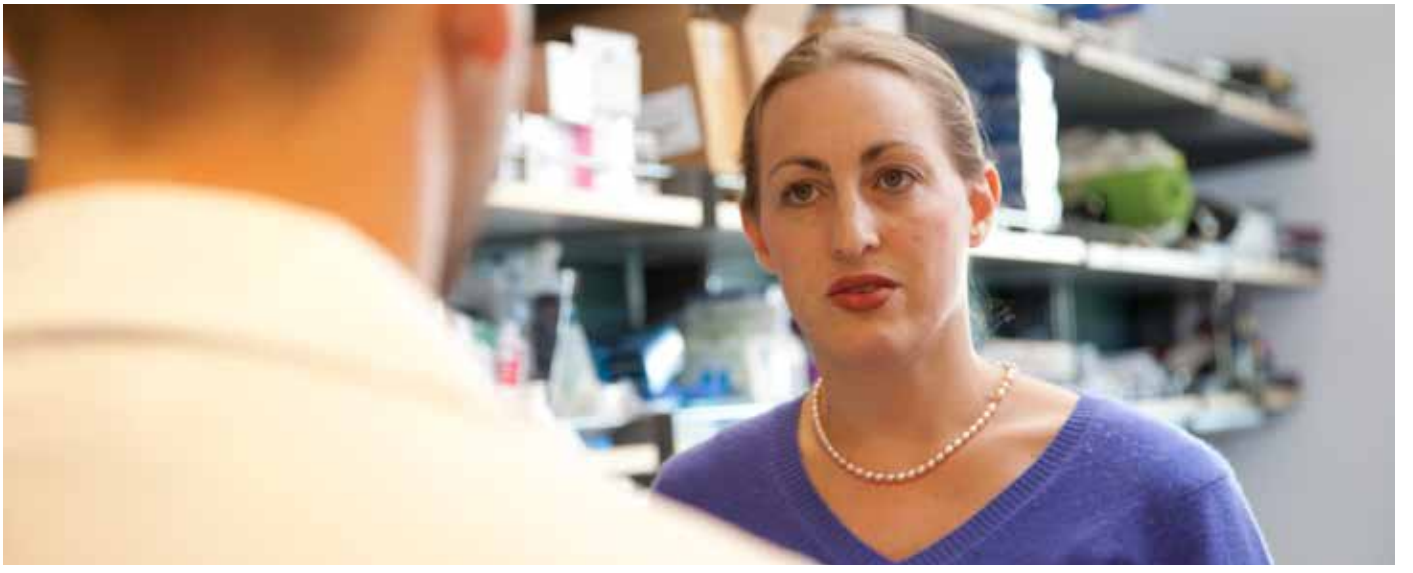
The connection seemed plausible because mouse models of Alzheimer's disease, in which mutated versions of amyloid precursor protein were introduced into the mouse genome, reduced the production of neural precursor cells, which are produced by brain stem cells and develop into various kinds of brain cells. In fact, reduced neural precursor cell production was the earliest sign of Alzheimer's disease, long before the development of amyloid plaques and neurofibrillary tangles. Because Clarke had shown that changes in USP16 levels could either diminish stem cell replication or promote it, they thought that the molecule might help correct this early decline in neural precursor cell production.

"We wanted to see if decreasing USP16 would normalize the brain stem cell defects we saw in the mouse model of Alzheimer's disease," Reinitz said. "What we found was that it did."

By manipulating the levels of USP16 in the Alzheimer mice, the researchers were able to restore normal levels of neural precursor cell production. But more importantly, they also showed that this also improved memory, which is the dominant feature of Alzheimer's disease. "Since we care a lot about cognitive impairment, we were gratified to see that treated mice improved significantly on object recognition tests and learning mazes," Reinitz says.

The researchers are aware that Alzheimer's is a complex disease, and that amyloid plaques and neurofibrillary tangles certainly are involved in the disease, they said. But manipulating USP16 levels may ultimately prove to be a useful tool in treating Alzheimer's, they added.

"USP16 seems to be one piece of the puzzle, a targetable component that has not been explored before," Reinitz said. "This, in combination with other therapies, may allow us to treat the worst aspects Alzheimer's disease."



Michelle Monje, PhD

Nerve activity can initiate the formation of tumors and foster their growth in neurofibromatosis

Researchers at the institute have shown, for the first time, that nerve activity is required for the formation of a particular kind of brain tumor in a genetically susceptible population. Institute researcher Michelle Monje, MD, PhD and her colleagues have shown that in a mouse model that carried a mutation in the neurofibromatosis gene NF1, nerve activity along optic nerves could initiate the formation of an optic glioma. The researchers also showed how the development and growth of the glioma could be controlled by controlling the activity of this nerve activity. Their research was published in the journal Nature.

Neurofibromatosis is a genetic disease that carries with it a significant risk that children with this disorder will form gliomas--cancerous tumors in the brain. Gliomas in the optic nerve are particularly common in children with NF1, affecting one in 6 children with the genetic syndrome. Monje, along with colleague David Gutmann at Washington University in St. Louis, studied how nerve activity affected the development and growth of tumors in a mouse model of the disease.

Activity in the optic nerve can be affected simply by regulating a mouse's exposure to light, which makes it to manipulate through controlled light exposure. "We found that tumors didn't form within the optic nerve when mice with predisposed to NF1-associated optic gliomas were not exposed to light during the critical period when these tumors develop," Monje said. "We were really surprised that tumor initiation was so dependent on optic nerve activity, despite a strong genetic predisposition to tumor formation in these mice."

The Monje lab had previously shown that the growth of another class of glioma (high-grade gliomas, including glioblastoma and diffuse intrinsic pontine glioma) was fostered by nerve activity. In this research, Monje and her colleagues showed that the growth of gliomas resulting from the presence of the NF1 gene mutation, which are low-grade gliomas, was also fostered by nerve activity.

The research has implications for the potential treatment of tumors associated with neurofibromatosis.. "These findings give us a new avenue to explore possible therapeutic interventions for these debilitating optic gliomas. We have a lot more work to do."

One possibility is to use pharmacological intervention to stop tumor initiation or growth. The Monje lab has previously shown that glioma growth is associated with the activity of a molecule called neuroligin-3 (NL3). "If we block neuroligin-3 signaling in mice, we see smaller tumors, but also fewer tumors," Monje said.

Aged skeletal stem cells interfere with healing and promote "inflammaging"

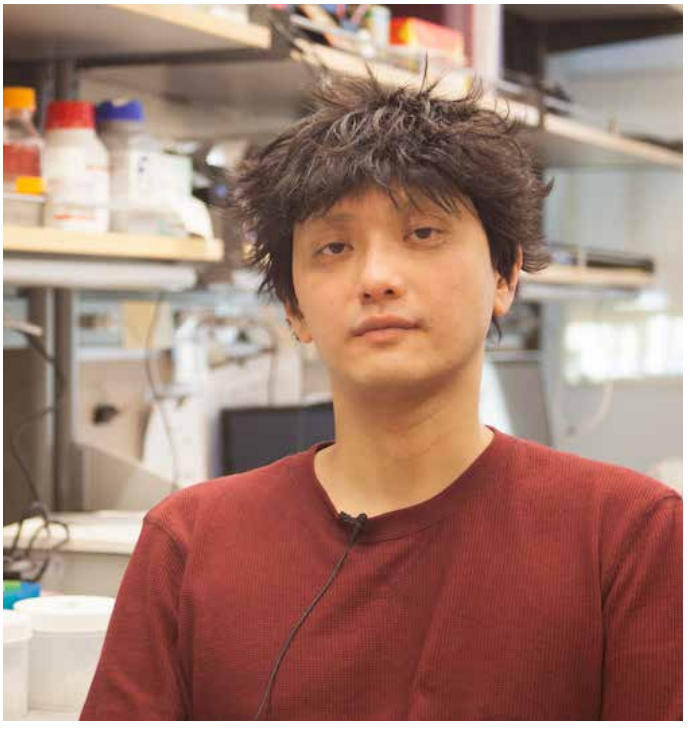
Researchers at the Stanford Institute for Stem Cell Biology and Regenerative Medicine have discovered how changes in aging skeletal stem cells may be an underlying cause of poor fracture healing, osteoporosis and various blood disorders as well as generalized inflammation and aging (sometimes called "inflammaging") of cells and systems throughout the body. However, the researchers are also discovering how they might reinvigorate aging skeletal stem cell so that they start acting younger again, potentially reversing these changes.

"Skeletal stem cells give rise to bone, cartilage, and special cells that provide a niche or nursery for blood and immune stem cells to develop," said Charles Chan, PhD, a member of the institute and an assistant professor in the Department of Surgery, Plastic and Reconstructive Surgery, and Immunology. "So if aged skeletal stem cells are not performing well, they can contribute to a wide variety of the disorders that we find in older people."

The Research was published in the journal Nature. Chan and professor Michael Longaker, MD, are senior authors on the paper. Longaker is the Dean P. and Louise Mitchell Professor in the School of Medicine and a member of the Institute for Stem Cell Biology and Regenerative Medicine. Postdoctoral fellow Thomas Ambrosi, PhD is a co- first author along with former medical student Owen Marecic, MD and former postdoctoral fellow Adrian McArdle, MD, PhD.

The researchers were first drawn to the question of how aging skeletal stem cell might contribute to problems healing bone fractures in the aged. Difficulty in healing bone is a leading cause of morbidity and mortality in aging adults. Building on their previous work in which they first discovered and characterized the skeletal stem cell, Chan and Longaker looked at how the behavior of the skeletal stem cells change as they age.

The researchers found that skeletal stem cells were much less active in older mice compared to younger mice. "When bone heals, it develops a callus at the fracture which is usually full of skeletal stem cells,"



Charles Chan, PhD

said Chan. "But in aged mice there are far fewer skeletal stem cells at the site of healing." Aged skeletal stem cells were also less able to form colonies or bone in the laboratory dish than young skeletal stem cells, they found.

Normally, bones are constantly in flux, with old bone tissue being resorbed and new bone being added to replace it and repair tiny fractures that appear over time. In young, healthy bones, this process is balanced. But in older bones, the researchers discovered that the genes expressed by aged skeletal stem cells were associated with reduced bone formation and greater bone resorption. This imbalance between bone formation and bone resorption ultimately leads to osteoporosis.

