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Fall 2011

Stem Cell Lines

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INSTITUTE

Hope for Spinal Muscular Atrophy Patients

There have been scientific studies with more scintillating, attention-grabbing titles, but "A screen for regulators of survival of motor neuron protein levels," contains encouraging news for the parents of children suffering from spinal muscular atrophy (SMA), as well as for HSCI's Therapeutic Screening Center.

SMA is a devastating genetic motor neuron disease, functionally very similar to amyotrophic lateral sclerosis (ALS), which is also known as Lou Gehrig's disease. SMA affects newborns and young children and claims the lives of 70 percent its victims before their third birthday. At this time there is no effective treatment or cure for SMA, which strikes about 1 in every 10,000 children born in the US.

At this point, two important things are known about the course of SMA — it occurs when levels of a ubiquitous protein called survival of motor neuron (SMN) drop to levels of 25 percent or less of normal, leading inexorably to the death of the patient; and, drugs that elevate SMN may be effective in treating the disease.

Led by Lee Rubin, PhD, Director of HSCI's Therapeutic Screening Center and a professor in Harvard's Department of Stem Cell and

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B.D. Colen/Harvard University

Lee Rubin, PhD, Director of the HSCI Therapeutic Screening Center

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Hope...

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Regenerative Biology, HSCI researchers are using motor neurons derived from stem cells to screen for drugs that may treat the disease.

"We've been looking for small molecules, drug-like molecules, that could be used to treat this in kids by elevating the levels of the survival of motor neuron protein," Rubin said.

"We did a very sophisticated and difficult screen" in HSCI's Therapeutic Screening Center, a facility more often found in a pharmaceutical company research center than in an academic institute, "which involved growing motor neurons derived from stem cells in culture, and imaging them using an automated high-throughput screening microscope. We were looking for any molecule that would raise the level of SMN," Rubin said.

The experiments resulted in the identification of approximately 150 molecules which raised the levels of SMN, and the team is now focused on subsets that are the most drug-like.

"In the paper in *Nature Chemical Biology* we described one of these drug-like substances that acts as a kinase inhibitor. It not only causes the level of SMN to stabilize but it increases it over time," Rubin said. "And when that happens, it does just what we were hoping for — it keeps alive motor neurons that would otherwise die."

"The next steps in this project are to start testing this compound and others in mouse models of spinal muscular atrophy, and then we would be very excited to start moving some of these substances into clinical trials," Rubin said.

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Harvard Stem Cell Institute

The Harvard Stem Cell Institute (HSCI) is a scientific collaborative established in 2004 to fulfill the promise of stem cell biology as the basis for the cure and treatments for a wide range of chronic medical conditions. HSCI is a unique enterprise that unites experts across the disciplines, schools, and departments of Harvard University and all its affiliated hospitals. HSCI depends on the vision and generosity of individuals, foundations, and corporate donors to carry on its research, training, and programs. Extensive information about HSCI may be found on our website: www.hsci.harvard.edu.

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Disease Program Annual Meetings Convene

Cross-institution, cross-lab, cross-disciplinary interactions are key to the success of HSCI's disease programs. To spur these interactions, each program holds an annual "think tank" — a gathering of researchers to discuss their latest findings, assess the state of the science, and define the priorities of the program. Each program does this in its own way.

This year, the Cancer Program organized a meeting, limited to a small group of junior faculty, with the goal of identifying the bottlenecks in the research on cancer stem cells and to solicit proposals on the program's projects. One of the outcomes was to issue a call for proposals aimed at identifying critical genes and pathways that sufficiently distinguish cancer stem cells from normal stem cells and hence serve as candidate targets for therapy. As a result, four new projects were just approved by the Executive Committee.

The Cardiovascular Program took a slightly different focus and looked at approaches, tools, and techniques that researchers could share across labs within the program with the ultimate goal of regenerating myocardium and treating heart failure. Eleven investigators gave scientific presentations, not aimed this time at showing the latest data generated in their labs, but at sharing an approach or technology that worked well in their experiments. Many young researchers, working in the different labs affiliated with HSCI, attended the meeting and numerous new collaborations were established as a result of this interaction.

Following the success of several previous think tanks, this year the Nervous System Diseases Program focused its meeting on "development and routes to regeneration of corticospinal to spinal motor neuron circuitry." The meeting included a half-day symposium open to all participants during which local, national, and international speakers showed their latest results and laid out the main unanswered questions in the field. A subset of the group spent the next day and a half in closed interactive sessions considering the state of the field and developing ways to move forward collectively.

