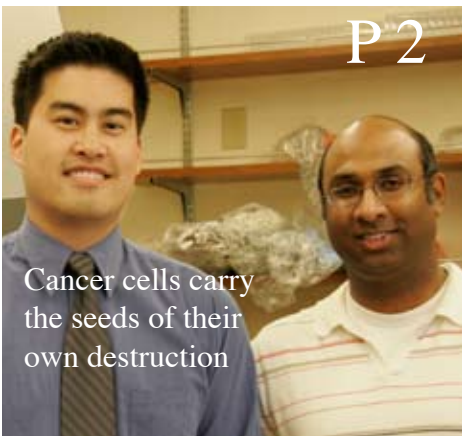


# New Evidence of the Important Role of Stem Cells in Cancer



FEB  
2011



P 2

Cancer cells carry the seeds of their own destruction



Anti-fungal drug slows tumor growth in mice

P 5

## Cancers with gene patterns most like stem cells have poor prognosis

Leukemia patients whose cancers express higher levels of genes associated with cancer stem cells have a significantly poorer prognosis than patients with lower levels of the genes, say researchers at the Stanford University School of Medicine. The finding is among the first to show that the cancer stem cell hypothesis — which posits that some cancers spring from and are replenished by a small, hardy population of self-renewing cells — can be used to predict outcomes in a large group of patients and one day to tailor treatments in the clinic.

“The clinical implications of this concept are huge,” said acting assistant professor of oncology Ash Alizadeh, MD, PhD. “If we’re not able to design therapies to target this self-renewing population of chemotherapy-resistant cells, the patients will continue to have a tendency to relapse.” And yet, although much laboratory evidence exists to support the idea, clinical evidence to support the cancer stem cell hypothesis has until now been sparse.

Alizadeh is a co-senior author of the research, published Dec. 22 in

Continued Page 2

# Stem cell theory of cancer supported

Continued from page 1

the Journal of the American Medical Association. Senior researcher Andrew Gentles, PhD, is the first author. Alizadeh and Gentles teamed up with assistant professor of hematology Ravindra Majeti, MD, PhD, and associate professor of radiology Sylvia Plevritis, PhD, to conduct a retrospective analysis of more than 1,000 patients with acute myeloid leukemia who were treated at centers in the Netherlands, Germany, Japan and the United States including Stanford Hospital & Clinics.

Alizadeh, Majeti and Plevritis are members of the Stanford Cancer Center. Majeti is the other co-senior author.

The cancer stem cell hypothesis has gained increasing credence as researchers from around the world have identified subpopulations of cells in a variety of solid and blood cancers that resist treatment and contribute to relapse in animal models. Eradicating these stem cells is necessary, many believe, for a complete cure. But studies in animals are still several steps removed from proving the idea's worth in humans.

"What's been lacking is clinical evidence that these observations in mice impact actual outcomes in human patients independently of existing prognostic factors," said Majeti. "We wanted to know, 'Do genes associated with leukemia stem cells confer a bad prognosis for a patient?'"

In September, Majeti and Alizadeh showed that targeting a protein called CD47 found on the surface of cancer stem cells in combination with another antibody could eliminate human non-Hodgkin lymphoma in laboratory mice. CD47, which has been dubbed a "don't eat me" signal that protects the cells from elimination by the host's immune system, has also been found on stem cells in several other cancers, and investigations aimed at eventually testing a similar combination antibody therapy in humans are ongoing. In this study, the researchers were interested in learning whether leukemia stem cells play a similarly



Majeti

important role in acute myeloid leukemia, which is one of the most aggressive blood cancers in adults. "We've made very little progress in the treatment of AML over the past 40 years," said Alizadeh. "We're still using the same drugs and therapies we've always used, even though about 70 percent of patients with AML die within five years of diagnosis."

The team used two cell surface markers formerly shown to identify leukemia stem cells to isolate these cells from tumor samples from seven patients. They then compared the overall gene expression patterns of the stem cells to other cells in the tumors and identified a total of 52 genes whose expression varies between the tumor stem cells and non-stem cells. Interestingly, the gene expression pattern is similar to that found on normal blood stem cells, which give rise to blood cells and the immune system. This similarity implies that the cancer stem cells not only can self-renew, but also that they, like normal stem cells, don't divide unless they're needed. Infrequent division may be one way the cancer stem cells escape many conventional treatments that target rapidly dividing cells.

