
# 2012 ANNUAL REPORT

## STANFORD INSTITUTE FOR STEM CELL BIOLOGY AND REGENERATIVE MEDICINE

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# MESSAGE FROM THE DIRECTOR

The year 2012 was a pivotal one. We now have several discoveries coming from the Institute that are either at, or will soon be at, the clinical trial stage. With this in mind, we need to beef up our ability to carry out stem cell therapies at Stanford. The key to any stem cell therapy is to identify and isolate pure populations of stem cells in a sterile facility so that those cells can be administered to patients.

I'm pleased to say that we now nearly have pledges of support to establish a Stem Cell Therapy Center at Stanford, which will allow us to isolate pure populations of stem cells and will make such clinical trials possible. This is a major partnership of Stanford Medicine: the Institute is partnering with Stanford Hospital, thanks to Amir Rubin, and with Lucille Packard Children's Hospital, thanks to Chris Dawes.

Our first cohort of patients at the new Stem Cell Therapy Center will be women with widespread, metastatic breast cancer. We will obtain their 'mobilized blood,' which contains blood-forming stem cells and also circulating cancer cells, and from that we will purify the stem cells of all cancer cells to safely regenerate their blood and immune cells after very high-dose chemotherapy. Years ago, a small clinical trial at Stanford using high-dose chemotherapy and these sorts of purified stem cell transplants led to 33% survival over the last 14 years among women with stage-four

breast cancer (while of those that received purified mobilized blood, only 7% were survivors at 14 years). We would like to use the facility to expand those trials, and to extend it to patients with widespread lymphomas and myelomas. These trials will be done only at Stanford.

We also plan to utilize the center to create stem cell therapies that regenerate the blood forming system of children with the “Bubble Boy” disease (severe combined immune deficiency, or SCID). Purified stem cells do not contain the T cells abundant in the blood and bone marrow of the donor, and which cause a severe ‘graft against host’ immune disease. This, also, will be done via our Institute and only at Stanford. If this clinical trial succeeds, it will be a promising and curative intent therapy for these children.

But our ability to purify stem cells for therapeutic use also opens the door to treating a vast array of other diseases using therapies that have already passed extensive proof-of-principle studies in mice. Purified stem cells may also be part of one-time treatments for autoimmune disorders like multiple sclerosis, lupus, and type-1 diabetes. In the future, purified blood stem cells from an organ donor may free all organ transplant recipients from the immune attacks that cause most transplant failures, and thus free these patients from a lifetime of anti-rejection drugs, which themselves have serious negative side effects.

We hope that in the coming year you will learn more about Stanford-based human clinical trials of promising new therapies, such as:

• An antibody against the ‘don’t eat me’ molecule CD47, which protects all human cancers tested so far from removal by the primary eating cells of the immune system, macrophages. The planned trials will be jointly carried out at Stanford University and with an alliance of United Kingdom cancer trialists at Oxford University to find which human cancers respond best.

• A genetically engineered, induced pluripotent stem cell (iPSC) that will be used to create healthy skin for children with epidermolysis bullosa (EB), a painful blistering disease that, in its most severe forms, is lethal. This trial will be one of the first to test iPSC use in human therapies. Although EB is rare, as a skin disease it offers the opportunity to visually monitor the progress of this stem cell therapy and may offer a clinical template for many other stem cell therapies going forward.

• An antibody therapy that allows safe, non-toxic blood stem cell transplantation. Currently blood stem cell or bone marrow transplantation can only take place after the native blood stem cells have been wiped out through high-dose chemotherapy. This is a grueling treatment that in itself has a 10% mortality rate, limiting the application of blood stem cell transplants to only the most lethal cancers. An antibody-based therapy that creates a small but viable niche for transplanted blood stem cells would make stem cell transplantation therapeutically useful for a wide variety of other diseases.

• A new understanding of how normal human embryos develop and the invention of a device for monitoring embryos created through in-vitro fertilization. The combination of new knowledge and this device is the basis for a clinical trial aimed at selecting IVF embryos most likely to result in a successful pregnancy and a healthy baby.

• Fundamental research from Stanford scientists Philip Beachy and Roel Nusse that has revealed signaling molecules, called Hedgehog and Wnt, made by some 'nurturing niche cells' to signal adjacent stem cells to proliferate or not to differentiate. Now large pharmaceutical and biotech companies have produced a plethora of 'look-alikes' that are currently in anti-cancer trials as well. Another signaling pathway has been found by Michael Clarke to be used by many breast cancers, and a biotech company is currently pursuing that target.

We are seeing iterative cycles of discovery coming from the basic science done by many of our investigators at the Institute. We have had advances in healing bone with stem cells, understanding how brain, blood, breast and bladder cancers develop, how scar formation can be inhibited, and how one type of cell can be directly transformed by gene transfer into another type—and therefore how skin cells may become the basis for creating replacement cells for many other failing organs like the heart or brain.  We hope that these and many other discoveries will become the basis for clinical trials and eventually approved clinical therapies.

We have partnered with the California Institute for Regenerative Medicine (CIRM) to fund many of these discoveries, preclinical tests and incipient clinical trials. We are thankful to them for this necessary grant support. But we will need philanthropic support to build up our Stem Cell Therapy Center and (in cooperation with our colleagues in the clinical departments) to hire and train stem cell clinical trial specialists.

This year we also laid a promising cornerstone for future growth with the admission of the first class of graduate students in the Interdepartmental Graduate Program in Stem Cell Biology and Regenerative Medicine. It is common in graduate programs to expect that if you offer spots to the best and brightest students from around the country, only about 30% will accept. This being our first class of graduate students, and having only the experience of other graduate programs to go by, we accepted a dozen truly outstanding students in hopes of getting 3 or 4 of them. Nearly every one of the students chose the Stanford program over all others, demonstrating the strength of this program (but also requiring a scramble for extra philanthropic support to help fund the education of our unexpectedly large first class).

This is a very exciting time for us and, I hope, for you–because your continuing support for the Institute, and your support for the creation of CIRM, have made all of this possible.

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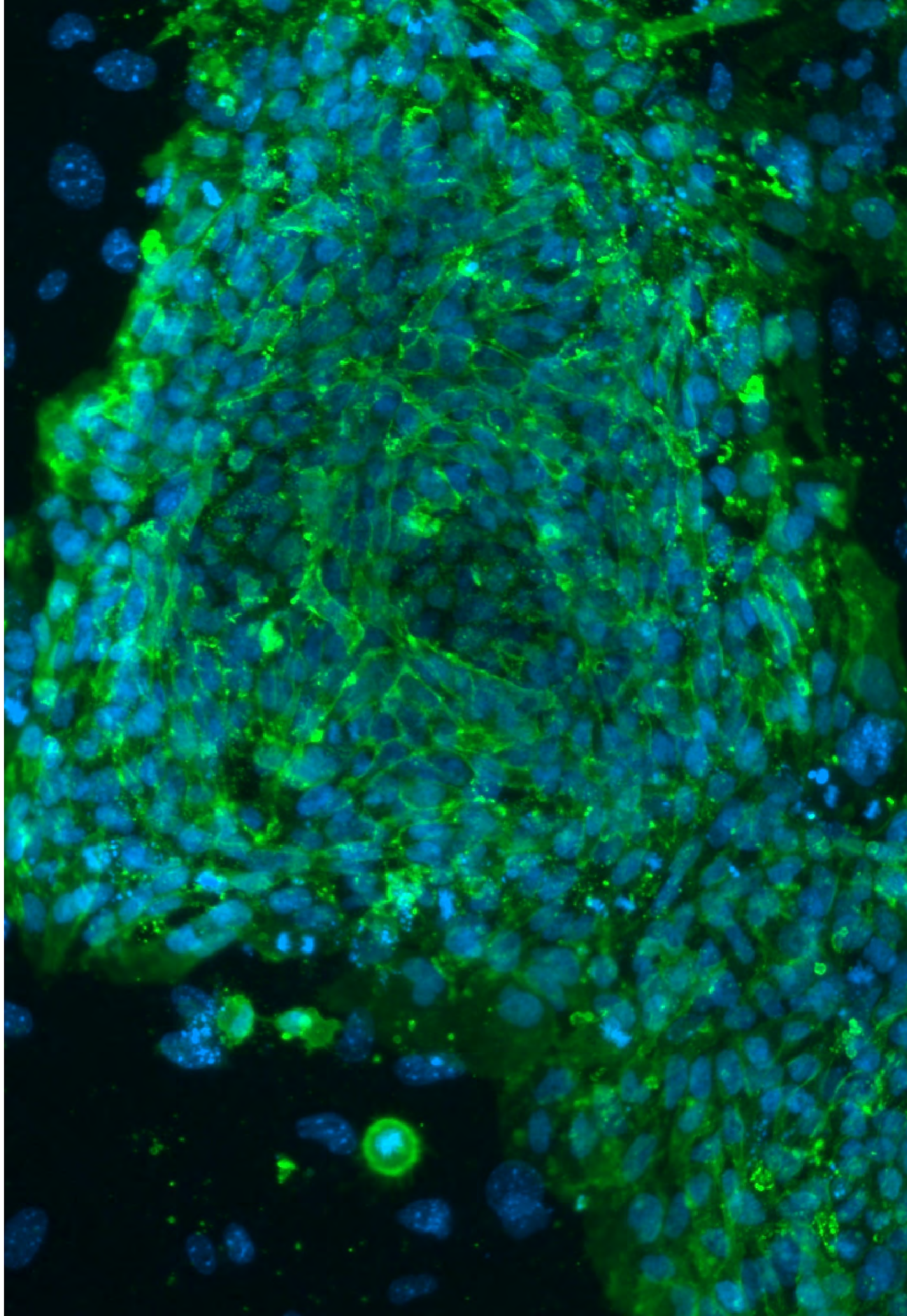
# RESEARCH AREAS

The institute conducts research across a wide variety of areas: embryonic stem cells, induced pluripotent stem cells, cancer stem cells, and adult (tissue-specific) stem cells. A primary mission of the institute is to translate basic stem cell science into the clinical therapies that are becoming the foundation of regenerative medicine.

# EMBRYONIC STEM CELLS

Embryonic stem cells naturally develop into every kind of cell in our body. Exploring the mysteries of how this occurs naturally, and how errors occur during the natural development process, is one focus of the embryonic stem cell researchers at the institute. This research will have significant influence not only on our understanding of the developmental process, but will also affect the medical approach to infertility. This year there were significant findings in many areas.



## Stanford Researchers Find Genetic Markers of Fertility in Women

Sonya Schuh-Huerta, PhD, a researcher in the laboratory of Renee Reijo Pera, PhD, is one of the Stanford scientists searching for genetic markers of fertility and ovarian reserve.

Researchers at the Stanford Institute for Stem Cell Biology and Regenerative Medicine have discovered genetic markers that may ultimately allow women to track and predict declining fertility. Ultimately, this study and further research may allow individual women to know in advance the approximate age when their fertility will decline, allowing them to plan accordingly.

“Many women now are delaying childbirth until their mid to late 30s, which is getting very near the edge of the usual fertility window,” says Sonya Schuh-Huerta, PhD. Some of these women are destined to have diminished fertility by the time they try to have children, but they won’t know that in advance, Schuh-Huerta says.

Testing for the number of maturing eggs in the ovary and levels of reproductive hormones can be a good indication of fertility, but women tend not to get these tests until they are already experiencing difficulties with conceiving, Schuh-Huerta says. “Ultimately, a test for specific genetic markers would be easier and could give them more information and more power to make reproductive decisions,” she says.


**Sonya Schuh-Huerta found genetic markers that could help women predict their window of fertility.**

The age of onset of menopause is highly determined by genetics, a fact that many women don’t know, says senior author Renee Reijo Pera, PhD. “We did a survey of undergraduates and most didn’t realize that their reproductive biology is relatively fixed,” she says. “They thought that if they didn’t smoke, ate right and exercised they could extend their fertile years.”

Reijo Pera and her colleagues set out to search for those genes that would indicate early or late menopause. They recruited healthy women of reproductive age, took DNA samples, and tested the women for levels of reproductive hormones, which is one indicator of fertility. Then they cast a wide net, sorting through all the genes in the body in what is called a genome-wide association study (GWAS)

to find those genes that were associated with early declines or rises in reproductive hormones. The study looked for these results in Caucasian and African American women to make sure that any genetic findings were valid in very different populations.