Investigating further, the group discovered that part of problem in fracture healing might be found in the connection between skeletal stem cells and the hematopoietic stem cells, or HSCs, that give rise to blood and immune cells. Skeletal stem cells produce not only bone and cartilage, but also special cells that create a nursery or "niche" for developing blood and immune stem cells in the bone marrow. They showed that aged skeletal stem cells produce a niche that al-

ters the development of blood cells. "Exposing HSCs to aged skeletal stem cells skews their development so that they produce more of the cells in the myeloid lineage, which includes a cell type that resorbs bone," Ambrosi said. The myeloid cells over-produced under the influence of pro-inflammatory aged skeletal stem cells also produce more inflammatory factors, which interferes even more with bone healing.

"This echoes what we see in the clinic, where we put a pin in a broken bone and it just doesn't heal," Chan said. "We think that the aged skeletal stem cells are not only making less bone, but they are influencing the development of blood stem cells in a way that that they produce more cells that resorb bone, and produce inflammatory signals that lead to the growth of fibrous tissue rather than bone."

By altering the normal development of blood and immune stem cells, aged bone cells may accelerate aging and disease in many other parts of the body as well. Inflammation is recognized as an important driver of aging in diverse tissues, and strong linkage between the two has led to the creation of the term "inflammaging." If aged skeletal stem cells are driving the creation of more inflammatory cells, they may ultimately be responsible for much of the increase in inflammation and the aging of tissues throughout the body.

"We really don't know where the influence of aged skeletal stem cells ends. They could end up being a primary driver of aging and disease generally"

Alterations in the normal development of blood stem cells has also been associated with the development of various disorders, such as atherosclerosis, so aging skeletal stem cells may play a role in the development of many other diseases. "We really don't know where the influence of aged skeletal stem cells ends," Chan saidsaid. "They could end up being a primary driver of aging and disease generally."

The researchers did find reason for hope, however. By doing single-cell genetic analysis of aged skeletal stem cells, they were able to see what genes were expressed less, and what genes were expressed more, as the stem cells aged. As mentioned previously, they saw an increase in many inflammatory factors, but they were drawn to an increase in one particular factor called Colony-stimulating factor 1 (Csf1). This molecule is necessary for bone healing, but it must be present in just the right amount—not too much and not too little. The age-related increase in the amount of Csf1 present seemed to be interfering with the healing process. The scientists also saw decreases in the expression of other genes, and in particular a decrease in the creation of a powerful skeletal stem cell stimulating signaling molecule called bone morphogenetic protein 2 (BMP2).

The group then treated the surface of a bone fracture in an aged mouse with a gel that contained BMP and an antibody that reduced the level of Csf1 to try to reset the behavior of the aged stem cells. The fracture healed much better, although they found that the levels of Csf1 had to be just right.

"We may be able to make aged fracture healing more like youthful fracture healing," said Longaker. "It's very exciting. "As our population is aging, the medical burden of aged fractures is also increasing, Longaker said. "If we can locally treat an aged fracture and rejuvenate the repair to be more robust, like it was when the patient was younger, this would have enormous benefits." For example, Longaker said, the patient could be walking and beginning their recovery more rapidly, thus avoiding complications such as a pneumonia or a blood clot in the leg.

The researchers also note that it is likely that the rejuvenation of aged skeletal stem cells will potentially reverse the skewed production of inflammatory immune cells and imbalances in the types of blood cells produced in older people. "We are hopeful that by addressing changes that occur as skeletal stem cells age, we may end up reducing age-related changes and disease throughout the body," Chan said.

Irv Weissman, MD

Institute researchers discover that blocking CD47 signaling protects against cerebral malaria in mice

Researchers in the laboratory of institute director Irv Weissman, MD, working with scientists the Food and Drug Administration, have discovered that a treatment currently in clinical trials as a cancer therapy is effective in combating the most deadly form of malaria in a mouse model. The research is being published today in the Proceedings of the National Academy of Sciences.

Malaria is currently one of the deadliest infectious diseases worldwide. This is partly due to the fact that malaria is endemic in many parts of the world, and partly due to the fact that there are few good treatments for severe forms of the disease. Every year, there are over 200 million cases and over 400,000 deaths worldwide. Cerebral malaria, when the parasite Plasmodium falciparum attacks the brain, is one of the deadliest forms of malaria.

The researchers in the Weissman lab had studied CD47, a don't eat me signal that keeps immune cells from attacking and devouring abnormal cancer cells. Anti-CD47 antibodies will block the CD47 signal on cancer cells and allow the immune cells called macrophages to attack the cancer. Such antibodies are currently in clinical trials as an anti-cancer therapy. But the researchers also had data indicating that CD47 signals would also stop the immune system from attacking cells infected by a pathogen. They decided to see what effect blocking CD47 signaling would have on an actual disease.

"Because there are naturally high levels of CD47 molecules on red blood cells, we wanted to start with a disease that targeted red blood cells," said Laughing Bear Torrez Dulgeroff, PhD, who is the first author on the research paper. "The obvious choice was the malaria parasite, which attacks by infecting those cells." When they looked at red blood cells infected by the malarial parasite in the lab, they found that the infected blood cells carried more CD47 protein, which was a good sign, indicating that infected cells might be removed better once the CD47 signal was blocked.

The results were promising. When mice with a highly infectious case of cerebral malaria were given anti-CD47 antibodies, 80 percent of them survived, compared with no mice surviving in the control group.

They also found something surprising. They expected that these mice were surviving because the immune cells were killing the malarial infected red

blood cells, but in fact the parasite load was not different between treated and untreated mice. "If the mice that were treated and survived have the same parasite load as those didn't get the treatment and died, what is the difference between them?" Torrez Dulgeroff said.

On closer inspection, the researchers discovered several important distinctions between the anti-CD47 treated and untreated mice, particular in the brain. For example, the blood brain barrier was disrupted on untreated mice, while it was much better preserved in the mice treated with CD47-blocking antibodies. "Once you get significant vascular leakage across the blood brain barrier, that leads to brain inflammation, and death. Anti-CD47 is able to reduce the inflammatory effects in the brain during infection." Torrez Dulgeroff said.

It is not clear exactly how blocking CD47 signaling would lead to better integrity of the blood-brain barrier and less brain inflammation. "Cerebral malaria is a complex disease and there are several of between the treated and untreated mice, so we can't say it's any one thing that is the reason why the treated mice are surviving," Torrez Dulgeroff said. But the results are exciting. "Going from zero to 80 percent survival is very promising," she said.

"It was surprising that a complex, multicellular parasite like malaria could also find protection from macrophages (via CD47)

The researchers say that further investigation might uncover important biological mechanisms underlying the protective effect of blocking CD47 signaling, For instance, researchers in the Weissman lab had shown before that virus-infected cells protected themselves from macrophages by producing higher levels of CD47. "But it was surprising that a complex, multicellular parasite like malaria could also find protection from macrophages in a similar way" said Weissman. "Now we wonder if the malarial parasite first infects red blood cell precursors in the bone marrow first, which could open new avenues to study the disease."

There is also the potential to test anti-CD47 antibodies or other drugs that act in similar was as treatments for cerebral malaria in clinical trials at some point in the future.

Embryonic cells use Yamanaka factors to defy developmental "gravity"

It has long been an accepted principle of development that cells give rise to other cells that have the same or less potential for producing various kinds of tissues. Embryonic stem cells can give rise to blood stem cells and neural stem cells, but neural stem cells will never give rise to blood and immune cells outside of the laboratory. Conrad Waddington nicely illustrated this process with his famous landscape showing a ball, representing a cell, that can roll down a hill and funnel into one valley or another (one cell fate or another). But once it has chosen the blood and immune path, for instance, a cell can't jump the ridge to the next valley over and become a muscle cell, for instance. In nature, it is thought, cells' fates are progressively restricted--anything else would be like a ball starting to roll uphill.

Now, however, researchers in the laboratory of institute scientist Joanna Wysocka, PhD,with some help from the lab of institute director Irv Weissman, MD, have shown that some cells in the early can effectively defy epigenetic gravity, rolling up Waddington's landscape and becoming more pluripotent instead of less. And perhaps just as surprising, the cells do it using factors that Shinya Yamanaka previously showed could create pluripotent cells in the lab. Yamanaka's work, which garnered him the Nobel prize, had never been shown to operate developing organisms. Wysocka and her colleagues published their work recently in the Journal Science.

The Wysocka lab has long been studying an early embryo cell group called the neural crest, focusing on a particular portion that gives rise to the face. Cells in the neural crest have already chosen their path down Waddington's landscape to become ectoderm, which gives rise to cells in the nervous system. This ectodermal tissue should not be able to give rise to muscle, bone and connective tissue, which usually come from another type of tissue called mesoderm.

"There were some ideas about how neural crest cells could give rise to the very different kinds of cells in

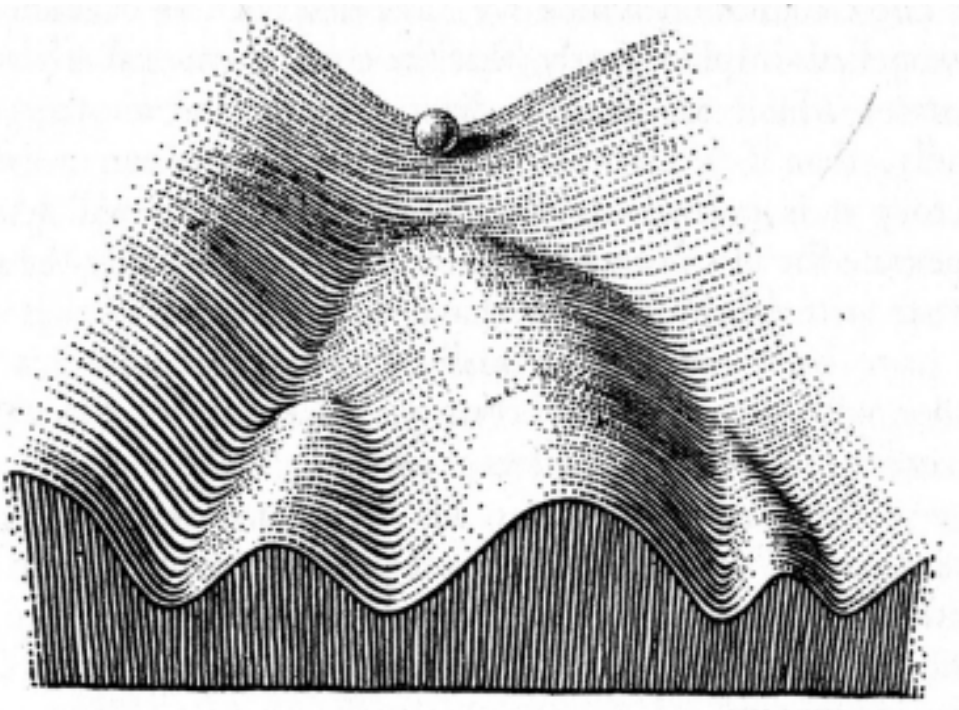


FIGURE 4
Part of an Epigenetic Landscape. The path followed by the ball, as it rolls down towards the spectator, corresponds to the developmental history of a particular part of the egg. There is first an alternative, towards the right or the left. Along the former path, a second alternative is offered; along the path to the left, the main channel continues leftwards, but there is an alternative path which, however, can only be reached over a threshold.

the face, such as perhaps there was small subset of cells that had retained their pluripotency and ability to produce mesenchymal tissues, "Wysocka said.