"The stronger the leukemia stem cell signal, the worse the patients did," said Gentles, who is a member of the Stanford Center for Cancer Systems Biology. "Their lives were shorter, they relapsed sooner and they were less able to respond to therapy."



Plevritis

"This finding adds to our clinical confidence that the cancer stem cell hypothesis is important to human disease," said Majeti. "It may also define features of the disease that will help us to determine whether individual patients should participate in clinical trials or if their initial treatment should be more aggressive than the standard approach."

The research was supported by the National Institutes of Health, the Burroughs Wellcome Fund and the Leukemia & Lymphoma Society.

## Calreticulin is an “eat me” signal on cancer cells

Researchers at the Stanford University School of Medicine have discovered that many cancer cells carry the seeds of their own destruction — a protein on the cell surface that signals circulating immune cells to engulf and digest them. On cancer cells, this “eat me” signal is counteracted by a separate “don’t eat me” signal that was described in an earlier study. The two discoveries may lead to better cancer therapies, and also solve a mystery about why a previously reported cancer therapy is not more toxic.

In the study published Dec. 22 in *Science Translational Medicine*, the researchers discovered that many forms of cancer display the protein calreticulin, or CRT, which invites immune cells called macrophages to engulf and destroy them. The reason most cancer cells are not destroyed by macrophages is that they also display another molecule, a “don’t eat me” signal, called CD47, which counteracts the CRT signal.

The characterization of the function of CD47 protein in cancer was previously published by the Stanford scientists. In the earlier work, they reported that an antibody that blocks CD47 could be a potent anti-cancer therapy. They demonstrated that the anti-CD47 antibody could eliminate disease in mice transplanted with human acute myeloid leukemia and cure a large proportion of mice with human non-Hodgkin’s lymphoma when combined with a second antibody. Although the result was exciting, it presented a couple mysteries. “Many normal cells in the body have CD47, and yet those cells are not affected by the anti-CD47 antibody,” said Mark Chao, a Stanford MD/PhD candidate who is first author of the new paper. “At that time, we knew that anti-CD47 antibody treatment selectively killed only cancer cells without being toxic to most normal cells, although we didn’t know why.” The researchers also questioned whether simply blocking CD47 would be enough to bring on a cell’s destruction. “It wouldn’t be likely that killing cells was the default action of the immune system,” said Ravindra Majeti, MD, PhD, assistant professor of



**Mark Chao and Ravi Majeti**

hematology and co-principal investigator on the project. “We postulated that there had to be an ‘eat me’ signal that the cancer cells were also carrying in addition to CD47.” CRT became the leading candidate for this signal because other researchers had previously shown that CRT and CD47 work together to govern a process of programmed cell death called apoptosis.

Indeed, when the scientists looked for CRT they found it on a variety of cancers, including several leukemias, non-Hodgkin’s lymphoma and bladder, brain and ovarian cancers. “This research demonstrates that the reason that blocking the CD47 ‘don’t eat me’ signal works to kill cancer is that leukemias, lymphomas and many solid tumors also display a calreticulin ‘eat me’ signal,” said Irving Weissman, MD, director of the Stanford Institute for Stem Cell Biology and Regenerative Medicine and the study’s other co-principal investigator. “The research also shows that most normal cell populations don’t display calreticulin and are therefore not depleted when we expose them to a blocking anti-CD47 antibody.”

The researchers also found that the most aggressive cancers were the ones making the most CRT. This raises hopes that some of the worst cancers may be the most vulnerable to therapies targeting CD47 and CRT.