“Normally, to test for fertility you can do a hormone assay and get a number, but if you talk to the heads of in-vitro fertilization clinics, they’ll say that hormone levels are highly variable,” says Reijo Pera. “We know when the hormones really crash that shows a decline in fertility, but there is a wide swath of hormone levels where we just don’t know exactly what they mean.”

The researchers hope that the new gene markers will help them better gauge fertility and interpret the hormonal tests. Interestingly enough, the genes discovered to be associated with reproductive hormones previously had no known connection to human reproduction, Schuh-Huerta says. “Some of them are active in early development, so they may have something to do with formation of early germ cells (eggs),” she says.

If a genetic test for fertility is developed, it could also help predict the likelihood of other health problems, since declining ovarian function is associated with cardiovascular problems, bone mineralization diseases (including osteoporosis) and various cancers, Schuh-Huerta says. And because such genes are present one’s whole life, clinicians would be able to make predictions about fertility and other health problems decades in advance.

The researchers are currently finishing a study that looks for genes associated with the number of viable egg follicles in the ovaries, which is another good indicator of fertility.  Other than information gained through studies in animal models, very little is known about genes directly linked with the number of eggs in the human ovary.

## Embryo-analysis technique developed at Stanford may boost in vitro fertilization success

Stanford University School of Medicine researchers have devised a two-part approach to identify developing human embryos most likely to result in successful pregnancies. The technique could transform the lives of infertile couples seeking to use in-vitro fertilization, or IVF, to start a family.

The research suggests that fragmentation — a common but not well-understood occurrence in the early stages of human development in which some of the cells in an embryo appear to break down into smaller particles — is often associated with a lethal loss or gain of genetic material in an embryo's cells. Coupling a dynamic analysis of fragmentation with an analysis of the timing of the major steps of embryonic development can significantly increase the chances of selecting an embryo with the correct number of chromosomes, the researchers found.

The findings extend beyond IVF and offer a glimpse into how human reproduction differs from that of many other animals. They also suggest that sperm selection could be much more important than previously believed.

"It is amazing to me that 70 to 80 percent of all human embryos have the wrong number of chromosomes," said Renee Reijo Pera, PhD, professor of obstetrics and gynecology. "But less than 1 percent of

all mouse embryos are similarly affected. We're trying to figure out what causes all these abnormalities."

Professor Reijo Pera, who is the director of the Center for Human Embryonic Stem Cell Research and Education at Stanford's Institute for Stem Cell Biology and Regenerative Medicine, is the senior author of the work, published online Dec. 4 in Nature Communications. Research associate Shawn Chavez, PhD, is the study's first author.

Regardless of the source of the chromosomal errors, nearly all result in miscarriage. For natural conceptions, this often happens before the woman realizes she is pregnant. Each embryo transfer in IVF, however, is eagerly anticipated and costs thousands of dollars. To improve the odds of a successful pregnancy, clinicians and parents frequently decide to transfer more than one embryo at a time — a decision that has its own risks for mother and any fetuses that may result. For example, instances in which there are multiple fetuses are more likely to result in miscarriages or to threaten the health of the mother.

Recently, Reijo Pera and her colleagues began to investigate ways to better predict embryonic developmental success within one or two days of fertilization. Not only would such an advance decrease the likelihood of miscarriage or the possible need for a selective reduction, it would also reduce the amount of time the embryo would have to be cultured in the laboratory before transfer. (Although it has not been conclusively shown, some researchers are concerned that epigenetic changes may accumulate in a cultured embryo and cause subtle, long-lasting effects in the fetus.)

The study extends previous findings in Reijo Pera's lab indicating that the timing of cell division and other developmental milestones as the embryo progresses from one to four cells can be used to

### Video: Techniques help predict which IVF embryos will work best


Shawn Chavez, PhD, describes her research on genetic fragmentation in the developing embryo.
View the video at http://stemcell.stanford.edu/video/2012.html

predict with 90 percent accuracy whether the embryo is likely to go on to develop into a 70- to 100-celled embryonic structure called a blastocyst. Achieving blastocyst status, which occurs about five days after fertilization, is a good, but not fail-safe, indication that an embryo might result in a successful pregnancy. That research was published in Nature Biotechnology in October 2010, and is currently the subject of clinical trials in several IVF clinics across California.

In the new study, the researchers decided to look more closely at the chromosomal composition of those four-celled embryos predicted by their previous method to be successful. The 75 human embryos used in the study were originally intended for use in IVF. They were donated for research by infertile couples to the Stanford RENEW Biobank. They are unusual in that they were frozen within hours of fertilization. Clinicians normally monitor the development of fertilized embryos for three to five days in an attempt to identify those that are the best candidates for transfer. Those remaining are then frozen for later use — either in future IVF cycles for that couple, or as research tools to learn more about human development. However, the clinics visited by the couples who donated the embryos for this study supported earlier freezing as a standard practice.

The researchers thawed the embryos and monitored the developmental milestones with time-lapse photography as they progressed over the course of two days to approximately the four-cell stage. They found that only 53 of the original 75 embryos progressed beyond the one-cell stage. They then dissembled the 53 embryos into individual cells, analyzed the chromosomal content of each cell, and compared the findings with each embryo's predicted chance of success. A normal human embryo has 23 pairs of chromosomes; each pair contains one chromosome from each parent.

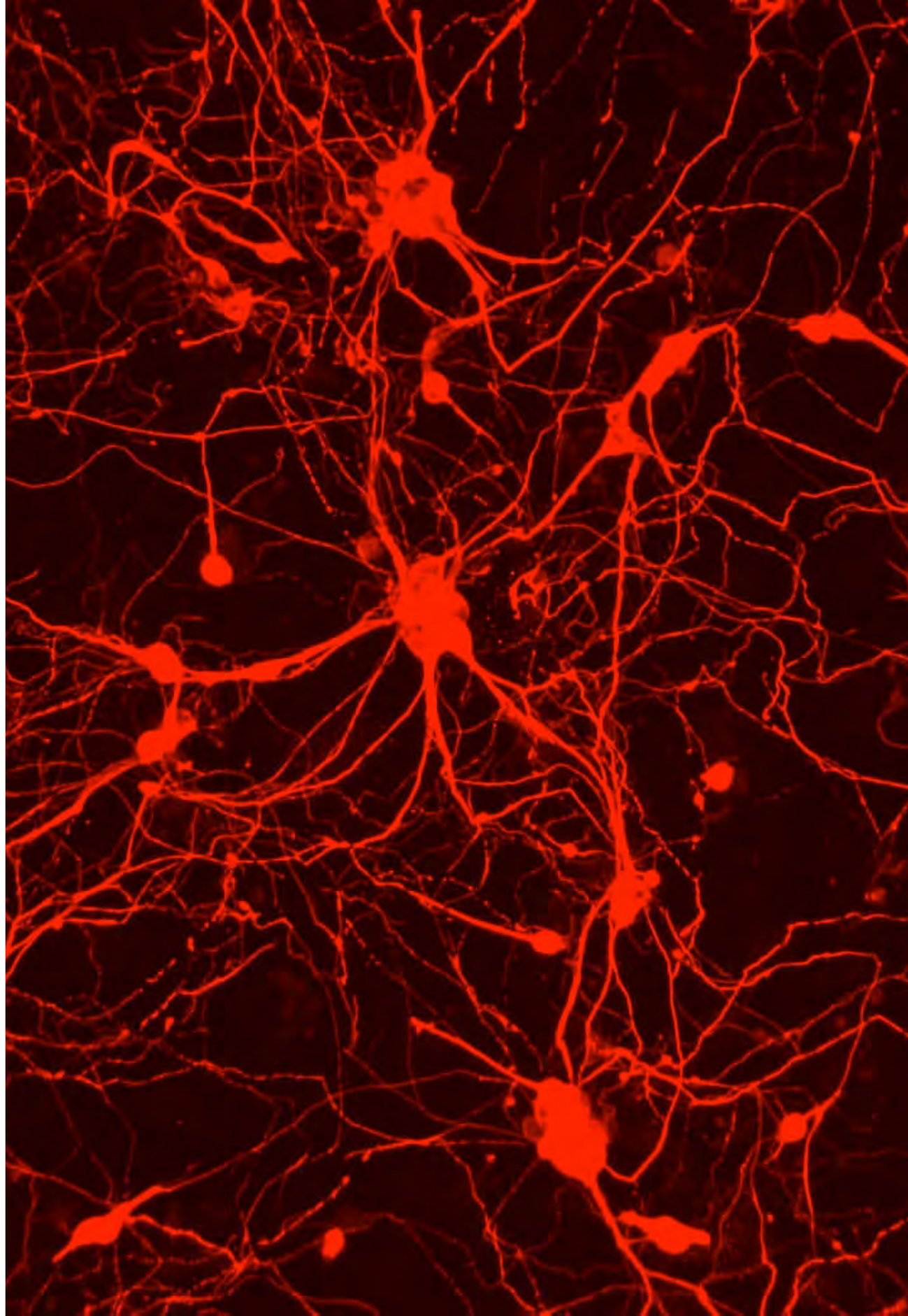
"We found that, although the parameters we defined earlier do very well in predicting blastocyst success," said Reijo Pera, "about 50 percent of those with normal developmental timing have the wrong number of chromosomes." That is, even though they would likely go on to become blastocysts, they were unlikely to result in healthy pregnancies.

The researchers found that they could increase their chances of picking an embryo with the correct number of chromosomes by combining their previous parameters with an analysis of a perplexing embryonic process called fragmentation, which is thought to possibly represent a breakdown of cellular components within an embryo.

The researchers concluded that, although neither developmental timing nor the presence of fragmentation was a foolproof way to pick a healthy, chromosomally intact embryo, a combination of the two approaches appears much more likely to be successful.

# INDUCED PLURIPOTENT STEM CELLS AND DIRECTLY TRANSFORMED CELLS

Although embryonic stem cells may be useful for treating disease on a small scale, their availability is limited, and there are questions about whether transplanted embryonic cells would tend to be rejected by the recipient's immune system. Researchers at the institute are finding new ways to create therapeutic stem cells out of common cells such as fibroblasts (skin cells). This approach carries the dual benefit of  being able to produce large quantities of therapeutic cells from the patient's own cells. All of these newly created cells would share the patient's genetic code and therefore would be less likely to be rejected by the immune system.



## Body may be able to 'coach' transplanted stem cells to differentiate appropriately, study shows

Pluripotent stem cells are nature's double-edged sword. Because they can develop into a dizzying variety of cell types and tissues, they are a potentially invaluable therapeutic resource. However, that same developmental flexibility can lead to dangerous tumors called teratomas if the stem cells begin to differentiate out of control in the body.

To prevent this outcome, researchers must first give the cells a not-so-subtle shove toward their final developmental fate before transplanting them into laboratory animals or humans. But exactly how to do so can vary widely among laboratories. Now researchers at the Stanford University School of Medicine have used an experiment in mice to hit upon a way to possibly skip this fiddly step by instead relying mostly on signals within the body to keep the stem cells in line.

"Before we can use these cells, we have to differentiate, or 'coach,' them down a specific developmental pathway," said Michael Longaker, MD, the Deane P. and Louise Mitchell Professor in the School of Medicine. "But there's always a question as to exactly how to do that, and how many developmental doors we have to close before we can use the cells. In this study, we found that, with appropriate environmental cues, we could let the body do the work."

Allowing the body to direct differentiation could speed the U.S. Food and Drug Administration's approval of using such pluripotent stem cells, Longaker believes, by eliminating the extended periods of laboratory manipulation required during the forced differentiation of the cells.

Longaker, who co-directs Stanford's Institute for Stem Cell Biology and Regenerative Medicine, is the senior author of the research, published online Nov. 19 in the *Proceedings of the National Academy of Sciences*. Postdoctoral scholars Benjamin Levi, MD, and Jeong Hyun, MD, and research assistant Daniel Montoro are co-first authors of the work.

