CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE

STEM CELL RESEARCH: CHARTING NEW DIRECTIONS FOR CALIFORNIA

October 1-2, 2005

Conference Report

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Abbreviations: *bone morphogenic protein, BMP; California Institute for Regenerative Medicine, CIRM; central nervous system, CNS; embryonic stem cell, ESC; extracellular matrix, ECM; fluorescence-activated cell sorting, FACS; Food and Drug Administration, FDA; Good Manufacturing Practice, GMP; graft-versus-host disease, GVHD; graft-versus-tumor, GVT; green fluorescent protein, GFP; Investigational New Drug; IND; lentiviral, LV; leukemia inhibitory factor, LIF; messenger RNA, mRNA; National Institutes of Health, NIH; pre-implantation genetic diagnosis, PGD; pancreatic-duodenal homeobox protein, PDX1; small interfering RNA, siRNA; somatic cell nuclear transfer, SCNT; standard operating procedures, SOPs; University of California, Los Angeles, UCLA; University of California, San Francisco, UCSF*

INTRODUCTION

In November of 2004, California voters strongly endorsed embryonic stem cell (ESC) research by passing Proposition 71, allocating $3 billion over 10 years to California institutions to support this research. Passage of the proposition, now formally known as The California Stem Cell Research and Cures Act, called for formation of the California Institute for Regenerative Medicine (CIRM) to disburse state funds in support of stem cell research and other vital technologies for the development of therapies and diagnostics for disease and disability. The proposition specifically states that preference be given to research that cannot be funded by the National Institutes of Health (NIH). Achieving the ambitious goals of the proposition requires a thoughtful and effective plan for expenditure of funds that will optimize the use of the scientific resources of California and give maximal scientific and medical benefit. This meeting, whose goals were to assess the current scientific challenges and opportunities in the field of stem cell biology and to recommend priorities for CIRM, is the first step toward realizing that plan.

Inspired by [the] possibilities … we are setting out on an arduous journey of discovery leading to therapy.

– CIRM President Zach W. Hall

In October 2005, scientists, patient advocates, journalists, representatives of public interest groups and members of the public converged in California to consider the pertinent scientific issues facing stem cell research. The ambitious two-day symposium – entitled *Stem Cell Research: Charting New Directions for California* – focused on the science of human ESC research, rather than on the political and ethical issues surrounding it. The president of CIRM, Dr. Zach Hall, outlined the mandate for the meeting when he articulated in his opening remarks that CIRM seeks "ideas for new projects, new approaches, new resources, new ways of organizing scientific efforts." He inquired, "What are the opportunities to be seized as quickly as possible?", and "What are the pitfalls to be avoided?" By inviting national and international experts to offer their advice, CIRM hoped to identify scientific opportunities that would significantly advance the field and expedite the development of stem cell-based therapies and diagnostics.

To set the stage for a clear understanding of the ideas that emerged from the symposium, this introduction offers basic information on stem cell biology and a brief description of the topics covered in the symposium's six scientific sessions. The introduction is followed by summaries of presentations from each of the sessions with recommendations developed during the meeting. This report ends with a glossary of technical terms related to stem cell research.

Stem Cells

Regarded by some as the most significant discovery in the biological sciences since the development of recombinant DNA techology, ESCs are distinguished by their unusually robust capacity for self-renewal and by their potential to differentiate into the approximately 200 different types of specialized cells found in the body. These versatile cells, which are derived from very early-stage, pre-implantation embryos known as blastocysts, have been isolated from mouse, dog, monkey, and human embryos. The traditional way to make ESC lines begins with eggs fertilized *in vitro*. The fertilized eggs divide in culture and form a hollow ball of cells - the blastocyst. The cells that form the outer shell or surface of the "hollow ball" comprise the trophectoderm, which become part of the placenta if implanted into the uterus. On the inside is a group of 60-200 cells, the inner cell mass, which will develop into the organism following implantation. ESC lines are derived from the blastocyst by removing the outer trophectoderm and culturing cells from the inner cell mass. It is also possible to derive ESC lines with a specific genetic background by using blastocysts tested for the presence of disease-specific genes through pre-implantation genetic diagnosis (PGD) or with a technique known as somatic cell nuclear transfer (SCNT). In SCNT, the nucleus in an unfertilized egg is removed and replaced by the nucleus of a somatic cell taken from a donor. The egg, now bearing the genetic blueprint of the donor, is allowed to divide in culture to the blastocyst stage. ESCs made from such a blastocyst will have the same genetic background as the donor who contributed the nucleus.

ESCs are pluripotent because they can differentiate into cells derived from each of the three primary germ layers - the ectoderm, mesoderm and the endoderm. One test for pluripotency is to inject ESCs into immune-deficient mice where they give rise to benign tumors called teratomas with cell types from all three germ layers. The other important property of ESCs,

self-renewal, allows isolated stem cells to expand their numbers in culture almost indefinitely while retaining their pluripotency. The scientific excitement generated by ESCs arises from these two key features: ESCs can be grown in culture to obtain large numbers of cells, and under appropriate culture conditions can be induced to undergo differentiation into specific mature cell types.

Adult or somatic stem cells have been identified in small numbers within a variety of differentiated tissues (for example, bone marrow, liver, skin, brain, cardiac and skeletal muscle). They differ from ESCs in that their capacities for both self-renewal and differentiation are more restricted. In general, adult stem cells are thought to give rise only to the types of cells found in the tissue(s) in which they reside. For example, neural stem cells present in the central nervous system may be able to produce only cell types found in the nervous system but not cells from other tissues. Because of their rarity *in situ*, these tissue-derived stem cells are difficult to extract and purify in large numbers. The inability to culture large quantities of adult stem cells limits their widespread usefulness in developing therapies.