**A longer version of this article can be found at:**  
<http://stemcell.stanford.edu/newsletter>

## Stanford joins first US clinical trial of embryonic SCs

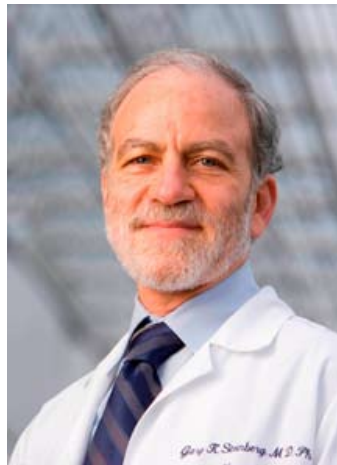
The first clinical trial of cells derived from human embryonic stem cells began in October 2010 in a paralyzed patient at the Shepherd Center in Atlanta. In January, Stanford University School of Medicine and Santa Clara Valley Medical Center became the third site to participate in the trial, which will enroll up to 10 patients with spinal cord injuries at up to seven institutions nationwide.

The FDA-approved, phase-1 trial is meant to test only the safety of the cells, which can develop into neural support cells called oligodendrocytes found in the brain and central nervous system. If the investigational treatment is shown to be safe for use in humans, larger clinical trials will be designed to test whether the cells are better able than conventional treatments to improve a patient's condition.

Because the cells must be administered within two weeks of the initial spinal cord injury, the trial is open only to those with very recent trauma and only upon physician referral.

The trial is run by Menlo Park-based Geron Corp., which developed and manufactures the cells for testing. The cells for this portion of the trial will be thawed and prepared at Stanford, and the surgery to implant the cells will take place at Valley Medical Center, which is one of the largest referral centers for acute spinal cord injury and rehabilitation on the West Coast. Physicians certified to conduct the procedure include Stanford neurosurgeons Gary Steinberg, MD, PhD, and Marco Lee, MD, PhD.

Steinberg is the principal investigator of the Stanford/SCVMC portion of the trial. He has been researching the use of stem cells in models of neurological injury or illness for more than a decade, and he heads a team



**Gary Steinberg**

of Stanford researchers that received a \$20 million grant from the California Institute for Regenerative Medicine to study how neural stem cells could be used to treat people with ischemic stroke — a condition that occurs when a portion of the brain is deprived of blood flow.

“Until recently, we have not had any hope of restoring neurological function in people with spinal cord injury or stroke, or those with brain tumors or Alzheimer’s disease,” said Steinberg, the Bernard and Ronni Lacroute-William Randolph Hearst Professor in Neurosurgery and Neurosciences. “But now we’re moving stem cell therapy into the clinic, which I feel is a tremendously important step. People are not mice or rats, and we can learn so much from clinical trials that we can never learn by studying animals.”

Steinberg and Stanford have a strong background in stem cell biology, while Santa Clara Valley Medical Center has the patients and an extensive rehabilitation facility for spinal cord injuries.

“Valley Medical Center treats more than 150 people a year with severe spinal cord trauma, many of which are caused by motor vehicle accidents, falls and sports injuries. Patients with this kind of injury are very familiar to our staff, and we have the training and experience to help them through their recovery,” said Stephen McKenna, MD, chief of SCVMC’s Rehabilitation Trauma Center. “By collaborating with Stanford, we can evaluate and enroll patients in the trial without altering their normal referral pathway.”

The strict timeline of the trial makes it impossible to predict in advance how many, if any, patients may be treated at each of the participating sites. Essentially, each site will prepare for the possibility that a person with a recent spinal cord trauma that meets the criteria will be referred to their center and will agree to participate in the trial. For more information about the study, including the major eligibility criteria, please refer to ClinicalTrials.gov at:

<http://clinicaltrials.gov/ct2/show/NCT01217008>.