The insight to what was going one came after the researchers stared doing single-cell RNA sequencing on cells in the neural crest, allowing them to look at what proteins particular cells were making at different times in development.

Famed writer Issac Asimov once said that the most exciting phrase to hear in science, the one that heralds new discoveries, is not 'Eureka!' but 'That's funny …' Antoine Zalc, PhD, a postdoctoral fellow in Wysocka lab and lead author on the Science paper, noticed something that at first seemed like an odd coincidence. "I was giving a talk on our data and I pointed out that, strangely enough, these neural crest cells were producing Oct4 and Nanog," Zalc said, naming two of the widely known factors that Yamanaka showed could be used to transform mature cells into pluripotent cells in the lab.

Wysocka prompted him to follow up on this observation, and after much more single cell sequencing and analysis done with Rahul Sinha, PhD and medical student Gunsagar Gulati of the Weissman lab, they showed that a small subset of cells in the neural crest could express Yamanaka factors themselves and reverse the differentiation process, turning themselves into cells that could give rise to the kinds of cells they were unable to produce before. For just a bit, Waddington's ball can indeed defy gravity and roll itself uphill.

"It's also amazing to me that the cells do this using the Yamanaka cocktail of factors," Wysocka said. "You would think that if increasing pluripotency is possible, there might be many other ways of doing it, but this suggest that there is something universal in the four factors that is hard to accomplish in other contexts."

The researcher now wonder what this means for our understanding of development and other biological processes. "This work is a game-changer," Weissman said.

"The developmental neural crest is somewhat unique, but I wonder if this reactivation of earlier gene programs happens in other instances, like in regeneration, for instance," Wysocka says.

"This is evidence of how teamwork between computational and bench biologists can lead to exciting discoveries.

Wysocka notes that there have been observations that sometimes Yamanaka factors like Oct4 have been spotted in cancer cells, and it has been discounted as an inconsequential sign of how dysregulated cancer cells have become. "We are now thinking that perhaps, in some contexts, expression of these factors in cancer cells might help them adapt and colonize new niches," she said.

The researchers note that further work must be done to understand the molecular process that is going on in the neural crest cells in reaction to the Yamanaka factors. Understanding more thoroughly how nature uses these factors may lead to new capabilities and more precise control of their use in the lab.

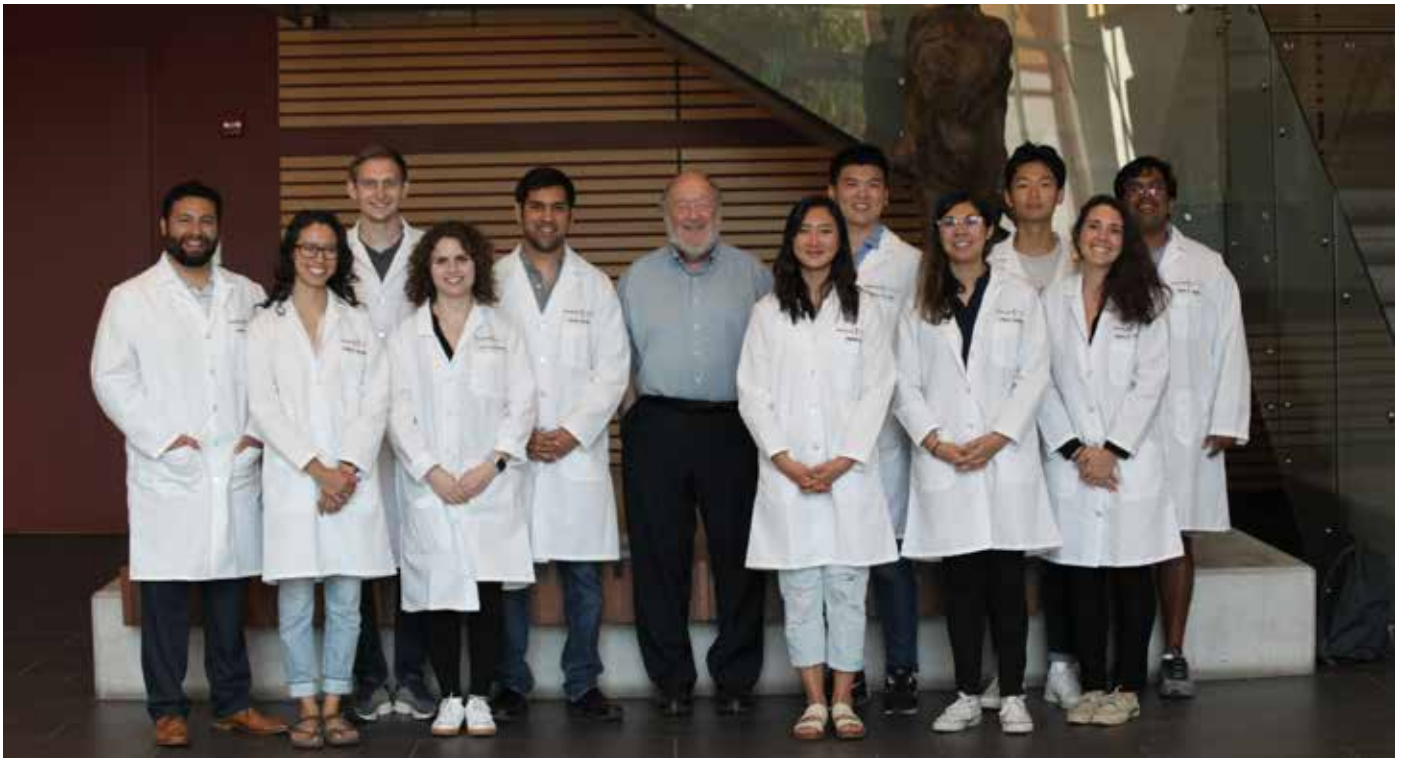
The researchers also note that this work is a testament to the benefits of interdisciplinary research. "Overall, this project greatly benefited from the collaboration between different labs and disciplines," said Gulati, who is pursuing an MD degree while also earning a PhD in cancer biology with a strong emphasis in computational biology. "It is evidence of how teamwork between computational and bench biologists can lead to exciting discoveries."

Other Stanford researchers involved in the work were Tomek Swigut, a senior scientist in the Wysocka lab, and Daniel Wesche, a graduate student in the ISCBRM program.

The departments of Chemical and Systems Biology and Developmental Biology were also involved in the research.

Joanna Wysocka, PhD

CIRM approves $5 million grant to train the next generation of stem cell scientists



Irv Weissman, MD, with graduate students

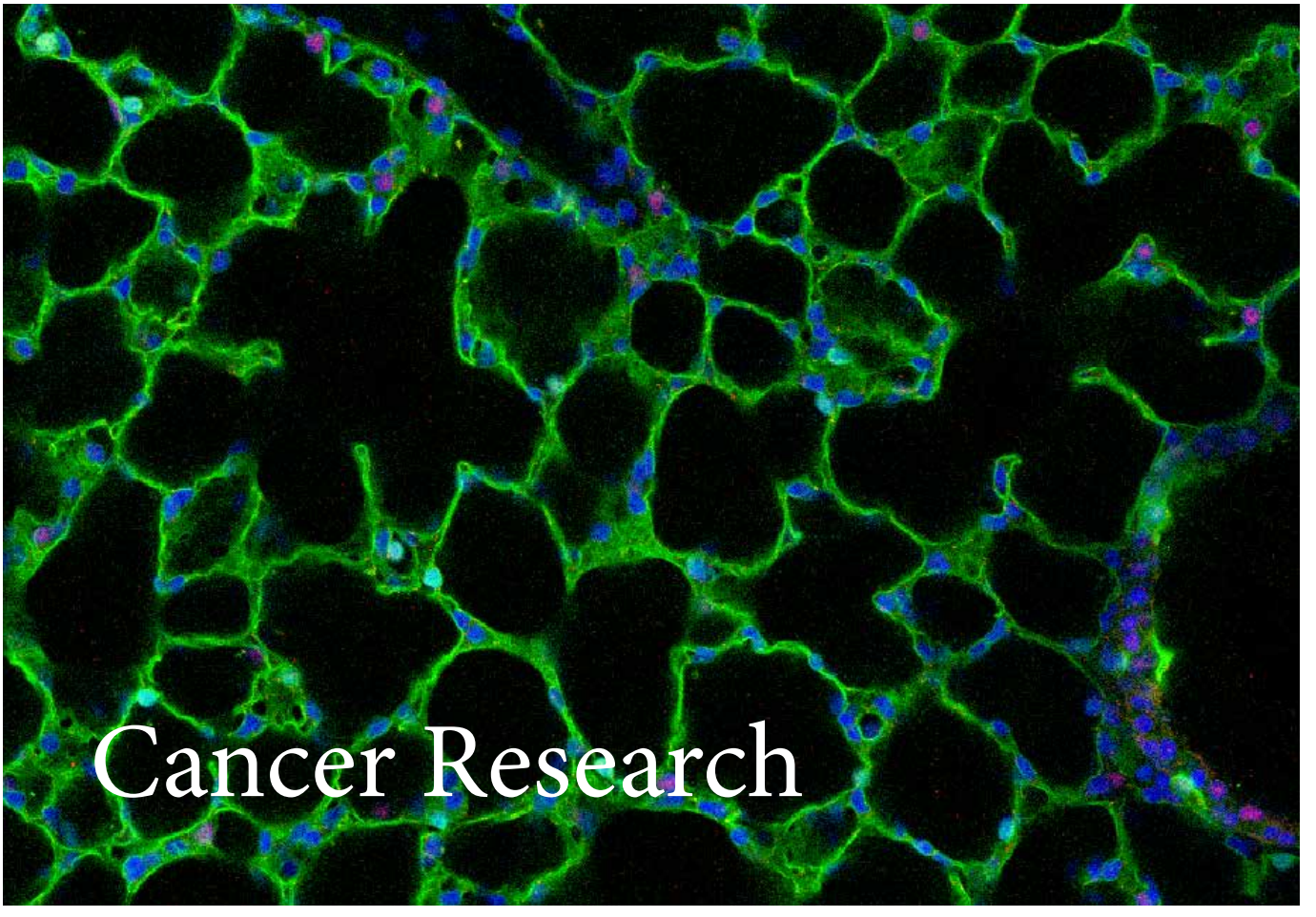
The board of the Californian Institute for Regenerative Medicine (CIRM) approved granting Stanford $5 million to fund the institute's CIRM Scholar Training Program. The program will help provide training for predoctoral students and postdoctoral fellows in stem cell research.