During development as well as in regeneration *in vivo*, it is believed that stem cells first give rise to progenitor cell populations that in turn produce mature cell types. In the adult, progenitor cells can be found in tissues and organs where they serve organ-specific functions by developing into the appropriate cell types required for tissue maintenance, replacement or repair.

The Sessions

The first session, *Cellular Therapeutics: Clinical State of the Art and Challenges for the Future,* reviewed the current state of cell replacement therapies for the treatment of diseases of the blood, diabetes, and neurological disorders. Dubbed the “poster child” for cell replacement therapy, bone marrow transplantation is an example of a therapeutic use of adult stem cells. Transplantation of bone marrow, which contains adult hematopoietic stem cells, has been used successfully to treat leukemias, lymphomas and genetic disorders of blood cells. In the treatment of type 1 diabetes, cadaveric tissue currently serves as a source of pancreatic islet cells for transplantation. For Parkinson’s disease, fetal brain tissue has been tested in clinical trials but with limited success. Each example serves as a proof-of-principle. Challenges remain, however. In this session, the speakers described conditions

that must be met if stem cell transplantation is to be considered for a specific therapeutic indication.

To use stem cells for cellular treatments, researchers must have standardized techniques to scale up the production of cells *in vitro*. Optimizing culture protocols and identifying molecules that keep human ESCs in a state of continuous self-renewal are crucial for the long-term maintenance of stem cell lines and the generation of the large numbers of cells needed for transplantation. **Session 2** – *Self-renewal of Stem Cells* – focused on understanding how self-renewal is regulated in stem cell populations, and outlined recent efforts to develop conditions for the maintenance of stem cell lines.

In most cases, the cells used for therapeutic treatments will not be stem cells themselves. Rather, the stem cells will be induced in culture to differentiate along a specific pathway to produce specialized cells for transplantation. Thus, an essential goal of ESC research will be to develop techniques for guiding differentiation along the correct lineage to obtain the desired cells. In **Session 3** – *Fate Decisions: Good and Bad Choices* – researchers outlined what is currently known about mechanisms that determine stem cell fate in culture and *in vivo*. This knowledge is essential if stem cells are to serve as building blocks for the *in vitro* engineering of tissues, such as cardiac muscle, insulin-producing islet cells, blood vessels and cells of the nervous system. To be able to direct ESC differentiation, scientists need to understand the biology and regulation of progenitor cells and how they produce differentiated cell types *in vivo*. Characterization of such fate-decision processes might also illuminate ways to recruit endogenous progenitor cells and stimulate tissue-specific cell differentiation at sites of injury or disease.

The very qualities – plasticity and capacity for self-renewal – that make ESCs ideal for replacement therapies also increase the chances that transplanted cells will: (i) generate unexpected types of differentiated cells, (ii) migrate to undesirable places, or (iii) spur tumor formation in the recipient. It is thus mandatory that scientists learn to regulate and control the behavior of ESCs and their progeny *in vitro* and assess the movement and function of transplanted cells *in vivo,* in order to develop protocols that avoid the potentially hazardous side effects of stem cell-based replacement therapies.

All protocols for treatment need to be tested and validated first in animal models of disease.

This topic was the focus of **Session 4** – *Bridging the Gap between Bench and Bedside* – where research that has moved beyond the laboratory bench and into preclinical studies in animals was presented. Preclinical studies, sometimes called translational research, are the critical link that allows scientists to ask how a cell preparation behaves *in vivo*. Such studies enable researchers to assess if a potential therapy has the desired activity and efficacy in an animal model of the disease under investigation, and to evaluate the likelihood of adverse effects. Preclinical studies are also useful in the refinement of therapeutic protocols prior to the initiation of clinical trails. In **Session 5** – *Stem Cells and Therapies: Lessons from the FDA and Industry* – scientists from industry and representatives from the federal government shared their expertise on requirements for advancing experimental cell replacement therapies to clinical trials. Most importantly, they reviewed the relevant regulations of the Food and Drug Administration (FDA) and requirements that must be met before embarking on clinical trials.

Although stem cells are frequently associated with cell replacement therapies, the first practical uses of stem cells may be in applications such as drug discovery, toxicity testing and the development of diagnostics. **Session 6** entitled *Stem Cells as Tools for Disease Research and Therapy* focused on research in which ESCs serve as novel tools for the molecular dissection of genetic-based diseases and for discovering new therapies, rather than for cellular transplantation. It is these tools which may offer the most significant near-term rewards from stem cell research.

Session 1: Cellular Therapeutics – Clinical State of the Art and Challenges for the Future

Presenters:

Robert Negrin, M.D., Stanford University

Jeffrey Bluestone, Ph.D., University of California, San Francisco (UCSF)

Olle Lindvall, M.D., Ph.D., University of Lund, Sweden

Moderators:

George Daley, M.D., Ph.D., Harvard University

Paul Berg, Ph.D., Stanford University

Key Points of the Session:

- Cell replacement therapy is an established therapeutic strategy that has been shown to be effective in blood disorders and diabetes, and has the potential for treating Parkinson's disease and stroke. In each of these instances, scarcity of suitable cells for therapeutic application is a limitation to widespread applicability. The use of ESCs to derive material for transplantation is a potential solution to this problem.
- Immune rejection remains a severe problem for cell replacement therapy, particularly for tissues outside the central nervous system. Selective drugs and protocols that augment or diminish particular immune cell populations are likely to be effective short-term strategies. Re-education of the immune system via transplantation of hematopoietic stem cells is a possible long-range strategy.
- The development of novel, non-invasive techniques for imaging cells *in vivo* after transplantation into animal models and humans would provide critical tools for understanding the behavior of engrafted stem cells and the biological basis of their effects so that optimum benefit can be obtained from cell therapy.
- Academic researchers need a clinical trial infrastructure – a support system to help with preclinical research, clinical trial design, quality control issues and meeting regulatory specifications – in order to move promising results to clinical application.