**A longer version of this article can be found at:**  
<http://stemcell.stanford.edu/newsletter>

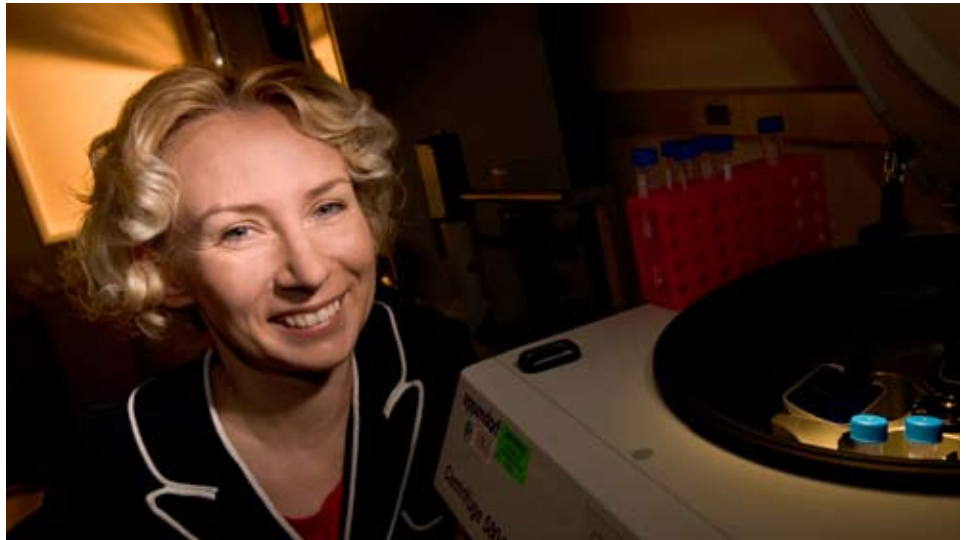
## Study identifies multitude of genetic regions key to embryonic stem cell development

More than 2,000 genetic regions involved in early human development have been identified by researchers at the Stanford University School of Medicine. The regions, called enhancers, are responsible for triggering the expression of distant genes when embryonic stem cells begin to divide to form the many tissues of a growing embryo.

“This is going to be an enormous resource for researchers interested in tracking cells involved in early human development,” said Joanna

Wysocka, PhD, assistant professor of developmental biology and of chemical and systems biology. “It will be very interesting to learn how these enhancers affect gene expression in each cell type.” Wysocka is the senior author of the research, published online Dec. 15 in *Nature*. Postdoctoral scholar Alvaro Rada-Iglesias, PhD, is the first author of the study.

The researchers also learned something interesting about human embryonic stem cells: They’re not too shabby at planning ahead. The cells prepare for the demands of future embryonic development by priming a subset of enhancers for activation with proteins and chemical tags. These “poised” enhancers are simultaneously kept in check by other modifications that keep them inactive. When the modifications (also known as epigenetic changes) are removed, the enhancers can quickly trigger the expression of genes needed to toggle from a mere stem cell to a developing embryo. The identification of the previously unknown enhancers, and the discovery of how they’re kept quiet until needed, represent a moment of research serendipity. Wysocka and Rada-Iglesias didn’t start out trying to identify enhancers involved in development. Instead,



**Johanna Wysocka**

they were looking for regions that activated genes involved in the maintenance of the embryonic stem cell state.

“We are interested in understanding how genomic information is integrated with epigenetic changes to produce cell-type-specific regulation — in this case in the embryonic stem cells,” said Wysocka. “Often this regulation is accomplished via gene activation mediated by a distant enhancer.”

In addition to Rada-Iglesias and Wysocka, other Stanford researchers involved in the work include postdoctoral scholars Ruchi Bajpai, PhD, and Samantha Bruggmann, PhD; senior research scientist Tomek Swigut, PhD; and medical student Ryan Flynn.

The work was supported by the California Institute for Regenerative Medicine, the W.M. Keck Foundation and the European Molecular Biology Organization. Information about Stanford’s Departments of Developmental Biology and of Chemical and Systems Biology, which also supported the research, is available at <http://devbio.stanford.edu/> and <http://casb.stanford.edu/>.

A longer version of this article can be found at: <http://stemcell.stanford.edu/newsletter>

## Mouse model for Duchenne muscular dystrophy implicates stem cells, researchers say

For years, scientists have tried to understand why children with Duchenne muscular dystrophy experience severe muscle wasting and eventual death. After all, laboratory mice with the same mutation that causes the disease in humans display only a slight weakness. Now research by scientists at the Stanford University School of Medicine, and a new animal model of the disease they developed, points a finger squarely at the inability of human muscle stem cells to keep up with the ongoing damage caused by the disorder.

“Patients with muscular dystrophy experience chronic muscle damage, which initiates a never-ending cycle of repair and wasting,” said Helen Blau, PhD, the Donald E. and Delia B. Baxter Professor and a member of Stanford’s Institute for Stem Cell Biology and Regenerative Medicine. “We found that in mice the muscle stem cells can keep up with the demands on them to cycle.”