### Video: Coaching cells to make bone


Dr. Michael Longaker talks about directing the development of pluripotent stem cells by controlling their environment.
View at http://stemcell.stanford.edu/video/2012.html

"Once we identify the key proteins and signals coaching the tissue within the body, we can try to mimic them when we use the stem cells," said Longaker. "Just as the shape of water is determined by its container, cells respond to external cues. For example, in the future, if you want to replace a failing liver, you could put the cells in a scaffold or microenvironment that strongly promotes liver cell

differentiation and place the cell-seeded scaffold into the liver to let them differentiate in the optimal macroenvironment."

In Longaker's case, the researchers were interested not in the liver, but in bone formation. Longaker himself is a pediatric plastic and reconstructive surgeon who specializes in craniofacial malformations. "Imagine being able to treat children and adults who require craniofacial skeletal reconstruction, not with surgery, but with stem cells," he said.

The researchers removed a 4 millimeter circle of bone from the skulls of anesthetized laboratory mice — a defect just large enough to stymie the natural healing properties of the bone's endogenous stem cells. They then implanted in the damaged area a tiny, artificial scaffold coated with a protein called BMP-2 that they knew (from previous experiments) stimulated bone growth. Each scaffold was seeded with 1 million human stem cells. They then waited and watched for several months as the bone regrew.

"We found that the human cells formed bone and repaired the defect," said Longaker. "What's more, over time that human bone created by the stem cells was eventually replaced by mouse bone as part of the natural turnover process. So the repair was physiologically normal."

The researchers credit the regrowth to the tandem nature of a macroenvironment of bone damage and a microenvironment of scaffolding coated with a bone-growth-triggering molecule.

The researchers tested the ability of both human embryonic stem cells and human induced pluripotent stem cells, or iPS cells, to heal the defects. (iPS cells are generated in the laboratory from fully differentiated cells like those found in skin, whereas embryonic stem cells are isolated from human embryos.) They found that the iPS cells seeded onto the BMP-2-containing scaffolds healed more than 96 percent of the defect within eight weeks of transplantation. Human embryonic stem cells were similarly successful, healing 99 percent of the defect within eight weeks.

In addition to repairing the defect, the technique also produced relatively few dangerous teratomas: Two out of 42 animals in the study developed the tumors. (Both teratomas occurred in animals that had received embryonic stem cells, rather than iPS cells.) In contrast, both types of stem cells readily formed teratomas when implanted under the kidney surface of an immunodeficient animal — a standard laboratory test to confirm stem cells' pluripotent potential.

"We still have work to do to completely eliminate teratoma formation," said Longaker, "but we are highly encouraged." He speculates that combining the technique with other strategies — perhaps by including other cell types to act as chaperones to the differentiating stem cells — may eventually overcome the problem.

"I want to see how broadly applicable this technique may be," said Longaker, who speculated that it may be useful to replace damaged cartilage in joints during arthroscopy. "Cartilage doesn't heal itself, so perhaps you can add some cells that can form replacement tissue in this macroenvironment while you're already looking in the joint.

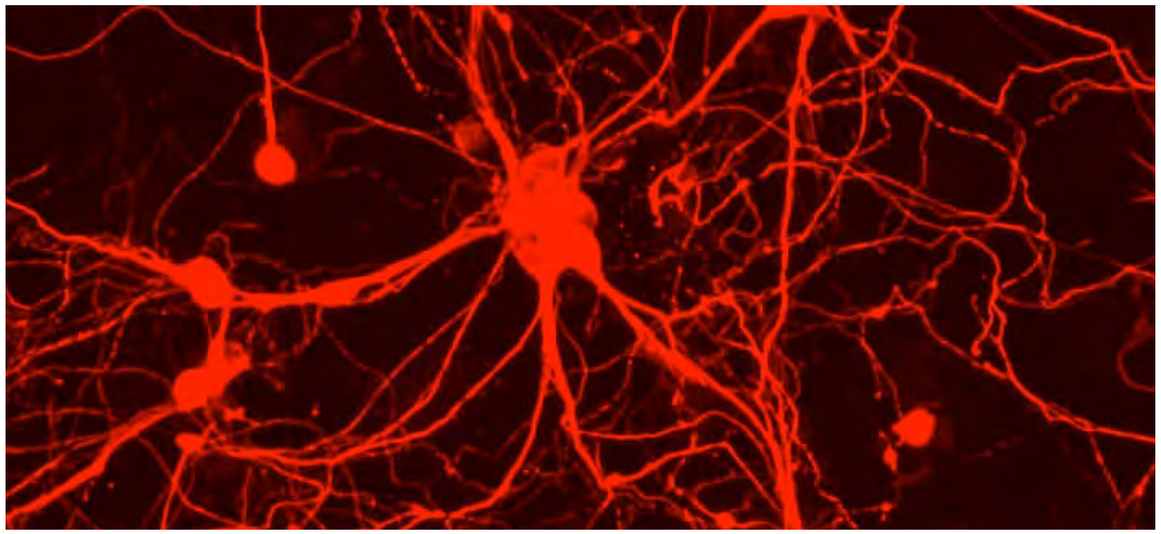
## Scientists turn skin cells into neural precursors, bypassing stem cell stage

Mouse skin cells can be converted directly into cells that become the three main parts of the nervous system, according to researchers at the Stanford University School of Medicine. The finding is an extension of a previous study by the same group showing that mouse and human skin cells can be directly converted into functional neurons.

The multiple successes of the direct conversion method could refute the idea that pluripotency (a term that describes the ability of stem cells to become nearly any cell in the body) is necessary for a cell to transform from one cell type to another. Together, the results raise the possibility that embryonic stem cell research and another technique called “induced pluripotency” could be supplanted by a more direct way of generating specific types of cells for therapy or research.

This study, published in the *Proceedings of the National Academy of Sciences*, is a substantial advance over the previous paper in that it transforms the skin cells into neural precursor cells, as opposed to neurons. While neural precursor cells can differentiate into neurons, they can also become the two other main cell types in the nervous system: astrocytes and oligodendrocytes. In addition to their greater versatility, the newly derived neural precursor cells offer another advantage over neurons because they can be cultivated to large numbers in the laboratory — a feature critical for their long-term usefulness in transplantation or drug screening.

In the study, the switch from skin to neural precursor cells occurred with high efficiency over a period of about three weeks after the addition of just three transcription factors. (In the previous study, a different combination of three transcription factors was used to generate mature neurons.) The finding implies that it may one day be possible to generate a variety of neural-system cells for transplantation that would perfectly match a human patient.



“We are thrilled about the prospects for potential medical use of these cells,” said Marius Wernig, MD, assistant professor of pathology and a member of Stanford’s Institute for Stem Cell Biology and Regenerative Medicine. “We’ve shown the cells can integrate into a mouse brain and produce a missing protein important for the conduction of electrical signal by the neurons. This is important because the mouse model we used mimics that of a human genetic brain disease. However, more work needs to be done to generate similar cells from human skin cells and assess their safety and efficacy.”

Wernig is the senior author of the research. Graduate student Ernesto Lujan is the first author.

While much research has been devoted to harnessing the pluripotency of embryonic stem cells, taking those cells from an

embryo and then implanting them in a patient could prove difficult because they would not match genetically. An alternative technique involves a concept called induced pluripotency, first described in 2006. In this approach, transcription factors are added to specialized cells like those found in skin to first drive them back along the developmental timeline to an undifferentiated stem-cell-like state. These "iPS cells" are then grown under a variety of conditions to induce them to re-specialize into many different cell types.

Scientists had thought that it was necessary for a cell to first enter an induced pluripotent state or for researchers to start with an embryonic stem cell, which is pluripotent by nature, before it could go on to become a new cell type. However, research from Wernig's laboratory in early 2010 showed that it was possible to directly convert one "adult" cell type to another with the application of specialized transcription factors, a process known as transdifferentiation.

Wernig and his colleagues first converted skin cells from an adult mouse to functional neurons (which they termed induced neuronal, or iN, cells), and then replicated the feat with human cells. In 2011 they showed that they could also directly convert liver cells into iN cells.

"Dr. Wernig's demonstration that fibroblasts can be converted into functional nerve cells opens the door to consider new ways to regenerate damaged neurons using cells surrounding the area of injury," said pediatric cardiologist Deepak Srivastava, MD, who was not involved in these studies. "It also suggests that we may be able to transdifferentiate cells into other cell types." Srivastava is the director of cardiovascular research at the Gladstone Institutes at the University of California-San Francisco. In 2010, Srivastava transdifferentiated mouse heart fibroblasts into beating heart muscle cells.

"Direct conversion has a number of advantages," said Lujan. "It occurs with relatively high efficiency and it generates a fairly homogenous population of cells. In contrast, cells derived from iPS cells must be carefully screened to eliminate any remaining pluripotent cells or cells that can differentiate into different lineages." Pluripotent cells can cause cancers when transplanted into animals or humans.

Skin cells expressing three particular transcription factors became neural precursor cells that were able to differentiate into not just neurons and astrocytes, but also oligodendrocytes, which make the myelin that insulates nerve fibers and allows them to transmit signals. The scientists dubbed the newly converted population "induced neural precursor cells," or iNPCs.

In addition to confirming that the astrocytes, neurons and oligodendrocytes were expressing the appropriate genes and that they resembled their naturally derived peers in both shape and function when grown in the laboratory, the researchers wanted to know how the iNPCs would react when transplanted into an animal. They injected them into the brains of newborn laboratory mice bred to lack the ability to myelinate neurons. After 10 weeks, Lujan found that the cells had differentiated into oligodendroytes and had begun to coat the animals' neurons with myelin.

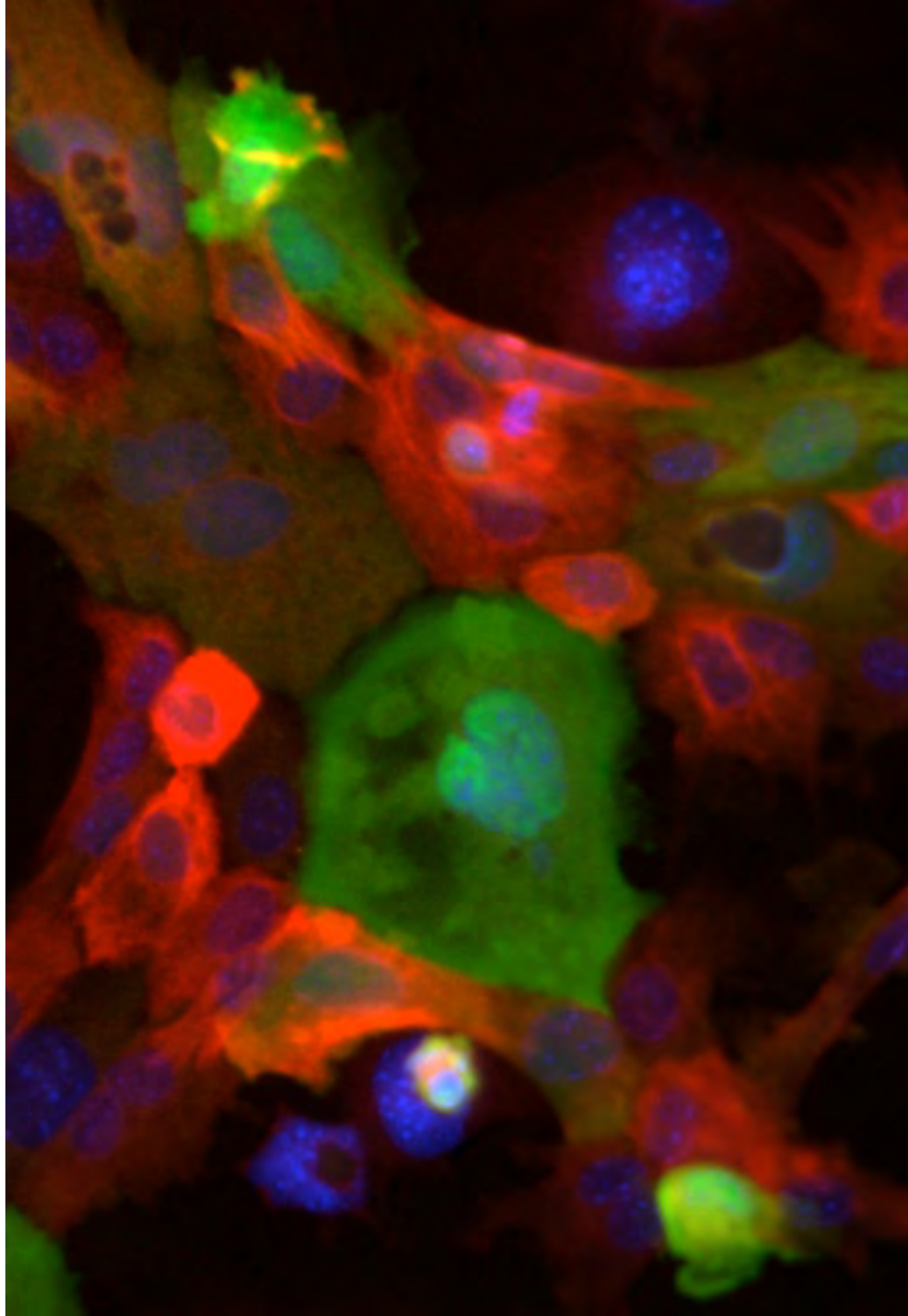
"Not only do these cells appear functional in the laboratory, they also seem to be able to integrate appropriately in an in-vivo animal model," said Lujan.