- Innovations in bioinformatics are needed to analyze the vast amounts of data generated from the analysis of large numbers of cell lines, diverse cell transplant preparations, and multiple treatment protocols.

Introduction

The symposium began by considering a major goal of stem cell research: the therapeutic use of stem cells to replace damaged or diseased tissues. The intent of the opening session was to evaluate the current clinical state of the art, to review how far researchers have come and to judge how far they must go to make the therapeutic use of stem cells a clinical reality for a broad array of diseases.

Session moderator George Daley initiated the discussion by outlining the characteristics of conditions that are most amenable to cell replacement therapy. These include diseases in which (i) there is loss of a single cell type; (ii) the anatomy of the replacement tissue is not overly complex; (iii) proof-of-principle for cell replacement therapy has been established; and/or (iv) organ or tissue transplants are effective, but the scarcity of cell source is a major drawback. The use of cell replacement therapy was then examined by the speakers in three clinical contexts: hematopoietic stem cell transplantation for treatment of blood cancers and hereditary blood diseases; pancreatic beta-islet cell transplantation in diabetes; and fetal-derived brain tissue transplantation in the treatment of Parkinson's disease and stroke. Although other examples could have been chosen, these three provide an informative contrast in their respective stages of clinical development and in the different problems that they pose.

The transplantation of adult hematopoietic stem cells through bone marrow transplantation is a well-established treatment that is currently practiced in clinical centers for hematological oncology worldwide. Robert Negrin pointed out that immunological intolerance is a common problem associated with all cell replacement therapies, and a key goal of researchers is to develop new protocols that prevent rejection of transplanted tissues. With respect to the treatment of type 1 diabetes, Jeffrey Bluestone described results of clinical studies in which cadaveric tissue served as a source of pancreatic islet cells for transplantation. While these trials provide important proof-of-principle for the efficacy of cell replacement therapy, the transplantation of cadaveric islet cells to treat type 1 diabetes is not practical for widespread

use given the scarcity of cadaveric tissue and the great numbers of patients with type 1 diabetes. Finally, Olle Lindvall presented data on the use of fetal brain tissue in the treatment of two neurological conditions – Parkinson's disease and stroke. These studies reveal some of the current limitations of stem cell transplantation for treatments for neurodegenerative disorders.

Hematopoietic Stem Cell Transplantation

Although research on human ESCs is still in its infancy, the use of stem cells for cell replacement therapy can be traced back to 1949, when research by the U.S. military showed that transfer of bone marrow cells into an irradiated animal restored its hematopoietic potential. Bone marrow contains many cell types; we now know that the relevant cells for restoring function are the hematopoietic stem cells in the marrow. These adult stem cells are responsible for producing red and white blood cells throughout life.

Following the initial experimental observation in animals, 40 years of basic and clinical research were required before bone marrow transplantation became a common and widely available therapy. In its current clinical application, bone marrow transplantation replaces a patient's diseased immune system with a healthy one. The patient's original system is first destroyed by chemotherapy or irradiation, and new stem cells are introduced from a compatible donor by transplantation. Bone marrow transplantation can be autologous (using the patient's own cells that have been removed and sometimes manipulated before re-implantation), syngeneic (using marrow from an identical twin), or allogeneic (using marrow from a donor who is not a twin, and therefore has a different genetic background).

Among the diseases treated by bone marrow transplantation are leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, aplastic anemia, and multiple myeloma, as well as genetic diseases of the immune system. Robert Negrin reported that among the 2,727 bone marrow transplants performed at Stanford University between June 1986 and June 2002, approximately 50% of patients enjoyed long-term survival. Although bone marrow transplantation has a proven record of success in treating blood cancers, the approach still faces several challenges. For autologous transplants, failures usually result from recurrence of the cancer; for allogeneic transplants, most failures are caused by complications arising

from the therapy itself. In addition, bone marrow transplantation can be limited by the availability of a suitable donor.

One strategy for reducing recurrence in cancer patients who undergo autologous transplantation is to attempt to eliminate all cancer cells from the graft. This can be accomplished by purifying hematopoietic stem and progenitor cells from the patient's bone marrow or peripheral blood for subsequent transplantation. In cancer patients, hematopoietic stem cells isolated from peripheral blood have been shown to engraft more rapidly and, thus, to speed reconstitution of the immune system when compared to stem cells purified from bone marrow.

Immunologic responses complicate allogeneic bone marrow transplantation. Allogeneic bone marrow transplantation can help patients suffering from leukemia or lymphoma, but problems can develop from the immunological differences between donor and recipient. For example, the transplanted tissue could be rejected by remnants of the host immune system. In many cases, this risk can be overcome by proper management with immunosuppressants. A more serious risk arises when donor immune cells – also known as T cells – in the transplanted bone marrow recognize the patient’s tissues as foreign and mount an immune response against them. The resulting graft-versus-host disease (GVHD) can be fatal. Acute GVHD typically occurs within 100 days after the transplantation and is nearly always lethal. Chronic GVHD is a less debilitating, long-term condition that is treated by immunosuppressive drugs, such as cyclosporine and methotrexate. Negrin emphasized the challenge of suppressing GVHD while maintaining the beneficial effects of the transplant, which, in addition to replacing the diseased tissue, also includes the destruction of the tumor cells in the recipient. This response, called the graft-versus-tumor (GVT) effect, occurs when diseased cells in the host are recognized as "foreign" by the reconstituted immune system derived from the graft donor.