The Blood Program's symposium focused on the science of chromatin remodeling with Constanze Bonifer, PhD, from the University of Leeds (UK), as a distinguished guest. The event, open to laboratories working on blood research, saw four speakers from HSCI labs — Benjamin Ebert, MD, Alexander Meissner, PhD, Peter J. Park, PhD, and Yang Shi, PhD, — present their latest related work. The subsequent poster session featuring work from many more labs allowed other junior scientists to present their latest results and interact with more senior investigators.

As one of the think tank participants remarked, "New topics, experimental techniques, even whole sub-areas of the field were introduced to me which I would not have been exposed to merely by talking with individuals in my own group or department."

"Imprinted" Developmental Genes Gain New Roles in Adult Stem Cells

New work by HSCI Principal Faculty member Carla Kim, PhD, and colleagues suggests that a network of genes crucial in embryonic development may also keep a tight rein on adult stem cells in the lung and other tissues, particularly as these cells rally to repair tissue damage. The findings are the first to link this set of genes, called an "imprinted gene network," to tissue repair, and suggest that these genes may play fundamental biological roles in maintaining the "stemness" of adult stem cells.

The repair of tissues damaged by injury or illness relies on the ability of adult stem cells, including those in the lung (called bronchioalveolar stem cells, or BASCs) to grow and self-renew. But this ability needs to be tightly controlled; if regulation is lost, the stem cells may instead give rise to cancer.

Kim's discovery, published in the journal *Cell Stem Cell*, came about when studying Bmi1, a protein known to regulate adult stem cell function and tumor cell development in many organs. "Bmi1 is required for many kinds of adult stem cells to renew themselves," Kim explained, "and its expression is an essential factor in some cancers, including lung tumors."

To understand the full scope of Bmi1's influence over BASC renewal, Kim's laboratory made use of a mouse previously engineered to lack the protein. "We were surprised to find that the loss of Bmi1 lead to overexpression of a network of imprinted genes in lung stem cells," Kim said.

Every person's genome harbors two copies of every gene, one from their mother and one from their father. For the most part our cells use both copies equally, but in the case of "imprinted" genes (which constitute a small percentage of our genome), our cells only use one copy and mute the second.

The network that Kim and her team studied comprises 14 imprinted genes that are all active in certain tissues during embryonic development. The activity of these genes — each of which individually impacts other pathways — dials down as we reach adulthood.

To understand the importance of these genes in BASC function, Kim's team measured their expression in a model of lung injury. Imprinted genes from normal lung stem cells, and in particular one gene named p57, showed a distinct expression pattern where activity decreased shortly after injury, peaked a few days later, and dropped back to baseline levels once repair was complete. Without Bmi1, expression of these genes remained high even weeks after injury.

"In normal BASCs, the pattern of expression suggests that Bmi1 and this imprinted gene network make sure that when the lung stem cells are called in to repair an injury, they stop when the repair is complete," Kim said. "This is the first time anyone has found a link between imprinted genes and tissue repair."

B. D. Colen/Harvard University

Carla F. Kim, PhD, Leader of the Junior Faculty Cell Regulation Program

The results imply that Bmi1 acts as a second layer of control that fine-tunes the expression of imprinted genes. "We think Bmi1 helps make sure the active copies of these imprinted genes are only expressed just enough, and are turned off when the stem cell needs them to be completely silent," Kim explained. "If the imprinted genes are expressed too much or too little, the lung stem cells can't self-renew."

The findings also reveal a hitherto unknown role for imprinted genes in regulating the growth of adult stem cells from the lung and potentially other tissues as well, adding them to the list of "stemness factors" that help stem cells maintain their unique capabilities.

Insights on Somatic Cell Nuclear Transfer

Five years after first gaining institutional permission to attempt to produce stem cell lines via somatic cell nuclear transfer (SCNT), two Harvard researchers and a former Harvard postdoctoral fellow have closed the loop with a flurry of new studies and a commentary.

In papers in *Nature, Nature Communications*, and *Cell Stem Cell*, the researchers report the first creation of a stem cell line containing a patient's genome using SCNT; an experiment explaining why other attempts at SCNT have been unsuccessful; and a commentary reporting that it is impractical if not impossible to recruit ova donors without paying them.