# CANCER STEM CELLS

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

The old theory of cancer is that any cell in the body, given the right combination of genetic alterations, can “go rogue” and become cancerous. This theory also holds that most cancer cells can be the cancerous seeds that enable the disease to grow and spread.

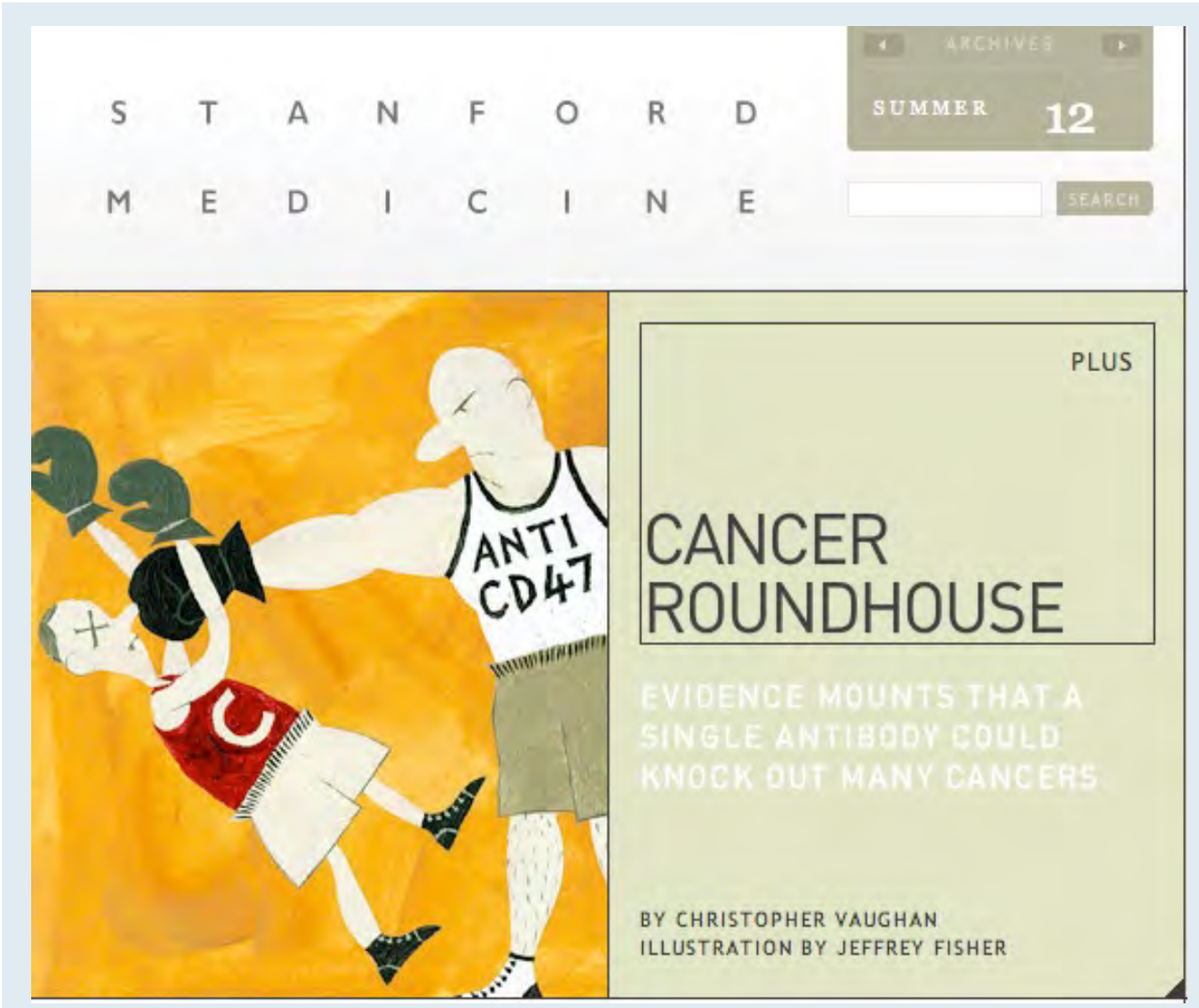




The stem cell theory of cancer proposes that cancers arise as a result of the slow accumulation of mutations in stem cells, the only kind of cells that live long enough to acquire all the right mutations. The stem cell theory of cancer also proposes that cancers are like any other organ in the body in that they are maintained and sustained by a small number of cancer stem cells, which are also the only cells that can spread the cancer. Under this model, curing cancer requires clinicians to destroy the cancer stem cells in particular. Once that is accomplished, any other non-stem cancer cells will die naturally.

In 2012, researchers made significant progress on many fronts. Investigators found that nearly all solid tumors carried excess amounts of a cell surface protein (CD47) that protects the cancer from the patient's immune system. Blocking the CD47 signal from human solid cancers transplanted into mice effectively reduces or eliminates the tumors.

Institute researchers also made important discoveries about the cells from which particular cancers arise, and about the process of accumulating the genetic mutations that are necessary for the development of a cancer.




Read the Stanford Medicine article about CD47 treatments.

http://stanmed.stanford.edu/2012summer/article7.html

## Single antibody shrinks variety of human tumors transplanted into mice, study shows

Human tumors transplanted into laboratory mice disappeared or shrank when scientists treated the animals with a single antibody, according to a new study from the Stanford University School of Medicine. The antibody works by masking a protein flag on cancer cells that protects them from macrophages and other cells in the immune system. The scientists achieved the findings with human breast, ovarian, colon, bladder, brain, liver and prostate cancer samples.

It is the first antibody treatment shown to be broadly effective against a variety of human solid tumors, and the dramatic response — including some overt cures in the laboratory animals — has the investigators eager to begin phase-1 and –2 human clinical trials within the next two years.

“Blocking this ‘don’t-eat-me’ signal inhibits the growth in mice of nearly every human cancer we tested, with minimal toxicity,” said Professor of Pathology Irving Weissman, MD, who directs Stanford’s Institute of Stem Cell Biology and Regenerative Medicine and the Ludwig Center for Cancer Stem Cell Research and Medicine at Stanford. “This shows conclusively that this protein, CD47, is a legitimate and promising target for human cancer therapy.”

The antibody treatment also significantly inhibited the ability of the tumors to metastasize throughout the animals’ bodies.

“This is exciting work and will surely trigger a worldwide wave of research designed to convert this strategy into useful therapies,” said Robert Weinberg, PhD, a professor of Biology at the Whitehead Institute for Biomedical Research in Massachusetts who was not involved in the research. “Mobilizing the immune system to attack solid tumors has been a longstanding goal of many cancer researchers for decades.”

Video: Antibody shrinks many types of solid tumors


Researchers talk about their results showing that the anti-CD47 antibody shrinks many kinds of human solid cancers in an animal model. View at http://stemcell.stanford.edu/video/2012.html

The research was published online March 26 in the Proceedings of the National Academy of Sciences. Weissman, who is the Virginia & D.K. Ludwig Professor for Clinical Investigation in Cancer Research at Stanford and a member of the Stanford Cancer Institute, is the senior author of the research. Postdoctoral scholars Stephen Willingham, PhD, and Jens-Peter Volkmer, MD, are the co-first authors of the study.

Previous work in Weissman's lab has shown that CD47 is normally expressed on the surfaces of circulating blood stem cells to protect them from immune cells called macrophages. Macrophages patrol the body looking for signs of trouble in the form of invaders or rogue cells, but they sometimes latch onto the wrong targets. CD47 prompts them to release cells they've grabbed by mistake.

Weissman and his colleagues also showed previously that some types of cancer cells — particularly those of blood cancers such as leukemia and lymphoma — have figured out a way to game the system and use this "don't-eat-me signal" to their advantage by expressing CD47 on their own surfaces. In 2010, they found that blocking CD47 with a specific antibody (plus adding another to further stimulate the macrophages' killing instinct) can cure some cases of human non-Hodgkin's lymphoma in mice. But it wasn't known until now how widespread or clinically important the phenomenon would be in human solid tumors.

In the current study, Willingham and Volkmer collected surgical samples of a variety of human tumors, including ovarian, breast, colon, bladder, brain, liver and prostate. To do so, they enlisted the help of clinical experts from across the School of Medicine, including those specializing in oncology, urology, obstetrics and gynecology, radiation oncology, neurosurgery, hematology, pathology, otolaryngology and hepatology.

They showed that nearly every human cancer cell they examined expressed CD47 — usually at higher levels (on average, about three times more) than did non-cancerous cells. Furthermore, people whose cancer cells express a lot of CD47 tend to have shorter life spans than people with similar cancers that express less CD47. This suggests that an analysis of the levels of CD47 expression in some types of tumors could be a valuable prognostic tool for patients and their doctors.

**"These results indicate that anti-CD47 antibodies can dramatically inhibit the growth of human solid tumors"**

Willingham and Volkmer then implanted the different human tumor cells into matching locations in the bodies of mice — breast cancer tumors into the mammary fat pads, and ovarian cancer tumors into the abdomen, for example. Once the tumors were well-established (after two weeks or more), they treated the animals with the anti-CD47 antibody.

The researchers saw that most of the established tumors begin to shrink and even, in some cases, disappear within weeks of treatment with the antibody. In one case, antibody treatment cured five mice injected with the same human breast cancer cells. When the tumor was gone, the treatment was discontinued; the mice were monitored for four months with no signs of recurrence.

"These results indicate that anti-CD47 antibodies can dramatically inhibit the growth of human solid tumors by blocking the ability of CD47 to transmit the 'don't-eat-me' signal to macrophages," concluded the authors.

"If the tumor was highly aggressive," said Weissman, "the antibody also blocked metastasis. It's becoming very clear that, in order for a cancer to survive in the body, it has to find some way to evade the cells of the innate immune system." The innate immune system is the body's first line of defense against pathogens like bacteria and

viruses. Unlike the adaptive immunity conferred by antibodies and T cells that recognize and battle specific molecules, cells of the innate immune system, like macrophages, respond non-specifically to a variety of threats.

The researchers' approach didn't work in every animal, though. A set of mice with breast cancer cells from one human patient experienced no benefit from antibody treatment. "There's certainly more to learn," said Weissman. "We need to learn more about the relationship between macrophages and tumor cells, and how to draw more macrophages to the tumors." He suggested that reducing the size of a tumor with surgery or radiotherapy before antibody treatment could make the treatment more effective. Another option, he added, would be to use a second antibody in addition to CD47 that would further stimulate the ability of the macrophages or other immune cells to kill the cancer cells.

While treatment modifications may be beneficial, the findings about the effect of the single antibody are promising in their own right and set the stage for advancing the research. "We believe these results show that we should move forward quickly but cautiously into human clinical trials for many types of solid tumors," Weissman said.

## Researchers prove that leukemias arise from changes that accumulate in blood stem cells

Imagine that a police bomb squad comes upon a diabolically designed bomb controlled by a tangled mass of different wires, lights and switches, some of which have a real function while others are decoys. The police don't know how to begin defusing the bomb because they don't know which parts are important. Then imagine the police discover the bomb-making factory and are able to see hundreds of these bombs at various stages of construction. With this information, they can reconstruct how the bomb was put together, and therefore how to disarm it.