Modification of immune cell populations in the transplant and host can reduce GVHD and enhance the anti-tumor effect. Experiments in humans and animals suggest that manipulation of immune cell populations – both in the host and in the donor cell population to be transplanted – can help to minimize the complications of GVHD and strengthen the desired GVT effect associated with allogeneic bone marrow transplantation. In mice, both effects are obtained by transplanting T cell-depleted bone marrow combined with purified

populations of regulatory T cells into irradiated animals. Because the types of T cells known to promote GVHD are removed from the cell preparation before transplantation, GVHD is inhibited, and the GVT effect is augmented. One difficulty with this approach is that it requires the isolation of pure preparations of regulatory T cells.

Another strategy that has been shown to inhibit GVHD while maintaining the GVT effect is to alter the immune environment of the host by increasing the number of regulatory T cells resident in the bone marrow prior to irradiation. This selective T cell enrichment, which has been carried out in both mice and humans, can be accomplished by immunotherapy along with an appropriate irradiation regimen. Results published recently in the *New England Journal of Medicine* by Negrin's group suggest that this method of augmenting hematopoietic stem cell transplantation can be used to treat human patients suffering from leukemia or lymphoma without inducing GVHD.

Hematopoietic stem cell research may offer broad opportunities for advancement of replacement cell therapy. In allogeneic hematopoietic stem cell transplantation, the immune system is effectively retrained so that tumor cells in the host are recognized as "foreign" by the reconstituted immune system derived from the graft donor. Several scientists have suggested that this approach might be effective in treating disorders such as autoimmune diseases and in combined bone marrow/solid-organ transplantation. If methods could be developed to derive large numbers of hematopoietic stem cells for transplantation - for example by the appropriate *in vitro* differentiation of human ESCs - re-education of the immune system may constitute a routine treatment for a wide range of immune disorders. Irving Weissman suggested that hematopoietic stem cell transplantation might represent a general solution to the problem of immune tolerance in cell replacement therapies.

Negrin pointed out that the focus on discovery research in academic settings encourages critical examination of and learning from clinical failures as well as successes. In order to yield such insights, however, clinical trials must be designed properly. Academic investigators often lack in-house support to design and embark on clinical trials, and are dependent on collaborations with big pharmaceutical companies because only companies have the infrastructure and expertise required for moving potential therapies from animal models to studies on patients. He made a strong case for the development and support of a clinical trial infrastructure "to empower academic laboratories to get ready for clinical and

preclinical trials," a theme echoed in a later session on the FDA and industry. Finally, Negrin emphasized that sophisticated new imaging technology would allow investigators to follow the fate of engrafted cells more precisely than is possible with current methods. Information gained from such explorations would be invaluable in elucidating the biological factors that control the behavior of these cells *in vivo*.

Cell Replacement Therapy for Treatment of Type I Diabetes

Type I diabetes is an autoimmune disease in which the insulin-producing beta islet cells of the pancreas are destroyed for unknown reasons, resulting in insulin deficiency and an imbalance in glucose uptake and metabolism. The disease usually arises in childhood and, in spite of treatment by long-term insulin administration, is associated with a barrage of debilitating secondary complications, including vascular disorders, diabetic retinopathy, and kidney disease. As with hematopoietic stem cell transplantation, experimental treatment of diabetes by cell replacement therapy has a long history. Transplantation of pancreatic islet cells to treat diabetic mice was shown to be successful over 30 years ago. Only recently, with improved protocols using cadaveric tissue, has transplantation in humans been shown to be effective. Jeffrey Bluestone reported that since 1999, scientists from around the world have transplanted islet cells isolated from human cadavers into more than 400 diabetic patients. At one year after transplantation, about 87% of the recipients still produce enough insulin to dispense with exogenous insulin treatment. After 5 years, 88% of recipients continue to produce insulin, but only 20% synthesize enough to be independent of exogenous insulin supplementation. With respect to cell delivery, one advantage of islet cells is that they do not have to be grafted in the pancreas to carry out their function. For treatment of type I diabetes, islet cells are commonly introduced - with radiological guidance and without surgery - into the patient's portal vein and ultimately reside in the liver.

Human embryonic stem cells could make islet cell transplantation a practical, large-scale treatment for type I diabetes. The major obstacle to widespread use of cadaver-derived islet cells for transplantation is their scarcity. The number of islet cells that can be obtained from current cadaver donors is sufficient for treatment of less than 0.2% of diabetic patients. Because the pancreas does not regenerate, using cells from living donors is not a viable option, and means are not currently available for growing new beta-islet cells in culture.

Human ESCs provide a possible solution to this dilemma. By expanding a population of ESCs and then inducing them to differentiate into islet cells, one could, in principle, have an almost unlimited source of cells for transplantation. Researchers have recently defined the sequence of discrete steps in the formation of islet-like cells from mouse stem cells and, under limited circumstances, these insulin-producing murine cells can be shown to reverse the disease after transplantation into diabetic mice.

Unfortunately, although some success in inducing human stem cells to form insulin-producing cells has been reported (see Odorico presentation, in *Session 4*), the normal differentiation of human islet cells *in vivo* is not well-understood, and has not been achieved *in vitro*. Bluestone stressed the importance of a careful and complete characterization of the differentiation pathway of human insulin-producing cells and made a plea for the development of more human ESC lines that can be used for these crucial experiments. He also addressed the need for research in non-human primates as an interim step in moving from experiments in mice to trials in human patients. Another safety concern emphasized by Bluestone was the importance of eliminating potentially tumor-producing residual ESCs in cell populations used for transplantation. Finally, Bluestone remarked that the creation of complex new research approaches that involve multiple stem cell lines, differentiation protocols, and immunotherapies has resulted in the generation of huge amounts of new data. Innovations in bioinformatics are needed for efficient and thorough data mining and analysis.