The difference is caused, the researchers found, by the fact that mice have significantly longer protective caps on the ends of their chromosomes. The caps, called telomeres, allow the cells to continue to divide and replenish the damaged muscle long after the human cells have reached their capacity for division.

The research marks the first time that muscular dystrophy has been shown definitively to be a stem-cell-based disorder, according to the scientists, who also generated the first-ever mouse model of Duchenne muscular dystrophy that closely mimics the human disease. Similar to human patients, the animals exhibit severe muscle weakness and shortened life span. The mouse model will allow clinicians and researchers to better study the disease and test new therapies.

“The results suggest that treatments directed solely at the muscle fiber will not suffice and could even exacerbate the disease. The muscle stem cells must be taken into consideration,” said Blau. Former postdoctoral fellow Jason Pomerantz, MD, co-corresponding author and now an assistant professor at the University of California-San Francisco, said, “if a treatment does not replenish the stem cell compartment, it will likely fail; it would be like pushing the gas pedal to the floor when there is no reserve.”

Blau is the senior author of the research, published online Dec. 9 in *Cell*.

A longer version of this article can be found at: <http://stemcell.stanford.edu/newsletter>

---

## hESC Classes in iPS Cell Techniques

The Center for Human Embryonic Stem Cell Research and Education (hESC) holds occasional, multi-day training classes in induced pluripotent stem cell technology and techniques. The course includes detailed lectures and practical protocols for deriving iPS cells, although there will be no hands on laboratory instruction. The class are free of charge and open to researchers associated with CIRM-funded institutions.

Apply at:  
<http://hesc.stanford.edu/education/apply.html>



## CIRM NEWS

News and Information from the  
California Institute for Regenerative Medicine

# CIRM awards six Stanford researchers \$10.5 million to remove roadblocks to stem cell research

Six Stanford University researchers have been awarded a total of \$10.6 million to address technical bottlenecks in the progress of stem cell science and aid in the translation of stem cell therapies to the clinic. The awards are part of \$32 million granted to seven not-for-profit and three for-profit institutions by the governing board of the California Institute for Regenerative Medicine during a meeting Jan. 27 in San Francisco.

The three-year grants represent the second round of the institute's Tools and Technologies Awards. "These awards are a crucial component of CIRM's commitment to accelerate the development of stem cell-based therapies for people of the world," said Alan Trounson, CIRM president. "CIRM funds all stages of therapy development, from basic research to translational awards, but any of these could be stalled by technological bottlenecks. In funding these innovative tools and technologies, CIRM is removing those barriers before they can delay cures."

Stanford researchers who received the awards include:

**Michele Calos, PhD**, professor of genetics, who received \$1.6 million to develop cellular models of Parkinson's disease.

**Ricardo Dolmetsch, PhD**, associate professor of neurobiology, who received \$1.9 million to develop an in vitro model for a rare form of autism.

**Sarah Heilshorn, PhD**, associate professor of materials science and engineering, who received \$1.4 million to optimize the use of three-dimensional hydrogels to make growing stem cells less costly and more efficient.

**Brian Rutt, PhD**, professor of radiology, who received \$1.9 million to work out new ways to label transplanted stem cells for tracking within the body.

**Marius Wernig, MD**, assistant professor of pathology, who received \$1.9 million to generate functional neurons from the skin cells of patients with a variety of brain diseases including schizophrenia, depression and autism.

**Irving Weissman, MD**, professor of pathology and of developmental biology and director of Stanford's Institute for Stem Cell Biology and Regenerative Medicine, who received \$1.9 million to devise ways to use antibodies to isolate specific populations of tissue-specific stem cells from a mixture of differentiated embryonic stem cells. With these grants, Stanford has now received a total of about \$186.5 million from CIRM.

CIRM was established in November 2004 with the passage of Proposition 71, the California Stem Cell Research and Cures Act. The statewide ballot measure provided \$3 billion in funding for stem cell research at California universities and research institutions and required setting up the agency, CIRM, to oversee allocation of the money.

To date, CIRM has awarded 364 grants worth more than \$1 billion in 18 rounds of funding.