For a team of researchers at the Stanford University School of Medicine, the bombs they need to defuse are killer leukemias. The researchers report that they have used advanced techniques to survey what's in the "bomb factory:" the stem cells that produce all blood cells. In the process, they have proven a controversial theory that blood cancers — and perhaps all cancers — arise only when mutations accumulate over long periods of time in stem cells.

The research, published in *Science Translational Medicine*, also sets the stage for the discovery of more effective therapies for defeating deadly cancers.

People with acute leukemias — cancers of the blood — are especially difficult to cure. Although doctors can drive leukemias into remission with chemotherapy, most of these cancers eventually come roaring back. About 60 percent of those who get acute

myelogenous leukemia will ultimately die from it, a statistic that has improved little in the past 30 years.

Cancer is caused in part by genetic mutations, but cancer cells are often full of these mutations, some of which are important and some not. “Each cancer-causing mutation is potentially a therapeutic target because we might be able to fix or block it, but we have to know which mutations to focus on,” said Ravi Majeti, MD, PhD, assistant professor of hematology and a co-principal author of the paper. His fellow principal co-authors are bioengineering professor Stephen Quake, PhD, and professor of pathology Irving Weissman, MD, who directs Stanford’s Institute for Stem Cell Biology and Regenerative Medicine and the Ludwig Center for Cancer Stem Cell Research and Medicine at Stanford.

**“The natural mutation rate is slow enough that only the stem cells are around long enough to accumulate all of the necessary mutations and other inherited changes in gene expression to develop the cancer”**

The researchers decided to test a hypothesis Weissman made more than a decade ago: That the progression from a normal stem cell to a leukemia stem cell occurs mostly in blood stem cells.

Weissman hypothesized that rare mutations and gene translocations accumulate in a line of blood stem cells to the point that the cancer or leukemia can break free of growth constraints and spread, eventually leading the altered blood stem cell to produce a progenitor cell from which leukemia arises. This hypothesis was investigated by graduate students Max Jan and Ryan Corces-Zimmerman, and postdoctoral scholar Thomas Snyder, the three co-first authors who conducted genetic analyses of 80-500 individual blood stem cells from each of six leukemia patients.

In the leukemia patients, even normal-seeming blood stem cells had one or more mutations because the cells were part way through the process of accumulating the mutations and other heritable changes in gene expression to become highly malignant. When the researchers compared mutations in these seemingly normal blood stem cells with the leukemia cells, they could reconstruct exactly which mutations led to the leukemia, and the order in which the mutations arose. They did this by looking for blood-forming stem cells with a single mutation, which they knew must be the first, then finding other stem cells with that first mutation plus one other, which they could then identify as the second. They continued to do this until they found examples of stem cells at each stage of mutation accumulation, leading up to the full set of mutations found in the actual leukemia cell.

The research confirms a once controversial theory. The traditional view has been that any blood cell could turn cancerous if it picked up the “right” mutations. Stanford scientists like Weissman have suggested that, in reality, only blood stem cells could accumulate enough of the those mutations to become cancerous. That’s because when blood stem cells divide into two, one cell retains its

stem cell properties in order to self-renew, while the “daughter” cell continues to divide. The blood stem cells are therefore present throughout life, while the stem cells’ progeny have life spans from days to weeks only.

“The natural mutation rate is slow enough that only the stem cells are around long enough to accumulate all of the necessary mutations and other inherited changes in gene expression to develop the cancer,” said Weissman. “I guarantee that in any room there are people who have blood cells with a cancerous mutation, but it doesn’t matter because in almost every case those cells die out naturally before they get the whole set of mutations that will give rise to an actual leukemia.”

Majeti, who is also a member of the Stanford Cancer Institute and the Institute for Stem Cell Biology and Regenerative Medicine, pointed out that having the correct model of how leukemias arise is important because it helps determine what kind of therapy might be most effective. “Because relapse is a clinical problem, we need to know if chemotherapy has somehow not killed all the leukemia cells, or perhaps it did kill all the leukemia cells, but new leukemias are arising from this pool of stem cells with preleukemic mutations,” Majeti said. “In the first case, we would want to do a better job of killing the leukemia cells, but in the latter case, for some patients it wouldn’t matter how well you do at killing leukemia cells if you don’t eliminate the mutated blood stem cells.” The next phase of the team’s research will focus on answering such questions, he added.

And although the research deals with leukemia, the implications could be much broader, Weissman said. “This confirms the hypothesis that for leukemias, all of the early mutation events occur in blood-forming stem cells, but it opens the possibility that the

same will be true for other cancers, and perhaps all cancers. The progression to the cancer might occur in the normal stem cells of any particular tissue, and the cancer would only emerge as the full set of mutations accumulate."

The leukemia findings are not only significant medically, but also showcase the benefits of conducting interdisciplinary research, said Quake, who located part of his advanced bioengineering research group to Stanford's Lokey Stem Cell Research Building so that studies like this could be done more often. "This research highlights how advances in high technology and advances in stem cell research can complement each other to address difficult problems, such as human leukemias, which could not be answered by technology or medicine alone," he said.

## Anti-CD47 antibody may offer new route to successful cancer vaccination

Scientists at the School of Medicine have shown that their previously identified therapeutic approach to fight cancer via immune cells called macrophages also prompts the disease-fighting killer T cells to attack the cancer.

The research, published in the *Proceedings of the National Academy of Sciences*, demonstrates that approach may be a promising strategy for creating custom cancer vaccines.

Various researchers have been working over the years to create vaccines against cancer, but the resulting vaccines have not been highly effective. Current approaches to developing the vaccines rely on using immune cells called dendritic cells to introduce cancer protein fragments to T cells — a process known as antigen presentation. The hope has been that the process would stimulate the body's T cells to identify cancer cells as diseased or damaged and target them for elimination. However, this process often only modestly activates the most potent cancer-fighting kind of T cell, called killer T cells or CD8+ T Cells.

The Stanford team discovered that there was another viable vaccine approach, using the macrophage pathway to program killer T cells against cancer. Irving Weissman, MD, professor of pathology and of developmental biology, and his team previously showed that nearly all cancers use the molecule CD47 as a "don't-eat-me" signal to escape from being eaten and eliminated by macrophages. The researchers found that anti-CD47 antibodies, which can block the "don't-eat-me" signal and enable macrophages to engulf cancer cells, eliminated or inhibited the growth of various blood cancers and solid tumors.

In the new study, the Stanford team showed that after engulfing the cancer cells, the macrophages presented pieces of the cancer to CD8+ (killer) T cells, which, in addition to attacking cancer, are also potent attackers of  virally infected or damaged cells. As a result, the CD8+T cells were activated to attack the cancer cells on their own. "It was completely unexpected that CD8+T cells would be mobilized when macrophages engulfed the cancer cells in the presence of CD47-blocking antibodies," said MD/PhD student Diane Tseng, the lead author of the study. Following engulfment of cancer cells,

macrophages activate T cells to mobilize their own immune attack against cancer, she said.

The Stanford group plans to start human clinical trials of the anti-CD47 cancer therapy in 2014. The new research provides hope that the therapy will cause the immune system to wage a two-pronged attack on cancer — through both macrophages and T cells. The approach may also give physicians early indicators of how the treatment is working in patients. “Monitoring T-cell parameters in patients receiving anti-CD47 antibody may help us identify the immunological signatures that tell us whether patients are responding to therapy,” said co-author Jens Volkmer, MD, an instructor at the Stanford Institute for Stem Cell Biology and Regenerative Medicine.

The research revives interest in an aspect of macrophages that has been neglected for decades: their role in presenting antigens to T cells. For many years, researchers have focused on the dendritic cell as the main antigen-presenting cell, and have generally believed that macrophages specialize in degrading antigens rather than presenting them. This research shows that macrophages can be effective at antigen presentation and are powerful initiators of the CD8+ T-cell response.

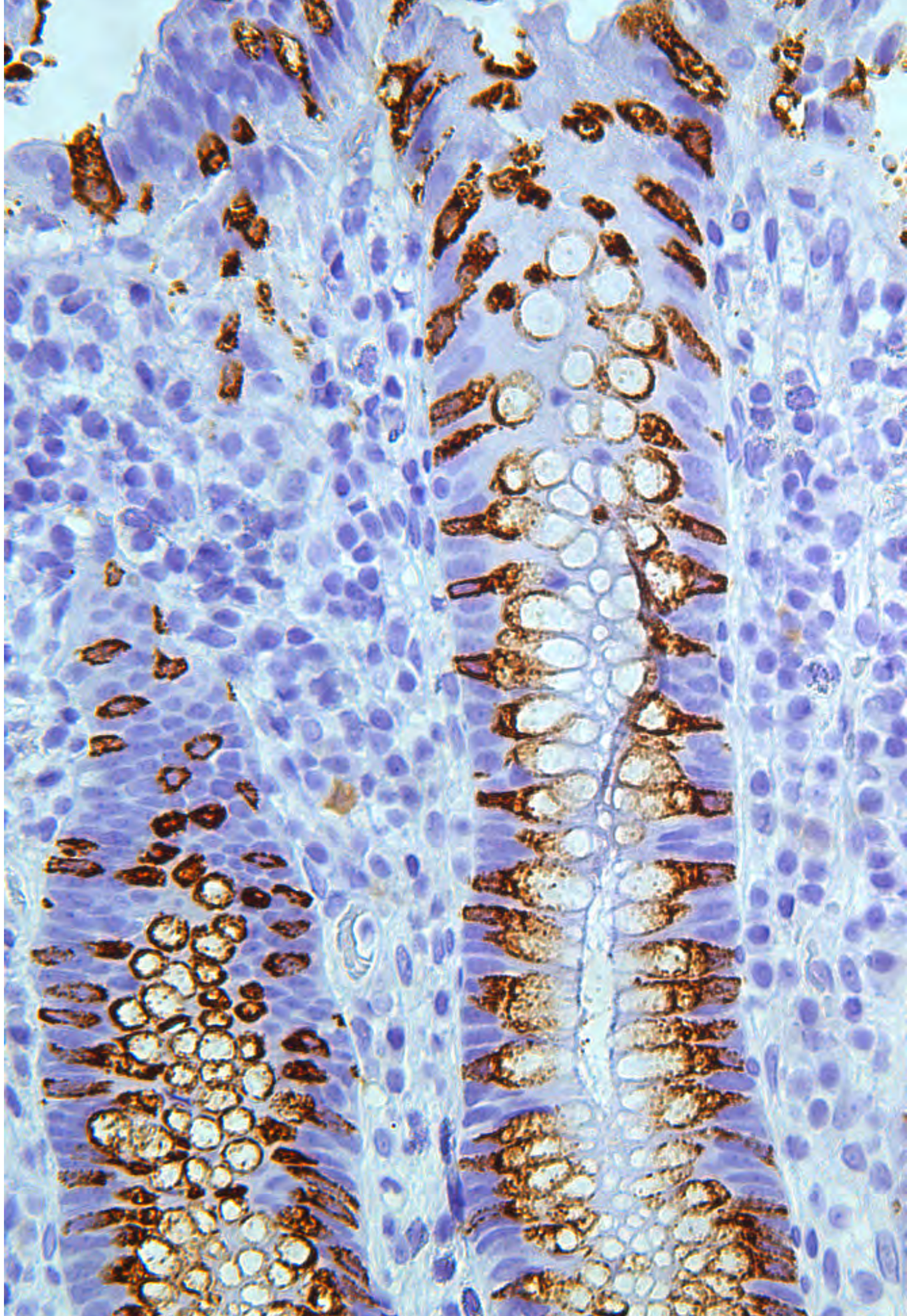
The fact that T cells become involved in fighting cancer as a result of CD47-blocking antibody therapy could have important clinical implications. The antibody might be used as a personalized cancer vaccine allowing T cells to recognize the unique molecular markers on an individual patient’s cancer. “Because T cells are sensitized to attack a patient’s particular cancer, the administration of CD47-blocking antibodies in a sense could act as a personalized vaccination against that cancer,” Tseng added.