Better solutions to immune rejection must be sought. The experience gained from beta-islet cell transplantation in type I diabetes revisits the problem of immune rejection in cell replacement therapies. Patients who receive transplants of cadaveric tissue must take immunosuppressive drugs that are toxic to the kidneys and carry risks for infection and cancer with long term use. The generation of patient-specific, beta-islet cells (e.g., through somatic cell nuclear transfer) is not a viable solution to this problem because these cells would still be susceptible to the same immune attack that brought on the original disease. One approach is to use drugs to re-educate the immune system so that it treats foreign proteins on the transplanted cells as "self". Another is the use of cell encapsulation devices to isolate and thus protect the transplanted cells from immune attack. The problem of immune compatibility is particularly significant when we consider the need to make replacement cells and tissue available to the racially diverse population in the United States.

In the discussion, Irving Weissman again pointed out that experiments in mice suggest a general solution to the problem of immune tolerance. In mice, transplants of hematopoietic stem cells produce permanent tolerance demonstrating that the recipient's immune system has been re-educated by the transplanted stem cells. If patients with type I diabetes were treated with hematopoietic stem cells and islet cells derived from the same human ESC line, the transplanted islet cells would be recognized as self by the newly educated immune system.

Restoring Brain Function by Cell Replacement Therapy

Implantation of fetal brain tissue shows proof-of-principle for cell replacement therapy in Parkinson's disease. Parkinson's disease is a neurodegenerative condition characterized by the progressive loss, through unknown causes, of a particular class of dopamine neurons that modulate circuits involved in movement. These cells reside in one part of the brain, the *substantia nigra* and project axons to contact target neurons in another part of the brain, the striatum. Because the disease strikes a specific group of well-defined cells in a discrete locus, Parkinson's disease has been a prime candidate for cell replacement therapy in the brain.

Human clinical trials in which mesencephalic tissue from fetal brains is transplanted into the striatum of Parkinson's patients have been conducted for over a decade. Olle Lindvall showed evidence that, in some Parkinson's patients, the grafted fetal neurons reinnervated the striatum, normalized dopamine release, became functionally integrated, and induced major, long-lasting improvement, even in the context of the ongoing disease process. As with the current cell replacement treatment for type 1 diabetes, fetal neuronal grafts are not a viable clinical treatment for Parkinson's disease because of the small quantities of tissue that are available.

Stem cell therapy for Parkinson's disease faces several challenges. Lindvall emphasized that current medications offer some help to Parkinson's disease patients; thus, stem cell therapy must be shown to be clinically competitive if it is to be useful. He outlined the steps that must be taken to reach this goal. First, standardized preparations of large numbers of dopamine neurons or their progenitors need to be produced from sources of human stem cells such as human ESC lines. Scientists then need to demonstrate efficacy in animal models of the

human disease by assaying three parameters: the extent of striatal reinnervation; the augmentation of *in vivo* dopamine release; and improvement of Parkinson-like symptoms. Lastly, the long-term survival of the cells will have to be demonstrated to evaluate continued efficacy of the treatment. Lorenz Studer and his colleagues have recently reported that, while they were able to generate midbrain dopamine neurons from human ESCs in culture, the cells survived poorly after transplantation into animal models. Researchers working with mouse models have observed a reduction of symptoms resembling those in Parkinson's disease when dopamine-producing neurons derived from mouse ESCs are transplanted into the mouse brain. It remains unclear how well the currently-used rodent models of Parkinson's disease mimic the pathology and symptoms of the human condition. Additional and better model systems are needed.

Review of the fetal brain engraftment trials suggests that the functional outcome was dependent on the extent of dopaminergic denervation in the patient. Implantation was relatively successful when the denervation was localized; when denervation was widespread, implantation at a single site was much less successful. These observations suggest that, for optimal results, each patient will require a unique protocol where sites of engraftment are identified on the basis of pre-operative imaging. In addition, because brain pathology in Parkinson's disease can eventually affect neuron types other than dopaminergic neurons, the specific cell types used for replacement may have to be determined by an individual's disease pathology.

Finally, Lindvall emphasized the need to understand the biological mechanisms that determine functional outcomes, and whether benefits are produced through correction of the biochemical defect, local innervation, effects of growth factors, or reconstruction of lost neural circuitry. At present, researchers do not as yet know the precise molecular fate or function of the transplanted dopamine-producing cells. This is a critical step that is necessary to move research into clinical trials.

Studies on stroke illuminate stem cell biology and suggest new therapies. Stroke arises from the blockage or rupture of blood vessels in the brain causing widespread loss of neurons and glial cells in the affected area. In limited animal studies and human trials, stem/precursor cell transplants have been shown to survive and to partly reverse behavioral deficits caused by

the stroke. The underlying mechanism of this reversal is unclear, and there is little evidence of actual neuronal replacement or enhanced neural circuitry.

One of the most interesting observations to come from animal studies on stroke is that injury can trigger the formation of new neurons. In rodents where a stroke is induced by occlusion of the cerebral artery, new neurons continue to be produced in the brain for more than four months after injury. These neurons presumably arise from the recruitment of endogenous neural stem/progenitor cells which were shown during the last decade to be present at a few sites in the adult brain. These results suggest a possible therapeutic strategy for the treatment of stroke and neurodegenerative diseases that is based on enhancing the response of endogeneous neural stem/progenitor cells to injury. To develop such a strategy, researchers will first need to decipher the endogenous mechanisms that regulate neurogenesis in the human nervous system.