Ironically, all three reports serve to underscore how astoundingly fast the field of stem cell science has advanced since 2006, when it looked as though SCNT provided the only path to the creation of disease-specific stem cell lines from patients. Interest in these stem cell lines was high because they could be used for studying disease development, for transplanting to treat diseases, and as tools for the development of conventional drugs.

Since that time, the creation of induced pluripotent stem cells (iPS) and the successful reprogramming of one type of adult cell into another type have provided researchers with alternatives to SCNT, and decreased interest in it. However, because it is still too early to know which approach will prove the most useful, groups have continued their research efforts in SCNT as well.

In their commentary in *Cell Stem Cell*, HSCI Co-Director Douglas Melton, PhD, and HSCI Principal Faculty member Kevin Eggan, PhD, report that without compensating donors — a practice restricted by Massachusetts law — they "could not recruit enough egg donors to enable controlled nuclear transfer experiments."

In fact, Melton and Eggan report that although 239 women responded to advertisements seeking ova donors, and 79 met all the study requirements, only one woman ultimately donated eggs, and the attempt at SCNT using those eggs failed. If SCNT research is to be successful, Melton and Eggan argue, researchers must be able to adequately compensate donors for the time, risks, and inconvenience involved in the donation process.

Because they were unable to recruit ova donors, Eggan, Melton, and Dieter Egli, PhD, who at the time was a postdoc in Eggan's lab at Harvard, attempted to perform SCNT using zygotes (very early stage embryos) donated by couples undergoing fertility treatment, from which the nucleus was removed and replaced with genetic material from patient skin cells. The researchers report in *Nature Communications* that though this procedure was successful using mouse zygotes, producing stem cells within hours of nuclear transfer, all attempts using human zygotes failed. What this suggests, they say, is that there is an as yet not understood natural barrier to human cellular reprogramming after SCNT.

Egli went on to establish his own research group at the independent New York Stem Cell Foundation (NYSCF) laboratory, where he is a senior research fellow. In New York state, lack of an ova donor payment statute made it possible for Egli to compensate donors for their participation and continue his SCNT work.

He now reports in the latest edition of *Nature* that along with his colleagues at NYSCF, Columbia University, and the Naomi Berrie Diabetes Center, the group has produced a stem cell line containing the genes of a diabetic patient, and has also pinpointed the cause of the SCNT method's previous failure.

Egli's work suggests that success was prevented due to aspects of one of the phases of the manipulations required during SCNT. Although this finding allowed Egli's group to produce a stem cell line, further work will be required before a precisely matched patient stem cell line can be made.

$1 Million Gift Supports Stem Cell Renewal Research in HSCI's Blood Program

As regulations on stem cell research and current budget crises stymie stable federal funding of this field, HSCI continues to find that the pioneering spirits of individual donors and private foundations are key ingredients in accelerating its mission. A major grant to HSCI's Blood Program by The Amelia Peabody Charitable Fund offers evidence that private sponsorship continues to be not only helpful but also critical to the work we trust will bring more effective treatments and cures.

In September 2010, HSCI received a letter from the Fund inviting it to put forward a proposal for research on "unique properties of adult stem cells" that could be used therapeutically. The HSCI Blood Program was a natural fit for such a request. Despite excellent characterization and four decades of clinical use, bone marrow and cord blood transplants benefit less than half of the patients with blood diseases. This is due in part to incomplete understanding of how stem cells self-renew. The HSCI Blood Program seeks to define the mechanism of self-renewal of blood stem cells by identifying molecular targets that turn on or off the self-renewal program. Turning on self-renewal is critical for expanding stem cells to regenerate damaged tissues, and turning off self-renewal is critical for interrupting the cancer stem cell perpetuation of malignancy; controlling both holds vast therapeutic potential.

A few weeks after submitting its proposal, the HSCI was informed that it was one of two finalists. In late January of this year, the HSCI hosted a site visit by the Trustees of the Fund, who toured two of HSCI's affiliated laboratories and met with the majority of the Principal Investigators on the proposal to gain clarity on the goals and abilities of this cross-institutional group, which includes researchers from four different Boston-based Harvard-affiliated hospitals. One of the main concerns for the Trustees was whether collaboration was truly possible in the highly competitive world of medical research. The HSCI's track record of successful collaboration and the rapport of the Blood Program scientists must have convinced them; in February we learned that the HSCI was being given a $1,000,000 grant for the Blood Program's work on stem cell self-renewal.