The grant is part of a larger block of more than $86 million CIRM is providing for training at various institutions throughout California. "The field of regenerative medicine is expanding rapidly and that's creating a rising demand for skilled workers to help keep up," says CIRM spokesperson Kevin McCormack. "CIRM has been a big supporter of training grant programs ever since we were created by the voters of California, and now we are kick-starting those programs again to ensure the field has all the talented workers it needs."

In the first year at Stanford, the CIRM Scholar Training Program will fund three predoctoral trainees, two PhD postdocs and two clinical fellows. In subsequent years, the program will grow larger and provide more trainees with a framework for professional development.

The CIRM training program will augment the educational mission of SCBRM by extending training opportunities to post-PhD and post-MD trainees interested in the translation of laboratory research findings, says Gerald Spangrude, PhD, executive director of education for the Graduate Program in Stem Cell Biology and Regenerative Medicine. This will help promote the clinical application of the latest findings in stem cell science and regenerative medicine, he says.

"The CIRM training grant will also provide additional training positions for pre-doctoral trainees in addition to our current predoctoral funding from the National Institutes of Health T32 training program," Spangrude said. "Our mission is to extend stem cell therapies to the clinical setting, and to train a workforce that represents California's diversity. The CIRM training grant will allow SCBRM to fund trainees in a research-intensive environment that will intimately result in translation of laboratory research to patients."



Cancer Research

The application of stem cell biology research and methods to cancer research is having a profound impact on our understanding of how cancer arises and propagates, as well as how to treat patients.

Institute data scientists identify who may be at risk from revolutionary cancer treatment

Aaron Newman, PhD

A class of cancer drugs called immune checkpoint inhibitors have been a boon to cancer patients, often leading to remission in cancers that were previously almost universally fatal. Unfortunately, many patients undergoing this treatment will also suffer severe immune-related toxicities, and there has been no reliable way to tell in advance which patients are at risk. Institute researchers have now used data science techniques to draw a picture of which patients are likely to experience this dangerous side effect.

"Checkpoint inhibition has revolutionized oncology, but unfortunately many patients given the strongest forms of this cancer therapy will experience severe side effects," said Aaron Newman, PhD, a member of the Stanford Institute for Stem Cell Biology and Regenerative Medicine. "We wanted to know if we could use data science to determine in advance who will be vulnerable to these toxicities."

Newman is the senior author on a paper describing this research, published January 13, 2022 in the journal Nature Medicine. Newman is an assistant professor in the Department of Biomedical Data Sciences and a member of the Institute for Stem Cell Biology and Regenerative Medicine. He is also a member of the Stanford Ludwig Center and a Chan Zuckerberg Biohub Investigator.

When immune cells are doing their jobs correctly, they are eliminating infected, precancerous or damaged cells. But immune cells also have the capacity to attack the body's own tissues, behavior that results in various autoimmune diseases. In order to keep such autoimmune attacks under control, immune cells use "checkpoint" genes that rein in immune cell activity. Unfortunately, cancer can activate this mechanism to suppress immune attacks on cancer cells.

Therapies that reverse that process and inhibit those checkpoints have saved or prolonged the lives of many cancer patients who previously had very low odds of survival. But about 60 percent of patients with melanoma who undergo the strongest forms of checkpoint inhibition therapy have severe side effects, forcing many of these patients to stop treatment. These side effects can affect any tissue in the body, and they can even be lethal. "In many cases, the cancer may end up advancing more rapidly not because it can't be treated, but because patients can-

not maintain regular treatment with immunotherapy," Newman said.

Adding to the difficulty was the fact that there was no way to tell who would experience this toxic side effect and who wouldn't. Newman and researchers from Stanford, Washington University in St. Louis, and Yale University set out to see if they could find a common pattern among patients who were treated and subsequently experienced severe toxicities. "We wanted to know, 'How can we improve this immunotherapy so that we maximize the benefits and minimize the risks?'" Newman said.

The group, including co-first author and former Stanford PhD student Alexander Lozano, analyzed blood samples from over seventy skin cancer patients who had undergone the therapy in the past and used clinical records to understand what side effects these patients experienced. They used a wide variety of analytical techniques such as single-cell RNA sequencing, mass cytometry, bulk RNA sequencing, and CIBERSORTx, a technique developed in Newman's lab to understand the cellular components of large numbers of bulk samples.

The group wanted to see if these analytical techniques gave an answer that converged on a suspected culprit in these toxicities. And behold, they found two. "We found that patients who before treatment had a high number of what are called activated CD4 effector memory T cells, and also had a great deal of sequence diversity in the receptors displayed by T cells that recognize antigens, were much more likely to have these severe immune toxicities after checkpoint inhibition therapy," Lozano said.

"Our working hypothesis is that patients who have this profile—high numbers of CD4 memory T cells and high T cell sequence diversity—have either a tendency toward developing autoimmune diseases or have subclinical autoimmune attacks on their tissue," Newman said. Since checkpoint genes are there precisely to put the brakes on immune cells so that they don't attack our own tissues, it makes sense that a therapy directed at suppressing these checkpoint mechanisms would increase the likelihood of our immune cells mounting an attack on our own, healthy cells, he added.

Since checkpoint genes are there precisely to put the brakes on imune cells so that they don't attack our own tissue, it makes sense that a therapy directed at suppressing these checkpoint mechanisms would increase the likelihood of immune attack on our own, healthy cells.

To further explore this idea, the researchers did the same sort of analysis on people who did not have cancer but did have different autoimmune disorders, including lupus and inflammatory bowel disease. They found that the same pattern of high CD4 memory T cells in the peripheral blood was also strongly associated with these autoimmune diseases.

For cancer patients undergoing checkpoint inhibition therapy, the results could mean that clinicians will be better able to pick a therapy that works but does not produce severe immune toxicities. "They might decide not to use the strongest immunotherapy, or they could more closely monitor patients likely to experience severe adverse effects," Newman says. "This is another example of how data science can lead to "precision medicine"—therapies and disease management that are customized for individual patients to provide the best possible outcomes."

Researchers sort out the puzzle of fibroblasts in cancerous solid tumors

Solid cancer tumors are hard lumps in our soft tissues because they are usually encased in and permeated with fibroblasts, the cells that make up scar tissue. In fact, even though cancer-associated fibroblasts (CAFs) are an integral part of solid tumors and are thought play a role in cancer's growth, that role is little understood.

Now a group of Stanford researchers have created a thorough portrait of CAFs' transcriptomic, epigenomic, and proteomic activity, leading them to conclude that there are actually three types of CAFs and that each reacts to cancer treatments in predictable ways that can affect treatment outcomes.

"We know that the environment around cancer cells can have a large influence on how cancer grows and responds to therapy," says institute researcher Michael Longaker, MD. "Cancer associate fibroblasts make up a large part of the tumor environment, but very few cancer treatments take their effects into account." Longaker is the Dean P. and Louise Mitchell Professor in the Department of Surgery.

Michael Longaker, MD

Part of the reason for that, the researchers say, is that there are mixed results about whether CAFs are good or bad actors. Sometimes CAFs can actively promote tumor proliferation, invasion and spread, but at other times, disrupting CAF activity can accelerate tumor progression. Now Longaker, along with Jeffrey Norton, MD, Howard Chang, MD, PhD and colleagues have created a clearer picture of how CAFs are acting in and around cancer. The researchers recently published their work in the journal Cancer Cell. Norton is the Robert L. and Mary Ellenburg Professor of Surgery, and Chang is the Virginia and DK Ludwig Professor of Cancer Research.

The researchers now see clearly that CAFs come in three varieties—one that is immunologically driven, one that is mechanically driven and a third type that can transition between these other two types, they say. One striking feature of these categories is that they are pretty much the same throughout evolution and throughout the body. "What's really surprising from an evolutionary standpoint is that across species–from mouse to man–and across tumor types–from breast to pancreas¬–this pattern is conserved," Longaker says. This is true despite the fact that most cells in different kinds of tumors can be very different.

The immunologically driven CAF is both "inward" looking, interacting with the immunological environment in the tumor, as well as "outward" looking, affecting the action of the immune system generally. "Cancer tumors affect the body's immune system, and this may be done through CAFs," says Norton. Mechanosensory fibroblasts, on the other hand, exert an effect on other cells in reaction to physical stresses between cells and tissues. In previous work, Longaker and his colleagues showed that mechanosensory properties in fibroblasts determined whether healing skin formed scars or not. In the tumor, the scientists say, CAFs under physical strain might for example aid the construction of new blood vessels to help feed a growing tumor.

The third "steady-state" type of CAF is not inherently immunomodulatory or mechanosensory, but

may be poised to become one of those types, depending on the situation. The researchers found that this tripartite arrangement can explain some of the seemingly contradictory findings showing that sometimes CAFs promote cancer and sometimes they don't. "We found that when you use immunotherapy against advanced skin cancer, for instance, it decreases the prevalence of immune-regulating CAFs, but there appears to be a compensatory transition of some steady-state CAFs switching to a mechano-sensory type," said postdoctoral fellow Michael Januszyk, MD PhD, one of the co-first authors on the Cancer Cell paper. The increase in mechanosensory CAFs might frustrate immunotherapy by prompting changes that make a tumor grow faster even as it is attacked by the immunotherapy, he said.

On the whole, the research supports the idea that CAFs are not just passive but are active components of cancerous tumors that should be taken into account when considering any therapy.

"For the past 50 years we have been developing new therapies to hit cancer cells, but now we are saying that we should also consider targeting the cells that surround and interact with the cancer," Longaker says.

The scientists credit their discoveries in part to the tremendous variety of expertise that the researchers brought to the problem. "We are able to have a big impact by leveraging experience in stem cell biology, cancer biology, genomics and proteomics," says Januszyk. "These discoveries were only possible because we used all these technologies together and pursued multiple heterogeneous modes of investigation."