Discussion and Conclusions

With cell replacement therapy, we are "at the stage of educated ignorance. We know what we don't know." – Paul Berg

Session moderator Paul Berg opened the discussion by pointing out that the clinical settings described in the opening session illustrate the enormous opportunities and the "big blocks of ignorance" in stem cell biology. Filling the significant gaps in knowledge will require information and a fundamental understanding derived from basic science. Berg also suggested that the field will be best served if "every hypothesis worthy of consideration" is pursued by CIRM. Unlike the National Institutes of Health (NIH), he continued, CIRM has "the freedom to make work move in every direction simultaneously." This approach includes the pursuit of research that might be termed "risky."

Larry Goldstein from the University of California, San Diego asked the panel to outline common bottlenecks that must be addressed in the context of any cell replacement therapy. The following themes were replayed throughout the two-day symposium.

First, many more human ESC lines are needed if researchers are to accomplish their basic and applied research goals. Second, for nearly all cell replacement therapies, methods are

needed to educate the immune system to avoid rejection of transplanted material; treatments that achieve selective immune suppression should enhance the clinical outcome for patients. One possible exception is the brain, which exists in a protected immune environment in which rejection does not occur.

Third, the clinical trial infrastructure needed to move studies on animal models to trials in humans must be developed for academic researchers. This fundamental support system should offer help with clinical trial design, quality control issues, and regulatory specifications of the FDA, and may even include access to Good Manufacturing Practice (GMP) facilities for industrial-level production of clinical grade material for transplantion.

Other recommendations include innovations in bioinformatics for efficient analysis and meta-analyses of the huge amounts of data generated by trials and the development of sophisticated imaging techniques that would allow more precise characterization of transplanted cells. Advanced bioinformatics and imaging capabilities will help researchers to interpret the results of preclinical and clinical trials.

Session 2: Self-Renewal of Stem Cells

Presenters:

Haifan Lin, Ph.D., Duke University School of Medicine

Andras Nagy, Ph.D., Mount Sinai Hospital

Martin Pera, Ph.D., The Monash Institute of Medical Research

Moderators:

Peter Andrews, Ph.D., University of Sheffield,

Janet Wright, M.D., ICOC

Key Points of the Session:

- Intrinsic (intracellular) and extrinsic (environmental or niche) mechanisms regulate self-renewal and differentiation. Researchers are seeking to elucidate the signaling pathways and effector molecules that control these processes in human ESCs.
- The extracellular matrix and supporting cells together form the local microenvironment or niche surrounding stem cells, and appear to regulate both self-renewal and differentiation. The effectors (proteins, small RNAs) and signaling pathways that determine niche cell functions need to be defined at the molecular level.
- Much of our understanding about niche function comes from studies on lower organisms such as the fruit fly, *Drosophila melanogaster*, and the roundworm, *Caenorhabditis elegans*. Supporting research that utilizes these organisms as models is therefore strategically important.
- A number of genetic diseases have similar properties in dogs and humans. Canine ESCs have now been isolated and cultured, and might constitute a novel model system for cell therapy and human disease research.
- The generation of homogenous stem cell preparations will require standardized methods and protocols that account for cell-to-cell variation; scaling up production to generate large numbers of cells for research or therapy will present a major technical challenge.

Introduction

One of the most basic and practical problems facing stem cell researchers today is how to produce large numbers of ESCs in culture while maintaining their pluripotent state The capacity of most cells to proliferate through cell division declines with age; ESCs appear to be an exception. Investigators are intensely interested in how stem cells maintain the ability to self-renew continuously for extended periods of time without undergoing differentiation.

In invertebrate model systems, stem cells divide to yield both an identical daughter stem cell and a non-identical daughter cell that will continue down a selected differentiation pathway. Haifan Lin described the roles of intrinsic and extrinsic regulators that drive this asymmetric mode of cell division.

An asymmetrical division by definition yields a mixed population of cells. A top goal of researchers in the stem cell field is to generate large, homogenous populations of unspecialized stem cells, progenitor cells, or lineage-specific differentiated cells for research or therapeutic applications. Using mouse genetics to dissect intrinsic signaling pathways in mouse ESCs, Andras Nagy described how positive and negative regulators play roles in preserving "stemness" and self-renewal as well as in generating the embryonic ectoderm, mesoderm and endoderm lineages.

To keep stem cells in a state of self-renewal for long periods of time in culture, researchers need a detailed understanding of the internal cellular machinery that controls proliferation. In parallel, bioengineers and cell biologists must define the external culture conditions that promote ESC self-renewal. Martin Pera explained the challenges of bringing these issues to bear on large-scale cell production of clinical-grade cells for therapeutic applications.

Molecular Characterization of Self-renewal

Stem cell self-renewal is directed by intrinsic (intracellular) genetic pathways that are influenced by extrinsic signals from neighboring niche cells. Scientists are striving to reveal the interplay between these two modes of regulation, as well as to define the cellular apparatus responsible for the asymmetric separation of contents during cell division, which balances self-renewal and lineage-specific differentiation.

The neighborhood determines cell fate. In the fruit fly, *Drosophila melanogaster*, the stem cell niche is a specialized regulatory environment composed of neighboring stromal cells and an extracellular matrix that modulates stem cell number and fate. According to *Drosophila* geneticist Haifan Lin, if researchers are to decipher the molecular mechanisms of stem cell self-renewal, it is essential to know where stem cells reside *in vivo* and what kinds of cells constitute the niche.

Although the niche concept was proposed more than 30 years ago for mammalian hematopoiesis, niche cells were first identified in the *Drosophila* ovary and shown to provide key regulatory signals that influence the behavior of neighboring stem cells. The role of the niche has also been studied in the germline or reproductive system of the nematode *Caenorhabditis elegans* where it has been shown to nurture the resident stem cells via Notch signaling pathways. Notch is a receptor for intercellular signals that specify cell fate. In mammalian systems, composition of the stem cell niche is being actively investigated in areas of cell genesis such as the germline, the bone marrow, the crypts of the gut, the hair shaft, and the subventricular zone of the brain.