# TISSUE-SPECIFIC STEM CELLS

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

For most of our lives, every organ and tissue in the body is regenerated by these tissue-specific stem cells. These tissue specific stem cells are called “adult” stem cells because they are more mature than embryonic stem cells, even though they are also present in children. Learning how these tissue specific stem cells operate will help us bolster our natural regenerative abilities.





## Face facts: Researchers discover long-ignored DNA segments that coordinate early face development

**Joanna Wysocka and Alvaro Rada Iglesias**

The human face is a fantastically intricate thing. The billions of people on the planet have faces that are individually recognizable because every person has subtle differences in the folds and curves that make up every face.

How is the face put together during development so that, out of billions of people, no two faces are exactly the same? Stanford researcher Joanna Wysocka and her colleagues have discovered key genetic elements that guide the earliest stages of the process. Their research, published in the journal *Cell Stem Cell*, provides a resource for others studying facial development and could give insights to the cause of some facial birth defects.

Since there is not enough genetic information in the body to define exactly where each cell will go, development of the face proceeds much like origami: genes provide instructions for folding, crimping, and movement of cells. As with origami, following a set sequence of simple instructions can result in a complex and intricate object.

Wysocka focused on the very first critical fold in the process of making an embryo, when the whole of the embryo is a flat sheet of cells that creases and closes over on itself to make a tube. Much of the tube eventually becomes the foundation of the brain and the spinal column, but one end sets the stage for the formation of the head and face. This process is driven by a small population of remarkable cells called neural crest cells. “We were interested in identifying the portions of the human genome that are responsible for the behavior of the neural crest,” Wysocka says.

What they have discovered is that modification of a collection of DNA sequences called “enhancers” can dial up or down the activity of the genes that govern cells which eventually become the face. It’s


almost as if they have discovered how the instructions for a beautiful piece of origami can be modified—slightly change how a fold is made and you may end up with something very different looking.

Of particular interest is the fact that although these enhancers affect how genes function, enhancers are not genes, nor are they always near the genes they affect. Enhancers exist in the vast non-coding regions of the genome that people used to call “junk DNA,” but which is now proving very important in genetic function. The enhancers can be silent or very active, depending on where a cell is and at what stage it is in the development of the embryo. “What’s really emerging is the idea that one cell type’s junk is another cell

type's treasure," says Wysocka.

What's useful about their discoveries is that researchers will now know much better where to look for the causes of disorders of facial development like cleft lip or cleft palate. In these disorders, sheets of cells from opposite sides of the face do not fuse fully during development, leaving a cleft or gap. "By identifying neural crest enhancers, our study can tell other investigators where to look for genetic variants that can explain these facial abnormalities or even why each human being has a unique face," says Alvaro Rada Iglesias, who is the first author on the paper and a member of the Wysocka laboratory."

Although only a handful of enhancers were already shown to be important in the regulation of early neural crest development, research by Wysocka and her colleagues has produced thousands of such enhancers that are active in determining the behavior of these cells. Moreover, this research showed that the information contained within those enhancers can be used to identify novel genes controlling neural crest and face formation."Our results will serve as a resource for other investigators," she says.

Wysocka expects that the usefulness of the data will extend far beyond facial development. By having the sequences of thousands of these enhancers, scientists can look at the kind of DNA patterns or "motifs" that are common in these enhancers and use that information to look for enhancers that regulate genes throughout development.

Video: DNA enhancers in development

View at http://stemcell.stanford.edu/video/2012.html

3

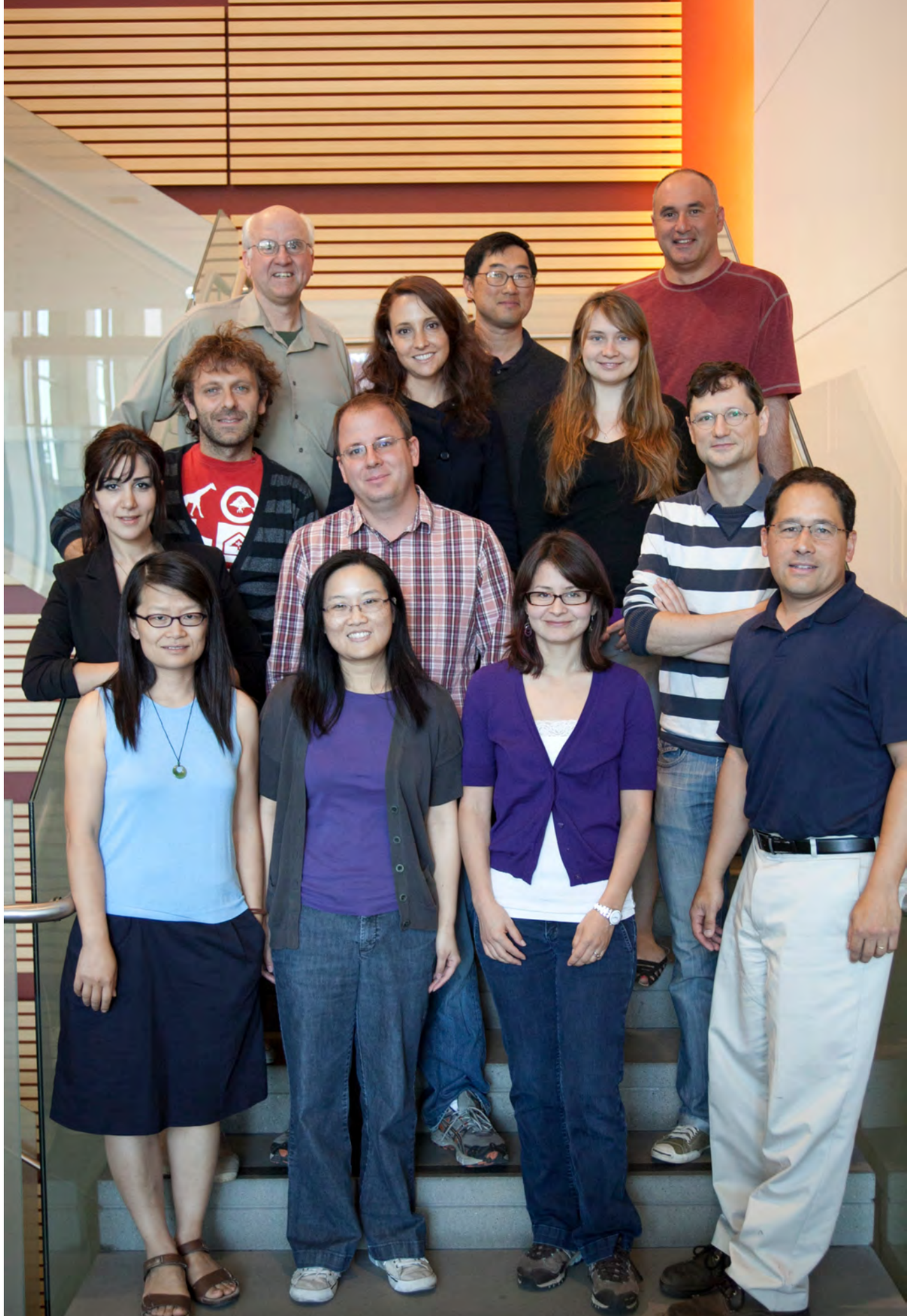
# CIRM DISEASE TEAMS

The California Institute for Regenerative Medicine (CIRM) has awarded large grants to select groups of researchers to accelerate advances on the treatment of specific diseases. These disease teams are designed in such a way that each elite, interdisciplinary team works together to use stem cell-based therapies to make progress on treating these disease within a four-year timeframe. (Photo: a patient with epidermolysis bullosa, a disease that causes skin

# SKIN DISEASE TEAM

Genetic skin diseases constitute a diverse group of several hundred diseases that affect up to two percent of the population. Common diseases include psoriasis and difficulty with wound healing. Patients with one genetic disease, dystrophic epidermolysis bullosa (EB), lack a collagen gene and suffer from debilitating blistering. This extremely painful blistering starts soon after birth and leads to chronic wounds and scarring that can be lethal by young adulthood. The disease is devastating and, despite all researchers' efforts, current therapy for EB is limited to caring for the wounds after blistering has occurred.





The CIRM funded skin disease team is composed of Marius Wernig, MD, assistant professor of pathology, and his colleagues Anthony Oro, MD, PhD, associate professor of dermatology, and Alfred Lane, MD, professor of dermatology. Their goal is to grow EB patients' own skin cells in a culture dish, alter them so that they can make normal collagen again, and then graft them back onto the patients' skin. This approach includes a number of complicated cell culture steps, including initially the generation of induced pluripotent stem (iPS) cells from the skin cells of the individual patient. These iPS cells share with embryonic stem cells the characteristic that they can give rise to all the cells in the body. Their great advantage is that genetic mutations can be fixed in this state.

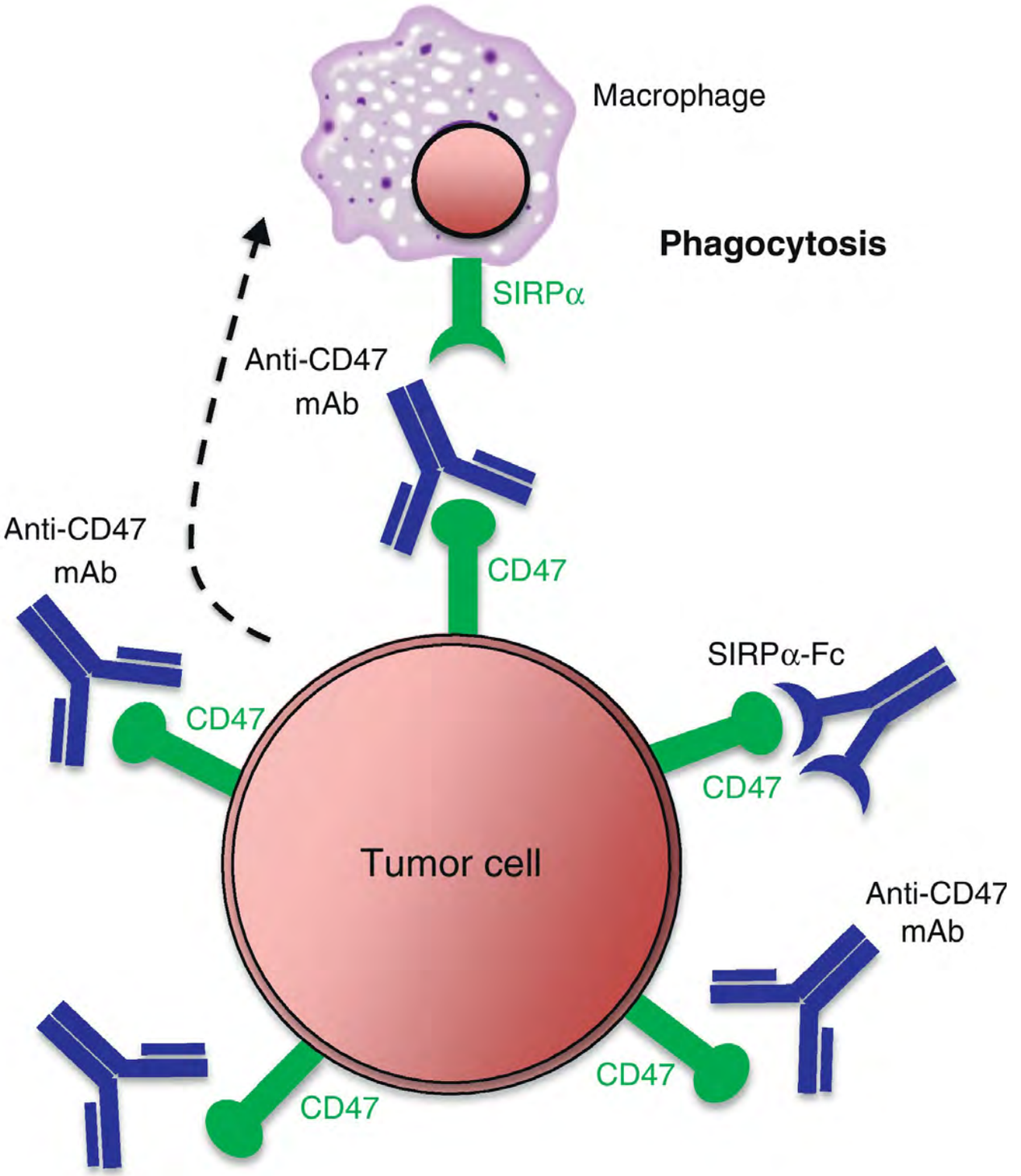
The team has already shown (i) that iPS cells can be generated from patients affected with this particular skin disease, (ii) that the genetic problem causing the disease can be corrected, (iii) that these corrected iPS cells can be differentiated into skin cells that look just like cells directly taken from human skin and (iv) that these iPS cell-derived skin cells can form human skin when transplanted on the back of a mouse. Thus, all the critical steps that are necessary for the development of this cell therapy are worked out. From a scientific point of view, therefore, there are no longer any principle obstacles to clinical application. Consequently, the project is moving now from the discovery to the application mode.