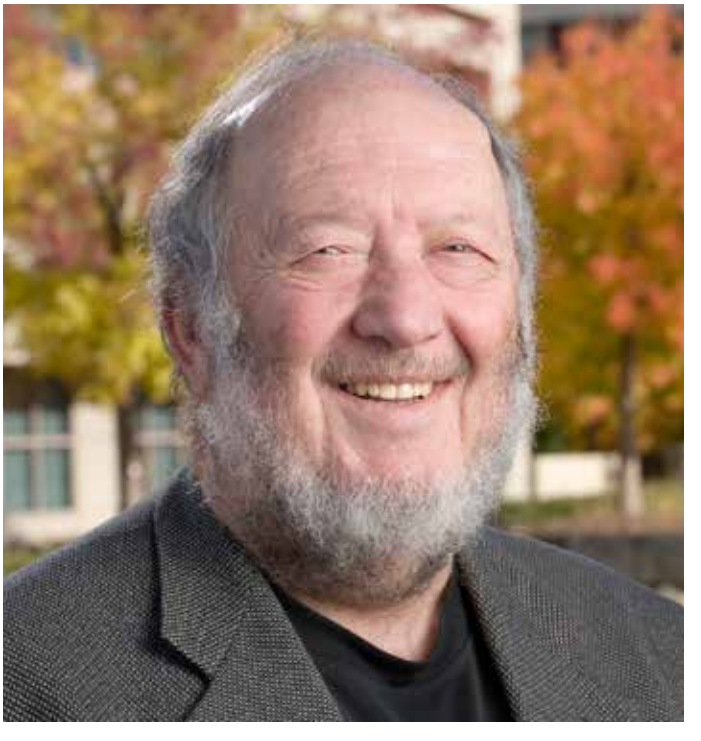
Anti-CD47 antibodies increase effectiveness of HER2+ breast cancer treatment

Researchers at Stanford have shown that blocking the CD47 signal on breast cancer cells increases the effectiveness of a standard antibody treatment for HER2+ breast cancer, even when the cancer has become resistant to the treatment.

"These results demonstrate that we can turbocharge existing treatments for HER2+ breast cancer by amplifying an underappreciated mechanism that the body uses to fight cancer," said Irv Weissman, MD. Weissman is the Virginia and DK Ludwig Professor for the Clinical Investigation of Cancer Research and the director of the Institute for Stem Cell Biology and Regenerative Medicine. The research is published in the Proceedings of the National Academy of Sciences (PNAS). Weissman and Mark Pegram, MD–the Susy Yuan-Huey Professor of Medicine–are senior authors on the paper, and Rosalynd Upton is first author.

Historically, patients with breast cancer that displayed the HER2 marker, which occurred in about 16% of breast cancer cases, had very low survival statistics. The clinical development of anti-HER2 antibody therapy called trastuzumab (which is sometimes referred to by the trade name Herceptin) led to large improvements in the treatment of HER2+ breast cancer and became a mainstay medication for those with this form of breast cancer.

Trastuzumab binds to HER2 on the cell surface, and can affect HER2+ breast cancer cells either directly



Irv Weissman, MD

through its inhibitory biological activity, or indirectly by recruiting immune system cells to attack the cancer cell. But though trastuzumab is highly effective to treat early-stage HER2+ breast cancer, the majority of patients with an advanced-stage, metastatic form of this cancer become resistant to the treatment. In such cases, their cancers ultimately progress, even if they initially responded to the treatment. Resistance to treatment occurs even though the cancer cells still display high levels of HER2 protein on their surface and should still be susceptible to antibodies that attack HER2.

Weissman and his colleagues wondered if the treatment of HER2+ breast cancer could benefit from the activation of an immune cells called scavenger macrophages, which are tasked with engulfing and destroying diseased or damaged cells. The Weissman lab and other researchers had already shown that CD47 could act as a "don't eat me" signal to macrophages, and that blocking the signal with anti-CD47 antibodies reactivated macrophages' natural cancer fighting activity. Weissman and his colleagues had shown that many cancers, including breast cancer, express high levels of CD47, which protects them against these immune monitors.

A recent clinical trial showed that blocking the CD47 "don't eat me" signal with an anti-CD47 antibody increased the efficiency of another antibody drug called Rituximab when used against another kind of cancer called diffuse large B-Cell lymphoma. This was true even when the lymphoma had acquired resistance to Rituximab alone.

"We wondered if we might see a similar beneficial effect if we blocked CD47 signaling when administering trastuzumab in the treatment of HER2+ breast cancers," Weissman said, "especially if cancer cells were resistant to trastuzumab treatment."

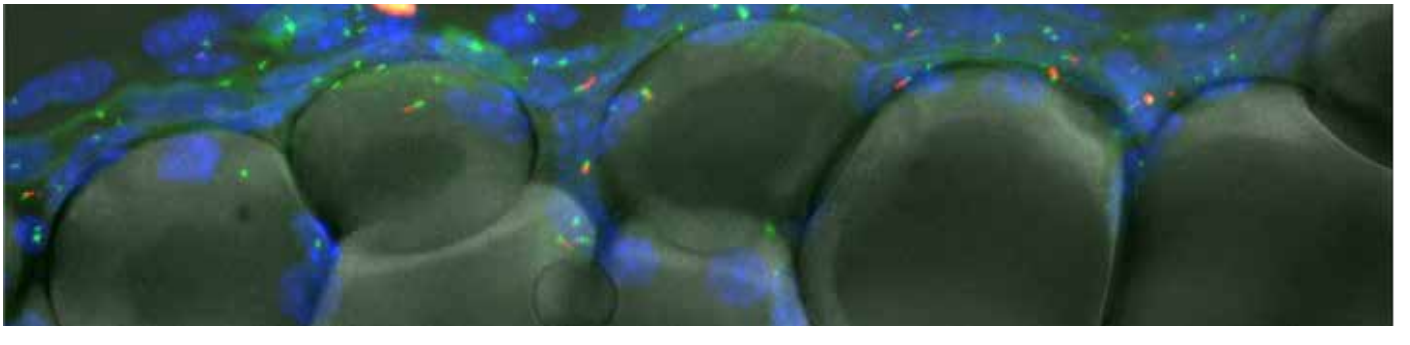
To test this idea further, researchers from the Institute for Stem Cell Biology and Regenerative Medicine, along with colleagues in the Stanford Cancer Institute, studied mice that had been engrafted with human HER2+ breast cancer cells that had become relatively resistant to treatment. What they found was that while trastuzumab or anti-CD47 antibodies by themselves had some therapeutic effect, the combination of the two therapies inhibited tumor growth significantly more and produced better survival statistics than either treatment alone.

Most of the research on trastuzumab has focused on the action of immune cells called natural killer (NK) cells to attack the cancer, but this study illuminates how trastuzumab also recruits macrophages to do the job. "Until now, the process of antibody-dependent cellular phagocytosis, in which macrophages engulf and kill cancer cells, has been an understudied mechanism of action of trastuzumab," said Pegram. "This study is evidence that this mechanism of action can be significantly enhanced by adding anti-CD47 antibodies."

What was most interesting to Upton, the first author on the paper, was that adding anti-CD47 antibodies made such a qualitative difference in cancer fighting. "That we could take cancer cells that were specifically resistant to trastuzumab, and by adding something else make those cells vulnerable again, was almost like magic." Upton earned her JD from Stanford Law School at the same time as she was earning her PhD in cancer biology. She is now clerking in the US Court of Appeals for the Federal Circuit.

The results support the further exploration of this approach in human clinical trials, Weissman said.

This work was supported by the National Institutes of Health (R35CA220434), the DK Ludwig Fund for Cancer Research, the Gunn-Olivier fund, the Israel Science Foundation, and the Mary Kay Foundation.



From loss comes hope: New treatment shows promise for currently incurable pediatric brain tumor

When Jace Ward came to Stanford in September 2020 to join a clinical trial for a novel therapy, he had been fighting a deadly brainstem tumor for more than a year. His diagnosis was diffuse intrinsic pontine glioma, or DIPG, which conventional cancer treatments can›t cure. The disease has a five-year survival rate of less than one percent.

Today a group of Stanford scientists who have spent the past decade unlocking the glioma's secrets are publishing data in Nature from the trial Ward joined. He was one of the first four patients with diffuse intrinsic pontine glioma or a closely related cancer affecting the spinal cord to receive immune cells engineered to fight the disease.

Though all the trial patients died of their disease or its complications, three of them experienced significant clinical benefits from the engineered cells.

"These four patients are heroes," said study's principal investigator, pediatric neuro-oncologist Michelle Monje, MD, PhD. "They taught us so much, and that knowledge is already being applied to help other kids."

The FDA approved the use of engineered immune cells, also known as chimeric antigen receptor T-cells, or CAR-T cells, to treat blood cancers in 2017, but the technology has not previously succeeded against solid tumors.

Although the research team has yet to achieve a cure for this type of glioma, they consider their findings a milestone.

"We see significant anti-tumor activity with these CAR-T cell therapies in this dreaded disease," said cancer immunotherapy expert Crystal Mackall, MD, who shares senior authorship of the new research with Monje. The findings are a hopeful sign for many types of brain tumors, not just this one, she added. "It's a small data set, but the evidence is there."

The case of Jace Ward

Ward was the trial's second participant. His experience with the CAR-T cells was characterized by several important breakthroughs, including monthslong reversals of his neurological symptoms, Monje said. "The fact that there were responses, transient though they may have been, brings hope."

Ward was 20 when he was diagnosed with the glioma

Jace Ward and his family

Pediatric neurooncologist Michelle Monge, MD, PhD

in early 2019, after a few weeks of disturbance in his peripheral vision. He and his family were shocked by the prognosis: Ward's neurologist in Kansas predicted he had six to nine months to live. He received an experimental chemotherapy agent on a compassionate-use basis and lived with the disease for almost 14 months before the opportunity to join the Stanford trial arose.

By the time he and his mom, Lisa, arrived at Stanford from their hometown of Wamego, Kansas, the tumor was progressing. Ward had started limping, could not use his right hand well, had weakness and loss of sensation on the left side of his face, and couldn't open his mouth wide enough to take bites of hamburgers or pizza, his favorite foods.

Jace Ward, second from left, with his family. Other family members with him, from left, are his sister, Brooke; his father, Roger; his mother, Lisa; his sister-in-law, Lauren; and his brother, Blake.

Ward knew the trial was unlikely to save his life. "He said, 'I know that I'm going to die, and I know this therapy will one day be the thing that cures other kids. Figure it out with me,'" Monje recalled. "This tough, football-playing 21-year-old said to me, 'I don't want it to be a 5-year-old who has to go first.'"