The *Drosophila* ovary and testis provide excellent model systems for studying the role of the niche in stem cell self-renewal and asymmetric cell division. The fly ovary contains two types of stem cells that are required for egg production in adult flies – germline stem cells, which differentiate into eggs, and somatic stem cells, which give rise to various kinds of somatic follicle cells which form the egg chambers of the ovary. The niche cells in the fly ovary are believed to secrete the proteins hedgehog, wingless, and decapentaplegic. Hedgehog regulates the maintenance and proliferation of the somatic stem cells. Wingless controls somatic stem cell maintenance and follicle cell differentiation. Decapentaplegic [a homolog of the mammalian family of bone morphogenic proteins (BMPs)] has a key role in maintaining the germline stem cell fate and thus helps to determine the number of germline stem cells in the fly ovary.

Mammalian stem cell niches are still poorly understood. In the intestinal crypts, BMPs and the Wnt signaling pathway appear to balance self-renewal and differentiation by antagonistic mechanisms. In the mammalian hematopoietic system, BMP signaling seems to control hematopoietic stem cell number through regulation of the size of the niche. According to David Scadden (see *Session 4*), osteoblasts – the chief bone-producing cells – are key

players in determining the number and function of hematopoietic stem cells. The cytokine and adhesion protein osteopontin, which exists within the matrix of long bones, interacts with osteoblasts to constrain the number of hematopoietic stem cells in the bone marrow. In each case multiple, parallel signaling pathways in the niche appear to regulate stem cell self-renewal.

Although extrinsic mechanisms of self-renewal are well-understood in several systems, Lin asserted that complete elucidation of the physiological functions of mammalian niche cells will require the identification of all the molecular players (genes, proteins, small RNAs, etc.) operating in both the niche cells and stem cells, as well as a detailed map of how the various signaling pathways are integrated. Recent evidence implies that niche cells not only play a role in regulating stem cell renewal but also influence differentiation. Investigators are working to define the signaling pathways in niche cells that help determine the fate of stem cell progeny.

Intrinsic mechanisms regulate self-renewal. Lin outlined three emerging areas of investigation related to intrinsic mechanisms of mammalian stem cell self-renewal: epigenetic influences, control of protein translation, and the actions of endogenous small RNAs.

Although every cell in the body has the same genome, differentiated cells clearly display distinct, specialized functions. Scientists believe that a cell's fate is dictated in part by the dynamic nature of chromatin which plays an integral role in the regulation of gene expression, chromosomal segregation, and other aspects of DNA metabolism. Chromatin consists of DNA interposed with a set of proteins that compacts DNA and defines the organization of the genome in eukaryotic cells. Regions of compaction produce structurally and functionally distinct chromosomal domains throughout the genome known as heterochromatin. Consisting largely of repetitive DNA, heterochromatin tends to be inaccessible to transcription factors and is thus transcriptionally silent. In contrast, euchromatin domains are regions of the genome that are diffuse or uncoiled during interphase and are thus accessible to regulatory factors; these domains contain protein-encoding genes and are transcriptionally active.

Although a variety of structural proteins and chromatin remodeling enzymes participate in its assembly, heterochromatin, once organized, is epigenetically heritable – that is, it persists

after DNA replication and mitosis and is independent of the DNA sequence. Heterochromatin is believed to play a central role in the maintenance of genomic stability and in the control of gene expression events that guide cell differentiation and development. Stem cell biologists are working to understand how heterochromatin affects the expression of genes in stem cells and to decipher how specific changes in chromatin structure are induced by niche signaling or by intrinsic factors. Understanding the function of chromatin remodeling enzymes is central to this line of investigation.

Stem cell fate and self-renewal are also controlled by regulating the translation of messenger RNA (mRNA) to protein. In *Drosophila,* a family of translational repressor proteins called Nos partner with the Pumilio protein; the resulting multi-protein complex adheres to the 3' untranslated region of selected mRNAs and represses their translation. Lin and colleagues have shown that if germline stem cells (cells that give rise to gametes) are depleted of Nos or Pumilio, they will differentiate into oocytes. This finding implies that Nos and Pumilio are required for the maintenance of germline stem cells, and translational control appears to play a role in stem cell self-renewal. Researchers are now working to characterize all of the key translational regulators of stem cell fate as well as their target genes. Whether or not the niche influences the behavior of translational regulators within stem cells is another fertile area of research.

Named as the "breakthrough of the year" by *Science* magazine in 2002, small endogenous RNAs constitute a new paradigm for genetic regulation in a variety of biological contexts, including stem cell self-renewal. There are many kinds of small endogeneous RNAs, and some are better understood than others. All, however, are thought to participate in reducing or silencing the expression of specific genes. The most well known are the small interfering RNAs (siRNAs) which are ~22 nucleotides in length and prevent expression of specific mRNA sequences as part of a multi-protein complex called RISC. Another group of endogenous RNAs, called microRNAs, regulate genes by modulating either mRNA stability or translation. These small, single-stranded RNAs are part of a multi-protein complex referred to as miRNP. Lastly, repeat-associated short interfering RNAs are thought to function in chromosomal maintenance and transposon silencing as part of the RITS complex (RNA-induced initiation of transcriptional gene silencing). Recently, it was shown that RITS helps proteins to assemble gene-quieting heterochromatin at centromeres and thus may have an indirect function in chromosomal segregation and asymmetric cell division. The

functional characterization of these diminutive regulatory RNAs is still in its infancy. Talented researchers, including many stem cell scientists, have flocked to the small RNA arena to investigate their role(s) in controlling genes and pathways of interest.