The next critical step is to translate what has been done so far in a research lab into a tightly controlled, FDA-approved manufacturing process. The team expects this process to be at least as difficult and important as the research phase. Safety will always be of the utmost importance in these trials, and the team is now discussing important safety criteria. Once the group and the FDA have come to a consensus on those criteria and the iPS cell-derived skin cells pass those tests, a Phase I clinical trial will be initiated. Thus, the world's first iPS cell-based clinical trial is on the horizon and it will be performed at Stanford.

The researchers' ability to reprogram and replace diseased skin would allow them to use this procedure to develop therapeutic reprogramming approaches for a variety of both common and life-threatening skin diseases. Moreover, genetically corrected pluripotent iPS cells could form the basis of future systemic therapies to treat common genetic disorders of organs other than the skin.

## Video: Cell therapy for Epidermolysis Bullossa


Marius Wernig, MD, talks about the work to create and transplant new skin cells in which the genetic defects involved in EB have been corrected.
View at http://stemcell.stanford.edu/video/2012.html




# CD47 DISEASE TEAM

CD47 is a protein found on the surface of some cells and protects cells from being ingested by the immune system's macrophage cells. This "don't eat me" signal to the macrophage is found on nearly every type of cancer, and is believed to be responsible for protecting cancer stem cells from attack by the immune system. In 2012, researchers at the institute showed that blocking the CD47 signal with anti-CD47 antibodies would shrink or eliminate human cancers in mice.

The CIRM-funded CD47 Disease Team Program is now focused on preparing a human clinical trial of this therapy. The team is producing pharmaceutical grade anti-CD47 antibodies and

structuring the clinical trials, which are slated to begin in early 2014. Through the past year, the team has been engaged in many activities, including:

• 'Humanization' of the antibody: The antibody has been optimized so that it looks like a normal human protein that the patient's immune system will not eliminate because it appears 'foreign' to them.

• Large scale production of the antibody: The team has made arrangements for the production of sufficient quantities of the antibody to complete the laboratory experiments and to move on to clinical safety trials with patients.

• Pre-clinical safety studies: The antibody has been tested in animals to ensure it does not cause serious limiting damage to any of the normal healthy tissues. Thus far, these assessments have revealed no major toxicity hurdles to further clinical development.

• Filing an Investigational New Drug application with the US Food and Drug Administration (FDA) to get regulatory approval to start the human trials.

• Discussions with the National Health Service and the Medical Research Council in the United Kingdom to conduct a simultaneous trial in the UK. With an extensive infrastructure for conducting clinical trials and access to a national population of cancer patients, the UK arm of the clinical trials will be a significant boon to research on the anti-CD47 therapy.

# STEM CELL TRANSPLANTATION

Successful stem cell therapy requires the replacement of diseased or dysfunctional stem cells with healthy ones. These healthy stem cells can come from either a donor or can be stem cells that are modified by gene therapy techniques. One important step in this process of repair and replacement is to eliminate the existing diseased cells so that physical space is created for the healthy ones, and competition for environmental factors that nurture and support the stem cells are removed.



The oldest and most commonly used form of stem cell therapy is bone marrow transplantation (BMT). Thousands of patients undergo BMT yearly to treat cancers or disorders of blood formation. Bone marrow contains many different kind of cells, and only a minority are the blood-forming stem cells. These stem cells are critically important as only stem cells can permanently generate new blood and immune cells. In a BMT, stem cells from a donor replace the recipient's diseased stem cells. Currently, the only way to eliminate the patient's own blood forming stem cells is to treat the recipient to accept donor cells with toxic agents such as radiation or chemotherapy.

The blood stem cell disease team will focus on developing a gentler, safer and more effective method for blood stem cell transplantation. The method involves using anti-CD117 antibodies to clear a space in the blood stem cell niche so that the body will accept donor hematopoietic stem cells. The disease team initially will focus on developing the technique for use in curing severe combined immunodeficiency (SCID). Children with SCID suffer a genetic deficiency that impairs the activity of T and B cells.

The only cure for the condition is bone marrow transplantation, but SCID children are not well equipped to deal with the rigors of chemotherapy and transplantation. An antibody-based approach to depleting blood stem cells, followed by transplantation with a donor's purified blood stem cells, may offer a safer option.

A successful therapy based on this model would open the door to treating a wide range of other diseases. Autoimmune disease such as lupus, multiple sclerosis, type 1 diabetes and others could be cured with a one-time treatment that depletes a patient's own blood and immune cell population and replaces them with donor stem cells.

The greatest causes of organ transplant failure and subsequent

**Autoimmune disease such as lupus, multiple sclerosis, type 1 diabetes and others could be cured with a one-time treatment**

health problems are immune attacks on the transplanted organ and the effects of drugs that suppress that attack. So in the future every organ transplant might be accompanied by a blood stem cell transplant that would induce immune tolerance to the patient's transplanted organ, freeing them from a lifelong need to take immunosuppressant drugs.

If it turns out that therapies with stem cells or iPS cells are vulnerable to rejection by the immune system, as is reported by some researchers, this therapy may be what's needed to induce immune tolerance to those cells, making regenerative medicine possible in the future.

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# STEM CELL THERAPY CENTER

The institute is now preparing to take the next major step in the translation from stem cell science to regenerative medicine. In cooperation with Stanford Hospital, the ISCBRM will be establishing a Stem Cell Therapy Center at Stanford University, a first-of-its-kind facility where tissue-specific stem cells can be purified at an academic medical center in sterile, clinically

appropriate conditions that make them suitable for human therapeutic use.

A stem cell therapy facility will open the door to treating people with a broad range of deadly diseases that are resistant to current therapies. Stanford stem cell scientists have conducted many proof-of-principle experiments in mice demonstrating unequivocally that a one-time treatment with purified stem cells can cure some chronic disorders for life. Diseases that initially can be treated this way are type-1 diabetes, multiple sclerosis, lupus, severe combined immune deficiency (SCID--also called the “bubble-boy disease”), sickle cell anemia, and Mediterranean anemia (thallasemia).

The Stem Cell Therapy Center will also facilitate the resumption of clinical trials to treat cancer patients with purified blood stem cell transplants after high dose chemotherapy. In the original Stanford trial, women with advanced, metastatic breast cancer were treated with very high doses of toxic chemotherapeutic agents in the hope that these high doses would be more effective at killing cancer stem cells than standard chemotherapy. This aggressive chemotherapy has the drawback of killing blood-forming stem cells in addition to the cancer, so the blood-forming system has to be repopulated through a stem cell transplant.

A small group of advanced breast cancer patients were treated in the first clinical trial over fifteen years ago. Of the women whose cancers showed sensitivity to chemotherapy (women who faced less than 5 percent chance of long-term survival with standard treatment) over 30 percent are still alive more than a dozen years later, most with no signs of cancer. The Stanford Stem Cell Therapy Center will play a key role in conducting a much larger clinical trial of this promising therapy.

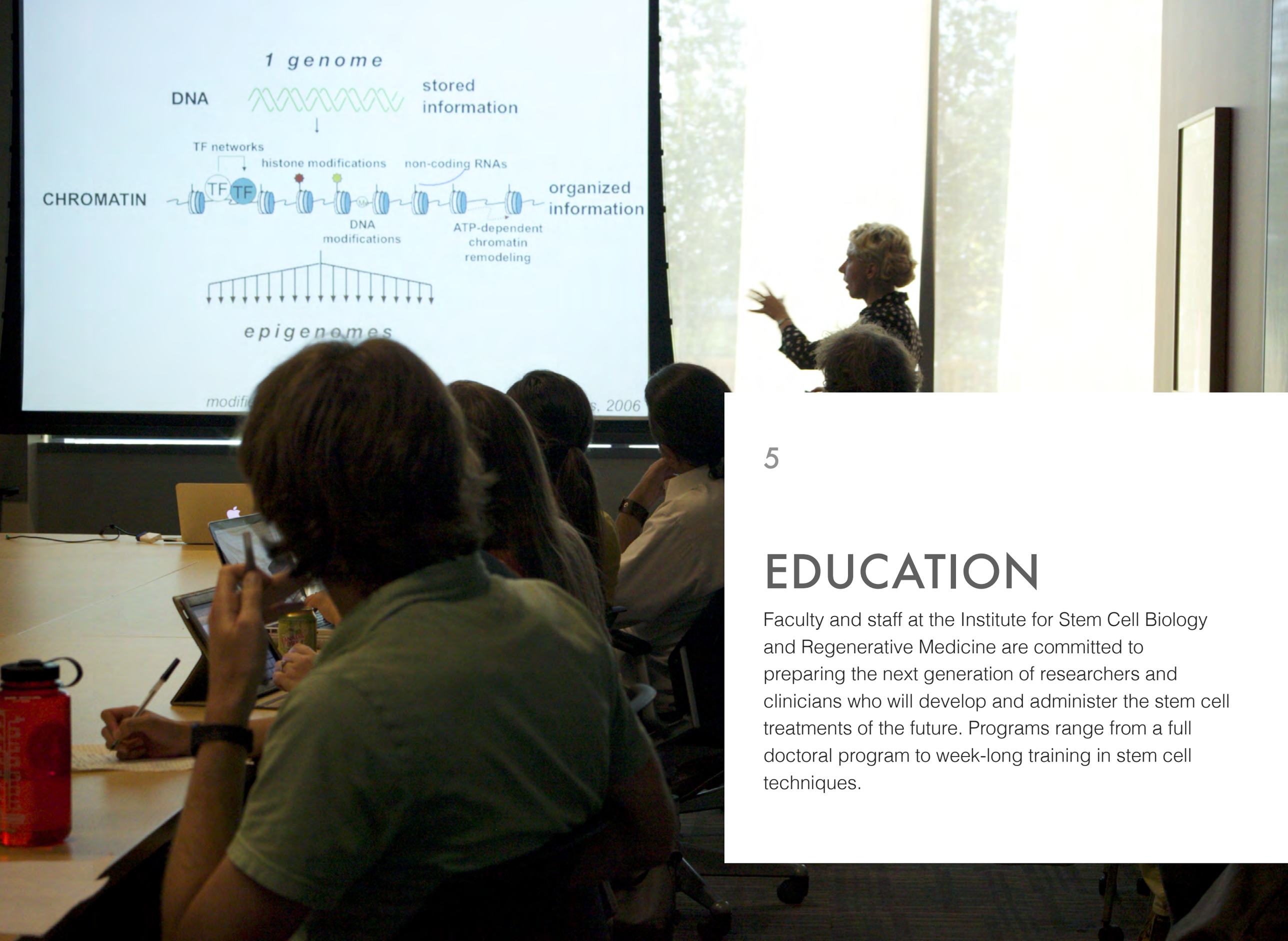
Video: High dose chemotherapy and purified stem cell transplantion for stage IV breast cancer

Ellyn Perez, the first patient given high-dose chemotherapy and purified stem cells for her metastatic breast cancer, talks about her treatment.
View at http://stemcell.stanford.edu/video/2012.html

Transplantation of the heart, lungs, liver, kidneys, skin and many other organs and tissues has become common throughout the world. But failure of the operation is most often the result of an immune system attack on the transplanted organ. Even when the operation is successful, transplant recipients have to take drugs for the rest of their lives to keep the transplanted organ from being rejected. But if the organ transplant is accompanied by a transplant of blood stem cells from the organ donor, tolerance to the new organ is induced, making transplantation operations more successful and freeing patients from lifelong bondage to anti-rejection drugs.