Ward saw his role as helping physicians understand the new treatment, his mom said. He and his family had been told that the trial carried significant risks.

"Jace said, 'Let me make the decision to do this,'" Lisa Ward said. "'It's not fair for parents to have to make this decision for their young child and live with regret. Let me go first.'"

Tumor donations for research

High-grade gliomas, a family of severe tumors, can occur at any time of life, and tend to develop in different regions of the brain depending on the age of the patient.

Among these are diffuse intrinsic pontine gliomas that occur in a few hundred new cases in the United States each year, mostly among school-aged children. Surgery isn't an option because the tumor cells grow intertwined with healthy brain cells in a critical region of the brainstem that's responsible for maintaining bodily functions such as breathing and heartbeat. A few chemotherapy drugs are under investigation, but none have been approved for the disease, and radiation buys only a short reprieve before the tumor worsens again.

When Monje launched her research career at Stanford Medicine in 2008, she and her colleagues decided to ask families whose children had the disease to donate their child›s brain tumor after death. Eighty-seven families have done so, which enabled scientists to study the tumors› biology. Among the discoveries Monje's team made as a result is that the tumor cells form electrically active synapses with healthy brain cells and use those connections to drive their malignant growth.

Anotherbig discovery came in 2018 when a student in Monje's lab characterized the molecular markers on the tumor cells' surfaces. The cells express vast quantities of a marker called GD2, the research showed. The molecule, a combination of lipids (fats) and sugars, is abundant on some other cancers. In fact, Mackall had already engineered CAR-T cells that could target GD2. Mackall's and Monje's teams collaborated on preclinical trials demonstrating that the engineered cells cleared diffuse intrinsic pontine glioma tumors in mice.

Engineering cells to fight cancer

Treating cancer with CAR-T cells involves removing immune cells called T-cells from the patient, engineering them in a lab to recognize cell markers abundant on tumors, then returning them to the patient. The altered cells include a protein -- the chimeric antigen receptor, or CAR -- that doesn't exist in nature.

The CAR protein binds the tumor and activates an immune response: The engineered T-cells multiply to create an armada of tumor fighters. These cells, and other immune cells they attract, attack and kill cancer cells, shrinking the tumor. The resulting immune response also includes surges of cytokines, inflammation-promoting molecules.

Although CAR-T cells can treat blood cancers, trials in solid tumors have faltered. One reason is geography: In blood cancers, malignant cells are distributed in blood and bone marrow, accessible to roving CAR-T cells.

But it's difficult for the cells to penetrate solid tumors. Additionally, the patient's immune system recognizes CAR proteins as foreign, and the body mounts an immune response to the therapy after the first dose. So one shot of the treatment is all that's effective, historically.

To try to boost the power of the engineered cells, Mackall's team developed approaches to prevent rapid exhaustion of CAR-T cells' replication ability. They've also fine-tuned the cells to respond only to high levels of molecular markers such as GD2, which keeps them from going after healthy cells with a few of the same markers.

"When you're creating a new therapy, it's more like invention than discovery," said Mackall, the Ernest and Amelia Gallo Family Professor of Pediatrics and Internal Medicine. "Inventions are iterative, with improvements over time. Now, 10 years into the CAR business, we know more about what these proteins need to be potent and safe."

The improved CAR-T cells showed promise in a mouse study that Mackall and Monje published in 2018. But it held warning signs: Inflammation in the tumor caused swelling in the brainstem of mice that received the CAR-T cells. Swelling in the brainstem can block drainage of fluid from around and within

Cancer immunotherapy expert Crystal Mackall, MD, PhD

the brain, a dangerous situation.

So when the scientists moved into clinical trials, they built in precautions. For instance, before treatment, a surgeon implanted a device under the scalps of study participants to drain the cerebrospinal fluid that bathes the brain, allowing for pressure to be quickly lowered if swelling occurred. The researchers also planned to hospitalize all participants in intensive care after they received the engineered cells.

The advance planning paid off: Though patients did develop swelling in the tumor, usually around a week after getting CAR-T cells, the team treated it safely and effectively.

Children with brainstem tumors are extra-vulnerable to swelling because the tumor already takes up space inside their skulls, Monje said: "This space is like a cup that's nearly filled up, and then if you put in one drop, and another drop, it can overflow. Just a little bit tips over [into the danger zone] if it's a large tumor."

'I can't die, I'm busy'

When Ward joined the CAR-T cell trial, he was a junior at Kansas State University, studying entrepreneurship and pre-law. He was always willing to speak

his mind and question authority figures, his mom said. After his diagnosis, Ward became a strong advocate for kids with diffuse intrinsic pontine glioma.

"Jace spoke at Congress, the National Institutes of Health and in rare-cancer virtual forums," Lisa Ward said. He was passionate about helping others access clinical trials, a daunting process that involves navigating complex eligibility requirements and often traveling cross-country.

In February 2020, Jace Ward was a speaker at the First DIPG Briefing to Congress, sponsored by the DIPG Advocacy Group.

Jace Ward worked on collaborative fundraising campaigns that raised more than $2.5 million to expand cancer research and treatment access. He also worked with his parents and others to launch a nonprofit that helps kids with brain tumors get expert opinions on clinical trials they can join.

"Jace would comfort his friends and family by saying, 'I can't die, I'm busy,'" Ward's mother said. "That became a mantra for him."

A week after Ward received his first infusion of CAR-T cells, given intravenously, he had fever and low blood pressure, signs of cytokine elevation, and his neurological symptoms worsened.

But by two weeks, he was experiencing a remarkable and unusual remission of neurological symptoms, opening his mouth fully and feeling more sensation on his face. His previously awkward, stiff-legged walking gait became almost normal. Within a month, his neurological exam was nearly normal, too.

He was thrilled to be able to open his mouth, Lisa Ward said. "He said that to eat a big hamburger or a slice of pizza was the best feeling in the world."

The scientists were excited for Ward as well as for what his results could mean for other patients. Not only is the tumor deadly, it severely debilitates patients during their lives, often causing paralysis, loss of hearing and ability to speak or swallow.

"To see a young man with rapidly progressive diffuse intrinsic pontine glioma regain an almost normal neurological exam is unheard-of," Monje said.

The researchers had suspected the tumors impaired, but didn't destroy, neural circuits, and that effective treatment might restore abilities the disease took away. Ward's response was the first proof.

"I felt for the first time that we were going to be able to cure this disease someday," Monje said.

Cancer therapy that will make kids feel better

"We didn't realize how dramatic the clinical improvement would be for these patients," Mackall said. Formal evaluations of patients' quality of life have been built into the next stages of the trial to thoroughly measure these benefits. "With cancer therapy, we're used to saying, 'It's toxic, you have to bear it,' but this is different. This therapy is going to make them feel better."

The researchers are also excited by growing evidence that CAR-T cell therapy may be especially useful for fighting many types of brain tumors. Mindful of the failures of other CAR-T cell trials for solid tumors, they decided to try something novel, infusing the cells directly into the cerebrospinal fluid. This approach had advantages: More CAR-T cells reached the tumor, and the cells were sequestered behind the blood-brain barrier, shielded from most of the patient's immune system.

Unlike studies with CAR-T cells in leukemia, where second doses are usually ineffective, the researchers found that patients could benefit from repeated doses of cells administered into the cerebrospinal fluid, likely because they escaped immune responses to the engineered cells. Patients also had less of the cytokines that cause undesirable side effects such as fever and low blood pressure.

When Ward's symptoms began to return a few months after his first infusion, the researchers infused CAR-T cells into his spinal fluid. Again, his tumor shrank, and he could walk and open his mouth widely.

In February 2021, Jace Ward, center, fulfilled a lifelong dream of seeing his home team, the Kansas City Chiefs, play in the Super Bowl. Jace's brother, Blake, left, and father, Roger, joined him at the game.

"The walking was huge after his second infusion: He went in in a wheelchair and walked out of the hospital," said Lisa Ward, adding that her son went from not walking at all to walking four or more miles a day in less than a month. He was also able to fulfil a dream of going to the Super Bowl in February 2021 with his dad, Roger, to watch their beloved hometown team, the Kansas City Chiefs.

"It was so freeing for him, such a good glimmer of hope," Lisa Ward said.

Ward ultimately received five cell infusions over a 10-month period, which the researchers believe extended his life by several months. They plan to give up to 12 infusions to future trial participants, and are working to open clinical trials of CAR-T cells for other kinds of brain tumors, including glioblastoma in adults.

On June 30, 2021, while at home in Kansas, Ward experienced a severe hemorrhage in an area of his brain where the tumor had caused fragile blood vessels to grow. He was hospitalized in St. Louis and died July 3, leaving behind his grieving parents, brother, sister, sister-in-law and nephew.

"Do I wish he were here with me, never knowing what DIPG was? Of course," his mother said recently. But, she has gained a sense of peace and purpose from their work together as advocates for patients. "Jace's desire to help children during his fight, his real need to speak for them, gave me such a glimpse of the man he had become."

And she knows her son's role as a medical pioneer, the first brainstem tumor patient to be able to report back to his doctors on the benefits of CAR-T cells, meant a great deal to him. "He was able to tell them things all along the way that changed the trial," she said. "I think he found great purpose in that."

Photos of Jace Ward courtesy of the Ward family.

Researchers develop a highly sensitive method of finding residual cancer after treatment

Institute researchers have developed a new method of detecting cancer DNA in the bloodstream that can find nearly all residual disease after treatment.

"Older methods would miss 25-50% of the cases in which some cancer cells remained after treatment," said Maximilian Diehn, MD, PhD. "This new method seems to be able to catch virtually everybody with residual disease." Diehn and Ash Alizadeh, MD, PhD, call their method PhasED-Seq and tested it on most common lymphoma subtype, and also did proof-of-concept studies on solid tumors. Diehn and Alizadeh, both members of the Stanford Institute for Stem Cell Biology and Regenerative Medicine, were senior authors on the research, which was published in the journal Nature Biotechnology. David Kurtz, MD, PhD and Joanne Soo, MD were joint first authors on the paper.