From Skin Cells to Motor Neurons:
Researchers Find Success with Direct Cellular Reprogramming

B. D. Colen/Harvard University

A team of HSCI stem cell researchers has succeeded in reprogramming adult mouse skin cells directly into the type of motor neurons damaged in amyotrophic lateral sclerosis (ALS), best known as Lou Gehrig's disease, and spinal muscular atrophy (SMA). These new cells, which researchers are calling induced motor neurons (iMNs), can be used to study the development of the paralyzing diseases and to develop treatments for them.

Producing motor neurons this way is much less labor intensive than having to go through the process of creating induced pluripotent stem (iPS) cells, and is so much faster than the iPS method that it potentially could reduce by a year the time it eventually takes to produce treatments for ALS and SMA, according to HSCI Principal Faculty member Kevin Eggan, PhD, leader of the Harvard team.

The work builds on and advances work by Douglas Melton, PhD, Co-Director of HSCI, who pioneered direct cellular reprogramming, and Marius Wernig, MD, PhD, of Stanford, who used direct reprogramming to produce generalized neurons.

In a paper published in the scientific journal *Cell Stem Cell*, the Eggan team reports that the cells they are calling iMNs appear to be fully functional. When placed in the spinal cord of a chicken embryo, the iMNs settle into the cord and send out their projections to connect with muscles.

"That's a unique thing," Eggan said. "We showed [that] they have contact with muscle cells and make synapses with them."

"When the iMN cells were placed in a lab dish with muscle cells, they made what appeared to be normal contact," Eggan said, "and when we add curare to the dish, that contact stops over time — which is exactly what curare (a paralyzing agent) does in nature; it is an antagonist to the receptors on the muscle cells."

"One of the utilities [of this new method for producing motor neurons] is it makes a much more rapid way to grow motor neurons. This could allow us to test very rapidly whether a new therapeutic is likely to be effective," Eggan said. "Realistically, it takes about a year for us to create an iPS cell line; this [approach] takes weeks."

Explaining how this success was built on previous discoveries and how scientists build on each other's work, Eggan said, "We had been taking fibroblasts from mouse embryos and were able occasionally, rarely, … to turn those cells into motor neurons using a set of factors we developed. We were struggling. And at that moment, Marius Wernig came up with a system for what I would call making generic neurons; they were electrically active, they looked like neurons, but they didn't have the properties you could assign to any neural cell type. But when we combined our factors with his factors, it allowed us to go on and make motor neurons."

B. D. Colen/Harvard University

Douglas Melton Named a University Professor

Douglas Melton, PhD, Co-Director of HSCI, a leading stem cell and regenerative biologist, and one of the driving forces behind Harvard's ascendancy to world leadership in the field, has been named a University Professor, Harvard's highest professorial distinction.

"While the world knows Doug Melton as a scientist who has played a seminal role in the exponential growth of the new field of stem cell science, we at Harvard also know him as an untiring mentor to scientific leaders of tomorrow, and as an academic who is passionate about improving undergraduate education," Faust said.

The University Professorships were established by the President and Fellows of Harvard College in 1935 as a special way to recognize "individuals of distinction…working on the frontiers of knowledge, and in such a way as to cross the conventional boundaries of the specialties."

"I really don't know how to properly express my gratitude to President Faust for this honor," Melton said of his assuming the Xander University Professorship. "But far, far more important than any honor I am being granted is the unstinting support that President Faust, the University, and my colleagues have provided for our efforts to establish Harvard as a global leader in stem cell science and regenerative biology. They have stayed the course in the face of intense political opposition and fiscal difficulties, and without their faith in the science we would never be where we are today."

HSCI/California Junior Faculty Symposium

For the sixth time in as many years, an invited group of HSCI's Junior Faculty started off this academic year by meeting with some of their California colleagues to share their latest findings and new ideas. The group was able to take advantage of the Department of Stem Cell and Regenerative Biology's new meeting space in its renovated labs on the Harvard campus. Scientific equipment company Leica Microsystems and the journal *Science Translational Medicine* joined HSCI in sponsoring the meeting, with the goal of developing collaborations among leading young scientists in the country who share the mission of using stem cell research to find better treatments and cures for a host of degenerative and often fatal diseases.