We are now about to enter a new era in which truly regenerative medicine will be a standard part of medical practice. Stem cell researchers at Stanford and elsewhere will continue to discover and purify stem cells for the heart, lungs, vascular system, skin and other important tissues. The Stem Cell Therapy Center at Stanford University will speed the day when treatments for the vast majority of diseases and conditions will include some form of therapy with purified human stem cells.

The new Stem Cell Therapy Center will be housed in the Stanford Hospital.






# EDUCATION

Faculty and staff at the Institute for Stem Cell Biology and Regenerative Medicine are committed to preparing the next generation of researchers and clinicians who will develop and administer the stem cell treatments of the future. Programs range from a full doctoral program to week-long training in stem cell techniques.


Nine of the twelve graduate students who make up the first class of the Interdepartmental Graduate Program

# SCBRM INTERDISCIPLINARY GRADUATE PROGRAM

In 2012, the institute admitted the first class in its new interdisciplinary doctoral program in stem cell biology and regenerative medicine. Of the twelve students offered spots in the program's first class, ten quickly accepted, an acceptance rate that is far higher than is experienced by most doctoral programs.

The foundation of a doctoral program in stem cell biology and regenerative medicine is a recognition of the unique perspectives, orientation and training inherent to the discipline. While developmental biology is concerned with the development of tissue, the field of stem cell biology and regenerative medicine also concerns itself with the renewal of tissues by tissue-specific stem cells and the effects of aging, many of which can be traced to the loss of function of pools of tissue-specific stem cells.

The inclusion of regenerative medicine in the program also recognizes the discipline's interest in moving basic science findings from the laboratory into the clinic.

# hESC STEM CELL TRAINING

The Center for Human Embryonic Stem Cell Research and Education (hESC) is dedicated to expanding stem cell knowledge among scientists and individuals. Their new, state-of the-art facility is optimized for stem cell training, and they offer several basic and advanced laboratory courses throughout the year.

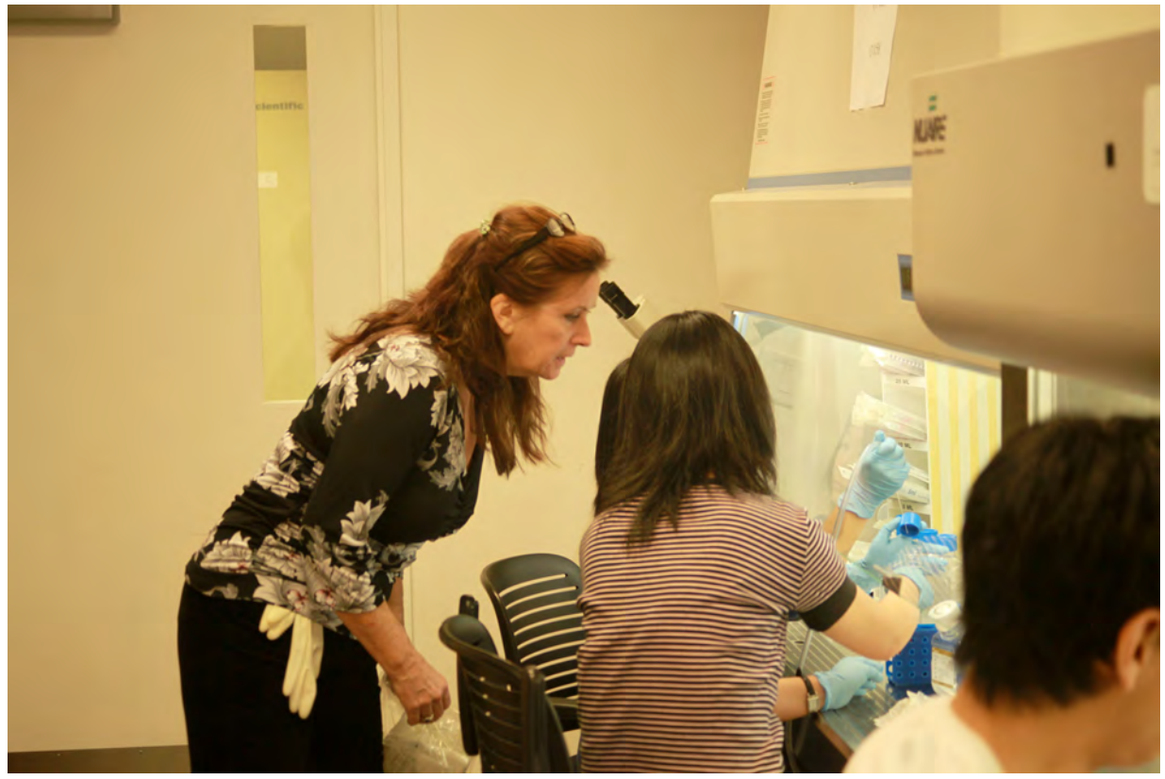
Demand for these courses has been overwhelming. Currently, courses are offered free of charge through a grant from the California Institute for Regenerative Medicine (CIRM) and



therefore can only take students from institutions receiving CIRM funding. Now, however, hESC is advancing plans to offer the course on a fee basis or as part of a partnership with other research institutions, thereby expanding the pool of people who are eligible to take the training. hESC has a facility for iPS cell (induced pluripotent stem cell) derivation from consenting patients. Most courses include some instruction in iPS technology, but laboratories that desire training in deriving human iPS cells can request a special training session. Courses offered are:

**Basic hESC Biology**: This course is one of the central components of the program. It provides the essentials of hESC biology to individuals with little or no previous experience with hESCs. Students learn the basic techniques required to culture, differentiate and analyze hESCs. Students leave the laboratory with basic protocols, appropriate frozen feeder cell preparations for several months of experiments, and established relationships for further assistance and troubleshooting as they begin their experiments in their own designated hESC laboratory space.

More recently the hESC, as part of the Progenitor Cell Biology Consortium (PCBC) of the NHLBI, has started offering courses focused on the differentiation of human pluripotent stem cells to the cardiovascular lineages. The course provides hands-on laboratory experience where students learn a variety of different protocols for the induction and the full characterization of endothelial cells and cardiomyocytes from pluripotent cells.



The hESC has also been instrumental within the newly established PhD program in Stem Cell Biology and Regenerative Medicine. PhD students are exposed to intensive training on stem cell biology and human development. The class is structured in three courses: 1) STEMREM 200, where the students get an intensive preparation on aseptic culture techniques and on maintenance of pluripotent stem cells; 2) STEMREM 201A, a series of lectures held by faculty members and instructors of the Institute focused on the biology of stem cells in the context of human embryonic, fetal and adult development; 3) STEMREM 201B, a laboratory-based course where students learn how to derive hiPSCs from neonatal fibroblasts using modified mRNAs and how to differentiate them into Neural Stem Cells and cardiomyocytes.

6

# PEOPLE AT THE INSTITUTE

## Irving Weissman, MD

**Director**

**Director, Ludwig Center for Cancer Stem Cell Research**

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

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

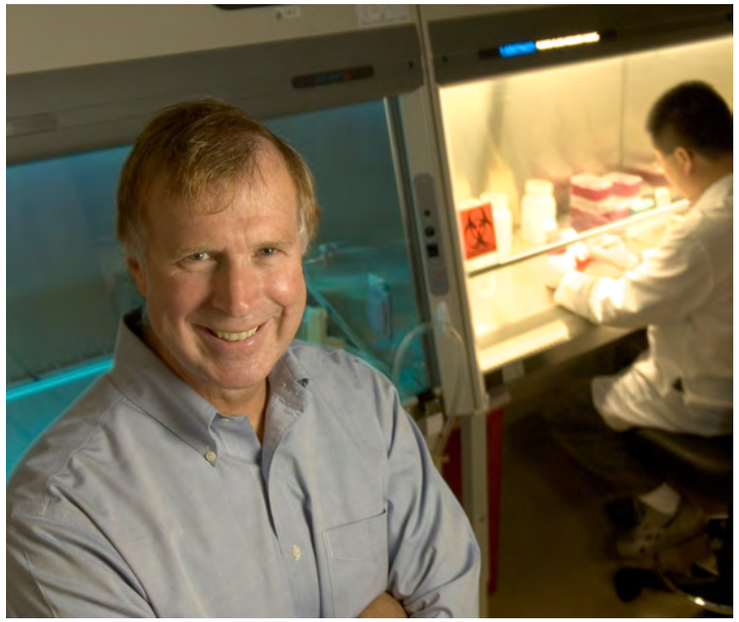
## Michael T. Longaker, MD, MBA, FACS

**Co-Director**

**Director, Program in Regenerative Medicine**

**Deane P. and Louise Mitchell Professor in the School of Medicine**

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



## Michael F. Clarke, MD

**Associate Director**

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

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

## Renee Reijo Pera, PhD

**Director, Center for Human Embryonic Stem Cell Research and Education**

**Director, Interdepartmental Doctoral Program in Stem Cell Biology and Regenerative Medicine**

Renee Reijo Pera focuses on understanding human embryo growth and development, and on characterizing the basic properties of human embryonic stem cells, especially programming and reprogramming in the human embryo and the human germ line. Studies have applications in basic science and in models of human disease including induced pluripotent stem cells and Parkinson's Disease. Her work has fundamentally changed the understanding of human preimplantation embryo development. Her laboratory has established techniques for differentiation of human embryonic stem cells to germ cells and somatic lineages, allowing genetic manipulation of these differentiation pathways.

## Philip A. Beachy, PhD

Philip Beachy studies the function of Hedgehog proteins and other extracellular signals in morphogenesis (pattern formation) and in injury repair and regeneration (pattern maintenance). The Beachy lab studies how the distribution of such signals is regulated in tissues, how cells perceive and respond to distinct concentrations of signals, and how such signaling pathways arose in evolution. He also studies the normal roles of such signals in stem-cell physiology and their abnormal roles in the formation and expansion of cancer stem cells.

## Marius Wernig, MD, PhD

Marius Wernig is interested in two major areas of stem cell biology. One focus is the epigenetic reprogramming of somatic cells into pluripotent stem (iPS) cells and this technique's translational applications for regenerative medicine. Another area of interest is the study of self-renewal mechanisms of mammalian neural progenitor cells, with the hope of identifying novel approaches to better understand brain cancer. Recently, he has published notable research on the direct transformation of human skin cells into nerve cells.

## Theo Palmer, PhD

The research of the Palmer lab examines how neural stem cells respond to cues in order to add and integrate new neurons into a functional circuit.

His studies of neurogenesis in the developing brain focus on the influence of maternal health or illness on fetal brain development. Studies of stem cells in the adult focus on the hippocampus, one of the few areas where neurogenesis naturally continues throughout life. The Palmer lab is now able to use human embryonic stem cells and non-embryonic, induced pluripotent stem cells to generate several types of human neurons.

## Ravindra Majeti, MD, PhD

Ravindra Majeti focuses on the molecular characterization and therapeutic targeting of leukemia stem cells in human hematologic disorders, particularly acute myeloid leukemia (AML). The Majeti lab is also interested in developing a similar characterization of normal human hematopoiesis and hematopoietic stem cells. A major focus of the lab is the identification of cell surface molecules preferentially expressed on leukemia stem cells and the development of therapeutic monoclonal antibodies targeting these proteins. Toward this goal, and with Irv Weissman, the lab is actively developing an anti-CD47 antibody for clinical trials in human AML.

## Maximilian Diehn, MD, PhD

Maximilian Diehn's research focuses on cancer stem cell biology and its implications for cancer therapy. He is interested in developing a deeper molecular understanding of cancer stem cells, including identifying pathways and genes important for their survival and self renewal. Additionally, work in the Diehn lab is aimed at overcoming resistance mechanisms to radiotherapy and chemotherapy in cancer stem cells. Dr. Diehn is a radiation oncologist and specializes in the treatment of lung cancer and stereotactic body radiation therapy.

# 2012 PUBLICATIONS

## 2012 Papers

### Beachy

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### Clarke

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**Diehn**

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**Majeti**

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