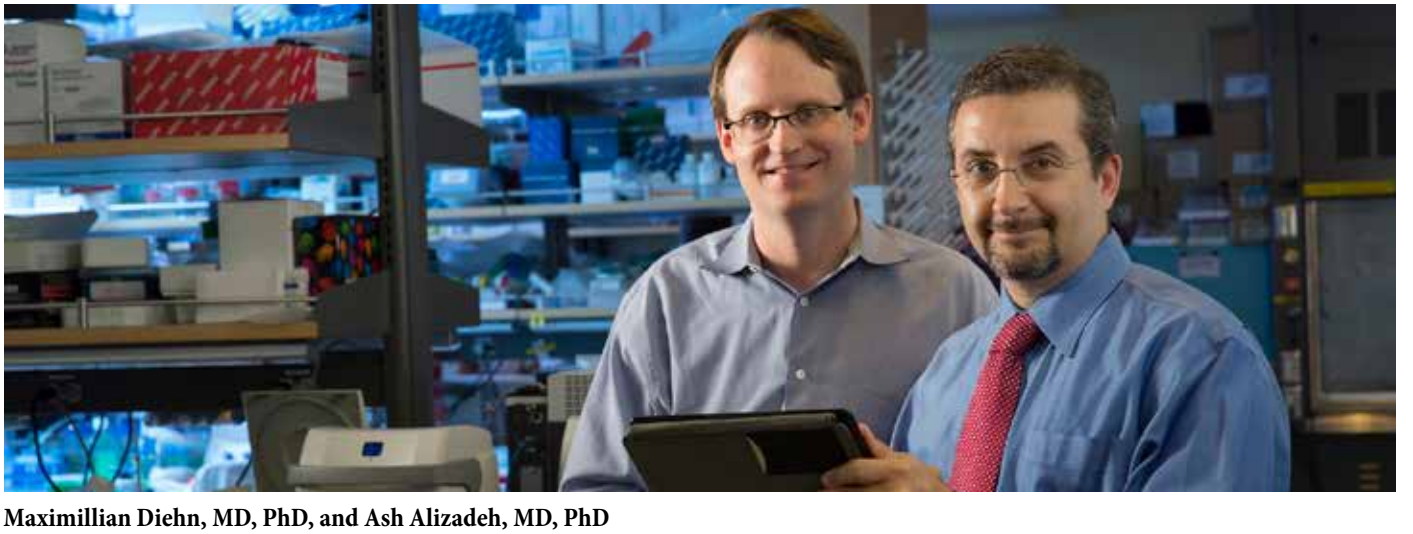
The researchers still need to confirm that the method works more broadly across diverse solid tumors and other blood cancers besides lymphoma. They also caution that the method needs further prospectively validation in clinical trials before it can be widely used for clinical decision-making.

Every year, cancer specialists have more therapies to choose from when treating cancer. Often these treatments work well, but the fear of every doctor and patient is that a therapy does not eliminate the cancer completely, even though there are no detectable signs of the cancer after treatment. When even a relative few cancer-initiating cells are left behind, the cancer can come roaring back, often stronger than before.

"The amount of tumor present in the body after treatment strongly predicts risk of later disease progression and death across cancer types and across different treatment types, so it's important that we can detect residual cancer as soon as possible after treatment," Alizadeh said. "We now know from several cancers that intervention based on the presence of minimal residual disease can improve outcomes, and this PhasED-Seq method now offers an ultrasensitive approach to find such residual disease."

When cancer cells die, they shed DNA into the bloodstream, and the circulating DNA that comes from cancer can be detected because some bits of DNA have particular cancer mutations. Alizadeh and Diehn assemble a panel of the mutations associated with a particular tumor type with a cancer found in individual patients. They have been refining methods of detecting these cancer-derived DNA segments with extreme sensitivity and accuracy.

"The old methods could detect cancer DNA at a level of about one part in 50,000 or so," Diehn said. With this new method we can detect on part in one or two million or so, so it's at least a 40- to 50-fold improvement."



Maximillian Diehn, MD, PhD, and Ash Alizadeh, MD, PhD

A new layer of gene control in blood development that is also modulated in cancer

Every cell contains all the genes in the genome, but which genes are used to make a cell's functional proteins often comes down to the presence of chemical methylation "marks" at key spots in the genome. These methylation marks control the expression of genes, especially as cells develop from stem cells into progenitor cells and through many more steps to mature cells.

These methylation marks particularly apear as 5mC, a master regulator of gene expression that can be removed by conversion into 5hmC. TET2 is one of a family of enzymes responsible for the conversion of 5mC into 5hmC, and it plays a key role in the normal formation of blood and immune cells. Importantly, mutations in TET2 are known to be an important initiating step in the development of multiple blood cancers.

Using several new technological approaches, Ravi Majeti, MD, PhD and his colleagues have investigated 5hmC in normal blood development and report on its modulation and association with gene expression across various types of cells, from blood stem and progenitor cells to mature blood cells. Further, this team, engineered TET2 mutations into human blood stem and progenitor cells to investigate the interplay between TET2 and 5hmC, and the effects of pharmacologic agents targeting this pathway. This work involved a collaboration between the Stanford team and Bluestar Genomics, who have pioneered assays of 5hmC across many tissues and diseases. The researchers reported their results in the journal Blood Cancer Discovery. The co-first authors were Yusuke Nakauchi, MD, PhD and Armon Azizi, BS. The senior author was RZ Cao Professor of Medicine Ravi Majeti, MD, PhD. Majeti is also assistant director of Ludwig Center for Cancer Stem Cell Research and Medicine, as well as a member of the Institute for Stem Cell Biology and Regenerative Medicine.

They discovered that 5hmC is modulated during blood development and links with gene expression programs, suggesting it plays a direct role in gene regulation. Notably, 5hmC levels were decreased in TET2 mutant cells, particularly at regions involved in red blood cell development. This was striking as the TET2 mutant cells were poor at producing red blood cells. When transplanted into mice, these TET2 mutant human cells were found to expand in a way that was similar to what is observed in human individuals who acquire this mutation in their blood.

Lastly, vitamin C, or the approved therapeutic azacytidine, are known to have activity against mutant TET2 and TET2-mutant cells, and the Stanford team showed that these agents not only reversed the effects of mutant TET2 on red blood cell production and expansion in mice, but also increased the level of 5hmC at the same regions affected by mutation of TET2. These results support the model that 5hmC plays a vital role in normal blood cell activity and the development of cancer, particularly with mutations in TET2, and provide important insights for potential therapeutic interventions.

Ravi Majeti, MD, PhD

Researchers identify key aspects of Tr1 cells, which may lead to greater success in cancer treatment.

Many patients with blood cancers, such as leukemias and lymphomas, have been saved through chemotherapy treatment, followed by transplant of blood stem cells to replace those destroyed by the chemotherapy. The transplanted blood stem cells come from a donor who has a similar immunological profile, but the match is never perfect. Mismatched immune cells from the donor that tag along with the stem cell transplant can attack the patient's tissues, causing a life-threatening syndrome called graft vs host disease (GvHD).

Researchers in the laboratory of institute co-director Maria Grazia Roncarolo isolated a kind of immune cell, the type 1 regulatory T (Tr1) cell, that can effectively suppress GvHD caused by donor immune cells transplanted together with blood stem cells. The California Institute for Regenerative Medicine (CIRM) recently awarded a grant to Roncarolo and her colleagues to support a clinical trial investigating the effectiveness of Tr1-based cell therapies in improving outcomes of childhood cancer treatments.

Now, in an article in Science Translational Medicine, Roncarolo and her colleagues reveal details about how to generate Tr1 cells, how they work, and how clinicians can effectively track the infused Tr1 cells in patient's blood. The first authors on the paper are Pauline Chen, MD, and Alma-Martina Cepika, MD, PhD. Roncarolo and Rosa Bacchetta, MD are senior authors.

A clue to how Tr1 cells work came in the discovery that in addition to soluble factors called cytokines, some surface inhibitory receptors expressed by Tr1 cells are essential for their suppressor function. Interestingly, these receptors are expressed also on another kind of regulatory T cell marked by a molecule called FOXP3. Regulatory T cells with FOXP3 are well known for developing early in life to protect us from autoimmune attack on our own tissues, whereas Tr1 cells are important for maintaining tolerance to novel antigens, including alloantigens, and for dampening undesired and excessive immune responses.

In a bit of good news for patients, the researchers showed in this paper that they could efficiently generate Tr1 cells by culturing specific types of immune cells from the donor with different immune cells from the patient. This results in a Tr1 enriched group of cells the researchers call T-allo10. This mixture can be generated and collected in advance of transplantation, thus increasing the odds of success for the cancer treatment.

"The long persistence of Tr1 cells in the patient's body is an important discovery..."

Another useful result to come out of this research is the finding that the T cell receptors of the Tr1 cells come in unique types, that can be tracked in the blood. "Usually, we have to insert a unique marker to track and identify cells and their offspring, which is OK for research but less desirable if we are injecting these cells into patients," said Cepika, "Because Tr1 cells have distinct patterns of their T cell receptors, it's like each Tr1 cell and their progeny have their own identifiable barcode." By being able to track the Tr1 cells, the researchers showed that they can persist in the bloodstream for a year.

"The long term persistence of Tr1 cells in the patient's body is an important discovery and a mayor advantage of this unique T cell subset" said Roncarolo. "These findings give support to the hope that Tr1 based treatments will not only help suppress GvHD immediately after transplantation, but permanently re-program the immune system of the patient to accept the stem cell transplants"

Honors

Weissman wins American Society of Hematology's most prestigious award

The American Society of Hematology (ASH) has announced that it is presenting institute director Irv Weissman, MD its highest honor, the 2022 Wallace H. Coulter Award for Lifetime Achievement in Hematology. The ASH says the intent of the award is to honor an "individual who has demonstrated a lifetime commitment and has made outstanding contributions to hematology, and who has made a significant impact on education, research, and/or practice."

In announcing the award, the ASH noted Weissman's achievements. "These include the isolation and transplantation of hematopoietic stem cells as well as the isolation of hematopoietic progenitor cells, central nervous system cells and solid tumor cells," the ASH said.

ASH president Jane Winter, MD, listed the many additional achievements by Weissman that played a role in giving him the award. "Your work has been foundational to our current understanding of the clonal evolution of leukemia," she said. "You discovered the CD47-SIRPalpha interaction to be a key modulator of programmed cell removal and then used this finding to develop a novel cancer immunotherapy."

Winter also noted Weissman's many efforts in support of stem cell science. "You were a tireless advocate for stem cell research and guided this important research through obstructive political climate," she continued. "This advocacy has made it possible for promising scientists to pursue their careers in this field. In addition, you have enhanced the field of hematology through your multiple generations of mentees who have gone on to make significant discoveries in science."

Joanna Wysocka gets 2022 ISSCR Momentum Award

Joanna Wysocka is being given International Society of Stem Cell Research's 2022 Momentum Award

Institute member Joanna Wysocka, PhD, has been given the 2022 Momentum Award by the International Society for Stem Cell Research (ISSCR). The ISSCR says that "the prize recognizes the exceptional achievements of an investigator whose innovative research has established a major area of stem cell-related research with a strong trajectory for future success."