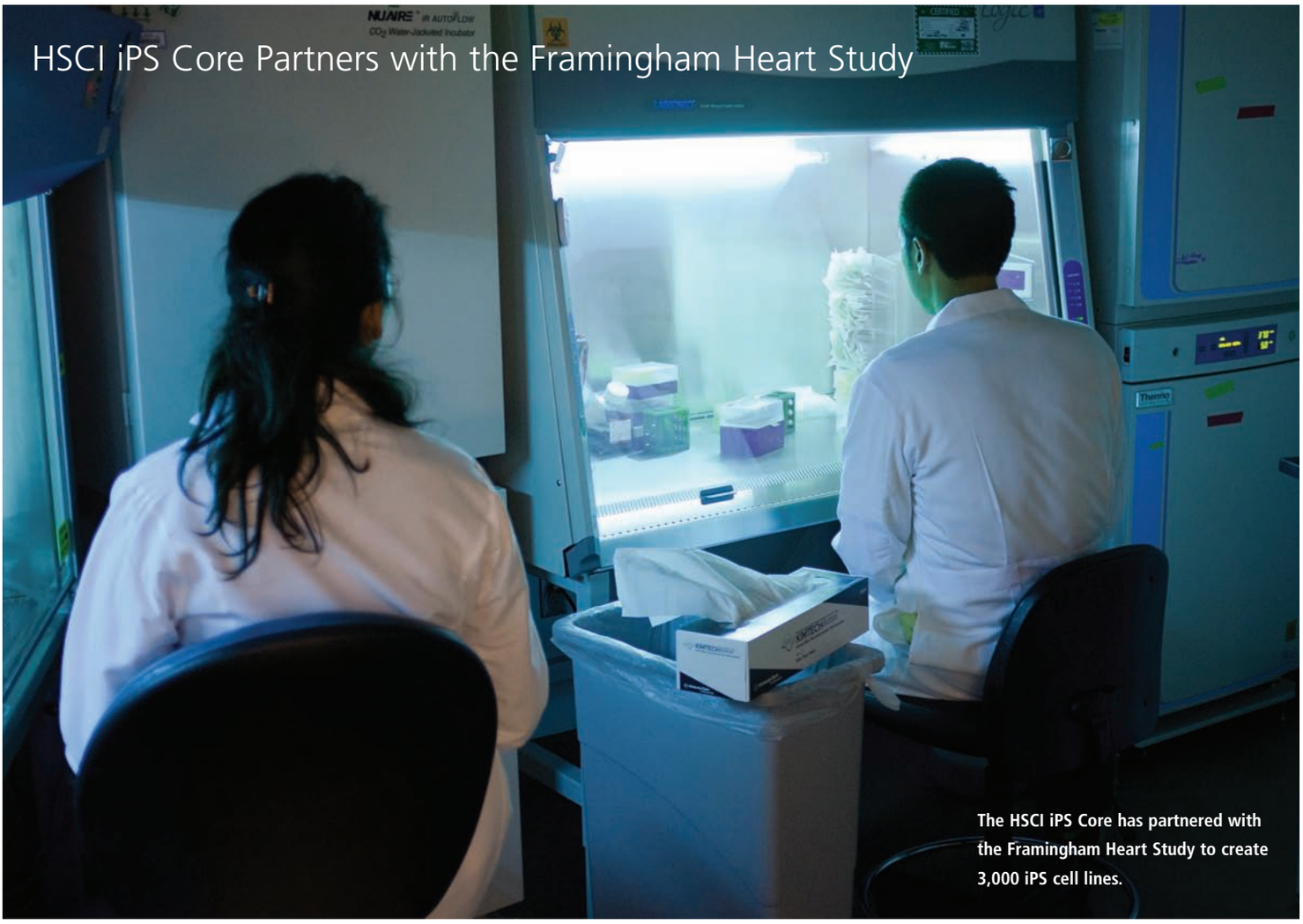
Coordinated by HSCI Principal Faculty members Trista North, PhD, Wolfram Goessling, MD, PhD, and Paola Arlotta, PhD, the two-day program was packed with presentations on current research — much of it not yet published — by 17 HSCI investigators as well as 15 scientists associated with the California Institute for Regenerative Medicine (CIRM) from San Francisco to San Diego. Also in attendance were journal representatives from *Cell Stem Cell, Nature Cell Biology,* and *Science*.

Discussion focused on the major scientific questions in areas such as pluripotency and self-renewal, epigenetics and reprogramming, development and regeneration of an array of organ systems, and a variety of other topics spanning the spectrum of current stem cell science.

Its evening reception was held at Harvard's Arthur M. Sackler Museum and had a private showing of the exhibit: *Prints and the Pursuit of Knowledge in Early Modern Europe*. The exhibit showed how artists contributed to scientific investigation as their images served both as instruments for research and as agents in the dissemination of knowledge.The group saw that although technology has changed in the intervening 500 years many of the underlying questions and challenges have not.

In keeping with the bi-coastal tradition, the 2012 meeting will be held in California and hosted by CIRM.

HSCI iPS Core Partners with the Framingham Heart Study



B. D. Colen/Harvard University

The HSCI iPS Core has partnered with the Framingham Heart Study to create 3,000 iPS cell lines.

Three years ago, when the subject of creating a core facility for the creation, storage, and distribution of induced pluripotent stem (iPS) cells was first discussed at HSCI, Principal Faculty member Chad Cowan, PhD, was skeptical at best.

"I was at the farthest extreme, saying it was crazy," Cowan recalled. "I said no one was going to use it; it wasn't going to be useful to the community; it just didn't make sense to me. My reward, of course, was to be named Director of the Core. And I've learned over the past two-and-a-half years that it's scientifically empowering. It's not only of use to our community, but also to scientists around the world, where we've distributed iPS cell lines to people studying Parkinson's, Alzheimer's, and a number of other diseases."

Recognizing the Core's unique expertise, the National Institute of Health recently funded a five-year collaboration between HSCI scientists and one of the best-known, most productive research endeavors in the world, the Framingham Heart Study (FHS).

Since it was established in 1948 in nearby Framingham, Massachusetts, FHS has made many landmark discoveries about the development and course of the various forms of cardiovascular disease, and has done so the old-fashioned way. By following large populations of patients over the course of their adult lives, examining and re-examining them, keeping track of all their major health-related behaviors, and studying how those behaviors correlate with the development of heart disease, FHS has identified the major risk factors for cardiovascular disease.

The Study began with 5,209 men and women between the ages of 30 and 62, and then in 1971, FHS recruited 5,124 of the original participants' adult children and their spouses to participate in similar examinations. Three thousand members of this offspring-cohort will provide the samples that researchers at the HSCI iPS Core will use to create iPS cell lines, which can then be used to study the molecular basis of diseases and conditions including diabetes, stroke, and heart attack.

As part of the NIH-funded project these newly minted cell lines will first be used in conjunction with the participants' medical histories to answer an important question about the genetics of heart disease.

The FHS is famous for determining the link between bad cholesterol and heart attacks and it is known that as many as 20% of people in the general population carry genes that predispose them toward high bad cholesterol. By using the iPS cells from participants who have either high or low levels of bad cholesterol and turning those cells into liver cells, which are the factories of cholesterol, the researchers will be able to see if the genetic links to high cholesterol have molecular outcomes that can be measured. A measurable molecular change could signal the possibility of a therapeutic target, which could be treated with drugs and help to lower the at-risk patients' bad cholesterol.

"It's a very ambitious plan, but it's exciting to see that the HSCI iPS Core was seen by the NIH as such a quality core and that they gave us an award of such magnitude to partner with the Framingham Heart Study," Cowan said.

HARVARD STEM CELL INSTITUTE

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Distinguished Speaker Forum

HSCI inaugurated its "Distinguished Speaker Forum" during the 2011-2012 academic year. An innovation based on the former "Seminar Series" which HSCI conducted between 2004 and 2011, the Forum will bring up to eight preeminent stem cell scientists to our community each year. While here, our guests will be hosted by one of HSCI's Senior Faculty and will deliver a mid-day public seminar, meet with individual scientists, and participate in the Department of Stem Cell and Regenerative Biology's classes and/or disease program think tanks. Please visit the HSCI website (http://.www.hsci.harvard.edu/evens) for information on seminar locations.

Constanze Bonifer, PhD, hosted by Daniel Tenen, MD - July 21, 2011

Deepak Srivastava, MD, hosted by Richard Lee, MD - September 28, 2011

Hans Clevers, MD, PhD, hosted by Ramesh Shivdasani, MD, PhD - October 13, 2011

Jonas Frisen, MD, PhD, hosted by Jeffrey Macklis, MD - November 8, 2011

Frank Costantini, PhD, hosted by Andrew McMahon, PhD - January 10, 2012

Kenneth Zaret, PhD, hosted by Gordon Weir, MD - February 14, 2012

Owen Witte, MD, hosted by David Scadden, MD - March 27, 2012

Brigid Hogan, PhD, hosted by Carla Kim, PhD - April 10, 2012

Ben Barres, MD, PhD, hosted by Lee Rubin, PhD - May 8, 2012

B. D. Colen/Harvard University

The newly renovated Sherman Fairchild Building.