Joanna Wysocka, PhD

"I am deeply honored to receive the 2022 ISSCR Momentum Award," said Wysocka. "I am grateful to have our research contributions recognized by my peers in the ISSCR, which is at the forefront of innovative and rigorous stem cell science.

"I am thankful for the talented and dedicated colleagues who have embarked on their scientific journeys with me and for the amazing research environment at Stanford," Wysocka added. "The award motivates me to work harder, and hopefully our best is yet to come."

Wysocka's research is has had significant impact in understanding how gene expression is controlled as cell fate takes shape in a developing embryo. She discovered a protein that keeps key genes in the early embryo silent but poised for action when they are needed. She and her colleagues discovered that the modification of a collection of DNA sequences called «enhancers» can dial up or down the activity of the genes in the neural crest, thereby governing which cells eventually become the face. She worked with institute member Vittorio Sebastiano, PhD, to show that in early embryo development, retroviral genes are activated to guide development, and then silenced. Recently, she and her colleagues documented 70 genes that control variation in the development of both face and brain structure.

"Joanna Wysocka is an outstanding mid-career investigator who has an established record of innovation and impact," said Melissa Little, ISSCR President. "Her exceptional achievements were recognized early in her career with the 2010 Outstanding Young Investigator Award, and it has been inspiring to follow her career and contributions to the field. Congratulations Joanna on this prestigious and well-deserved honor."

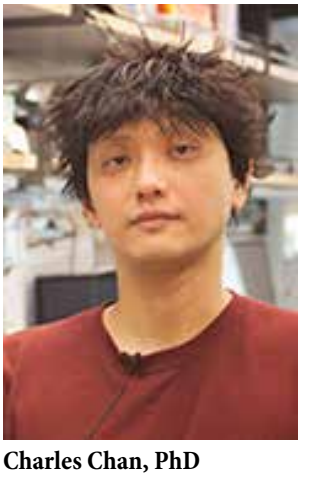
Wysocka is the Lorry Lokey Professor and a Professor of Developmental Biology in the School of Medicine, as well as an HHMI investigator.

Five institute researchers win Stinehart Reed awards

A record 17 institute researchers submitted proposals this year for in search of funding via Stinehart Reed awards. After thorough review by a panel of outside experts, five of those proposals garnered the prestigious awards, which are only available to institute members.

For ten years, institute researchers have found support from the Stinehart Reed foundation for projects that show great promise of producing high-impact discoveries. These research projects are often too speculative or new to have accumulated supporting data that could lead to larger grants from established funding sources like the NIH. The only restrictions on the research, besides needing to be proposed by an institute member, is that the work be generally in the fields of stem cell science or regenerative medicine. Most years, Stinehart Reed grantees receive $200,000, paid at the rate of $100,000 a year over two years.

This year, the Stinehart Reed winners proposed projects over a broad range of scientific projects, addressing problems afflicting all ages, from newborns to seniors.



Charles Chan, PhD

Perhaps no other subject inspires the public interest as much as the idea of regenerative therapy for aching joints. But so far, what passes for "stem cell therapy" for worn knees and hips have only been unproven, unpromising treatments using blood cells or mesenchymal cells. A few years ago, institute researchers Charles Chan, PhD, Michael Longaker, MD and colleagues discovered the human skeletal stem cell, from which all bone, cartilage and stromal cells come. Now, Chan has won a Stinehart Reed grant to build on this discovery by activating existing but inactive skeletal stem cells in the joint and guiding their growth so that they create regenerate cartilage. Current treatments can only generate a scar-like material called fibrocartilage," Chan said. "What we've shown in mice is that we can generate true cartilage in the joints." The Stinehart Reed award will help Chan and his colleagues do the some of the work necessary to bring this potential treatment to human clinical trials.

Irv Weissman, MD

Irv Weissman, MD was awarded a grant to study clonal competition among brain stem cells. It turns out that not all stem cells are equal. Some stem cells acquire mutations that allow them to multiply more and survive better than other stem cells of the same type. For instance, supercompetitive mutant blood stem cells can push out other blood stem cells until, in some case, a person's whole blood and immune system arises from just one clone. It is thought that such clonal competition in blood stem cells help lead to leukemias and diseases like atherosclerosis. Weissman and his colleagues hypothesize that a similar competition is taking place among neural stem cells, and that supercompetitive neural stem cells are helping give rise to brain cancer as well as neuropsychiatric and neurodegenerative disorders.

Marius Wernig, MD, PhD

Marius Wernig, MD, PhD, proposed investigating the activity of a brain cell receptor called TREM2, which has been tied to neurodegenerative diseases. In the central nervous system TREM2 (which stands for Triggering Receptor Expressed in Myeloid Cells 2) is exclusively found on microglial cells, which act as immune and housekeeping cells in the brain. Despite the seeming importance of TREM2, what we know about the receptor comes from studying a very different mouse molecule.

Wernig proposes to study human microglial cells and their interaction with human neural tissue inside a mouse model. He want's to understand why human TREM2 is so important is stopping neurodegeneration, and to find it's role in the communication that happens between different cell types in the brain.


Roel Nusse, PhD

Roel Nusse, PhD, wants to understand what controls the growth of the liver in early postnatal life. The liver is a unique organ in that it not only grows to a certain size and then stops, but if a portion is cut away, the liver will regenerate itself until it reaches that size again. Nusse has postulated that liver size is governed by the number of functional units call lobules. But what controls the number of lobules that develop? Nusse and his colleagues, which include institute member Kristy Red-Horse, PhD, Nusse lab member Dicle Azzizoglu, PhD, and UC Santa Cruz researcher (and former institute trainee) Camilla Forsberg, PhD, want to study the molecular mechanisms that govern lobule growth. "The understanding we gain from this work will directly inform the ongoing organ repair and vascularization efforts in regenerative medicine," Nusse said.


Natalia Gomez Ospina, PhD

Krabbe disease is a rare and often fatal neurodegenerative disorder in children, caused by a genetic defect in an enzyme that the body uses to break down fatty molecules called lipids. Shortly after birth, old lipids start building in the brain, killing nerve cells. Institute researcher Natalia Gomez-Ospina, PhD has won a Stinehart Reed award to help create blood stem cells and progenitor cells that are engineered to produce the enzyme missing in Krabbe disease. Eventually, clinicians may be able to take cells from these kids, engineer the cells to make the missing enzyme, and give them back to the patients to treat the disorder.

Institute director Irv Weissman notes that over ten years, Stinehart Reed awards have advanced stem cell research along a number of fronts, and the most recent awards are likely to be equally successful. "Stinehart Reed awards have been a huge boon for our institute members, and for stem cell science," Weissman said.

Anthony Oro, MD, PhD, elected to the National Academy of Medicine.

Institute member Anthony Oro, MD, PhD, a professor of dermatology, and Ludwig Stanford researcher Crystal Mackall, MD, became two of the scientists elected of the National Academy of Medicine this year.


Anthony Oro, MD

Oro is also the Eugene and Gloria Bauer Professor of Dermatology, and the co-director of the Stanford Center for Definitive and Curative Medicine and of the Stanford Maternal and Child Health Research Institute. Oro was elected for "solidifying the first link between Hedgehog signaling and human cancer, and building chromatin maps identifying how environmental factors drive tumor epigenetic plasticity and drug-resistance," the Academy said. "He built developmental chromatin maps to uncover disease mechanisms and enable clinical manufacturing of pluripotent cell-derived tissues for incurable skin diseases."

Oro is among the 90 regular members and 10 international members elected this year to the academy, which provides policymakers, professionals, business leaders and the public with independent, scientifically informed analysis and recommendations on issues related to health and the biomedical sciences.

New members are elected by current members through a process that recognizes individuals who have made major contributions to the advancement of the medical sciences, health care and public health.

Charles Kwok Fai Chan, PhD, is named DiGenova Endowed Faculty Scholar

Charles Kwok Fai Chan, PhD, a member of the institute and an assistant professor in the Department of Surgery, has been named the DiGenova Endowed Faculty Scholar by the Stanford School of Medicine.

The funding accompanying the title comes financial support provided by a gift from Anthony DiGenova, who was profiled in a recent article:

Anthony (or Tony, as he preferred to be called) DiGenova grew up in a large family in the North Beach neighborhood of San Francisco. The son of a barber who owned a shop where the Bank of America Center now towers, Tony dreamed of going to Stanford University. Although he did not attend, he loved Stanford football and always rooted for the Cardinal when they played the Big Game. As a young man, Tony had the foresight to invest whatever money he had in real estate. Through the years he bought apartment buildings in San Francisco, Alameda, Oakland, and Sacramento. When he passed away in 2017 at the age of 94, he left part of his estate to Stanford Athletics, but the largest distribution of the trust that he built up over his lifetime went to stem cell research at Stanford.

Chan was effusive in his praise in response to the honor. " My team and I are incredibly grateful for Mr. Anthony DiGenova's visionary generosity and to the Trustee's of the DiGenova Foundation for this award," he said. "Their gift will make it possible for our group to build on our recent breakthroughs in stem cell-mediated cartilage regeneration and bring the technology to the clinic as soon as possible. We are also immensely grateful to our colleagues in the Stem Cell Institute, and the whole School of Medicine for their kindness and steadfast support over the years!"

Former graduate student Amira Barkal wins Weintraub Award

Amira Barkal, MD, PhD, a former student in the Stanford Stem Cell Biology and Regenerative Medicine Graduate Program, is one of 13 graduate students from across the nation to be given a Harold M. Weintraub Graduate Student Award for work she did while a student in the Stanford Graduate Program in Stem Cell Biology and Regenerative Medicine. The prestigious award is given by the Fred Hutchinson Research Center in Seattle following a highly competitive review of graduate students from programs around the country. The award recognizes "outstanding achievement during graduate studies in the biological sciences." The work of nominated students is reviewed on the basis of quality, originality, and scientific significance.

While a Stanford graduate student in the laboratory of Institute Director Irv Weissman, MD, Barkal did research on CD24, a cell surface protein that modulates immune reactions. She is now a resident physician in internal medicine at Brigham and Women's Hospital in Boston.

"I'm thrilled to be a Weintraub awardee," Barkal said. "I want to thank the Weintraub Award selection committee, the one-and-only Irv Weissman, and all of my mentors at Stanford and Brigham and Women's Hospital."

Barkal and the 12 other awardees will be granted an honorarium and will give a talk about their work at an awards symposium in Seattle on May 6, 2022.

Amira Barkal, MD, PhD