Culture Condition Conundrums

In animal systems, in vitro genetic manipulations have been crucial to the study of "stemness". Asymmetric cell division and self-renewal of ESCs are choreographed in part within the stem cell itself by a host of growth and transcription factors, enzymes, receptors, and morphogens that form complex regulatory circuits. Andras Nagy outlined the intrinsic signaling pathways in mouse ESCs, including the well-known proteins Oct 4, Nanog, and STAT3. These and other positive and negative regulators play roles in the preservation of "stemness" and self-renewal as well as in the generation of the embryonic ectoderm, mesoderm and endoderm lineages.

Much of what is known about self-renewal in mouse ESCs was deciphered through the use of genetic manipulations. These techniques included not only classical transgenic approaches, but also point mutations and gene knock-out and knock-in techniques. Some of these approaches are discussed by Inder Verma in *Session 6*. In addition, intricate conditional and inducible gene manipulation approaches have led to the generation of cell lineage- or developmental stage-specific alterations under temporal control. The vast number of Cre recombinase-expressing mouse lines - often referred to as "Cre-Zoo" animals - has greatly contributed to these accomplishments by allowing Cre recombinase to be expressed in specific cell types, in some cases in an inducible manner.

Nagy stressed that it remains unclear whether a signaling circuitry similar to that in mouse ESCs regulates self-renewal and cell fate decisions in human ESCs. To decipher intrinsic mechanisms of self-renewal in human ESCs, the sophisticated genetic techniques available for the mouse system must be developed for their human counterparts. Another challenge is that human ESCs are hard to grow, transfect, and clone. By learning more about mechanisms of self-renewal and by achieving efficient homologous recombination in human ESCs, it may be possible to create "smart" lines that replenish themselves more robustly. Nagy's goal is to design human ESCs with engineered genetic switches that lock cells in the self-renewal state or promote or abort lineage-specific differentiation. These genetic switches

could complement small molecule manipulators discovered by high-throughput screening (see the Schultz presentation in *Session 6).*

Until scientists can genetically manipulate human ESCs at will, mouse ESCs will continue to be the most widely used model system for research on cell-based therapies. Rodent models, however sometimes fail to mimic the human condition, and it may be desirable to create novel animal models that are closer to humans evolutionarily. Non-human primates, although closest to humans, are expensive, and availability is limited. In search of a new model, Nagy found that dogs suffer from a number of genetic disorders with properties similar to the corresponding diseases in humans, including diabetes, arthritis, and retinopathies. Canine ESCs have been isolated recently and shown to differentiate *in vitro* and to produce teratomas in immune-deficient mice when tested for pluripotency. While further characterization of canine ESCs is needed, early data obtained with these cells are promising. Canine ESCs might constitute a novel model system for cell therapy and human disease research.

Better definition of culture conditions is required to optimize stem cell maintenance. Martin Pera from the Monash Institute of Medical Research in Australia was one of the first investigators to generate human ESCs. In his presentation he reviewed the critical issues relating to the propagation and differentiation of human ESCs. Although numerous researchers around the world are attempting to develop defined systems for stem cell maintenance, the molecular systems responsible for creating and maintaining the stem cell state, as well as those that direct differentiation are still poorly understood.

In the first culture systems for human ESCs, the stem cells were grown on mouse embryonic fibroblast feeder cells in media containing fetal calf serum. These early conditions introduced animal products and, potentially, animal viruses into the growing human cell culture. Current research is aimed at the development of new systems of defined media that exclude non-human components. We now know that a variety of human feeder cells function as well as mouse feeders to support self-renewal of human ESC lines. Today, one of the most widely used systems for culturing ESCs is a serum-free medium that contains a serum replacement and basic fibroblast growth factor.

According to Pera, however, analysis of the human ESC culture literature reveals sizable gaps in our current technology. Although many candidate growth factors and culture systems are being examined, no system is completely defined or supports clonal growth of human ESCs. In some studies, essential quality control is lacking, most notably, an adequate evaluation of the ability of the culture system to support the long-term maintenance of diploid, pluripotent cells. In addition, the current culture systems produce cell populations that are heterogeneous for a variety of reasons described below.

The ability to generate large numbers of human ESCs with standardized culture protocols is essential if stem cells or their derivatives are destined for therapeutic treatments. Growing cells in small culture facilities and scaling-up to therapeutic quantities are "two different worlds," declared Pera. To develop protocols for the growth of human ESCs in bioreactors and to solve the unique problems associated with scale-up, biologists and bioengineers need to collaborate early in the process of designing the optimal culture medium and conditions. Realistic requirements for large-scale cell production and cost factors must be assessed for each therapeutic scenario as cell densities can differ dramatically with cell type. Scaffolds might provide additional surface area to achieve higher cell densities. Although valuable lessons have been learned in moving mammalian cell culture from bottles to large scale production in bioreactors, Pera has found that the growth of human ESCs is a more difficult problem than is the long-term perpetuation of many standard diploid cell lines.

The elimination of feeder cells and animal proteins from human ESC culture media is a key step toward defining the optimal culture conditions for self-renewal. Feeder cells produce uncharacterized maintenance factors and differentiation inhibitors. The stem cells themselves also may secrete soluble mediators of growth and differentiation. It is known that in the embryo, progenitor and differentiated cells - progeny of pluripotent stem cells - secrete factors that regulate cell fate. These events probably also occur in human ESC cultures, yielding a heterogeneous population of cells.

In addition to cells, there are other environmental variables such as solid, liquid and gaseous components that may affect cell behaviour in culture. Although the role of the gaseous environment required for stem cell maintenance is unclear, oxygen tension and pH control are known to be crucial for low-density, feeder-free and serum-free cultures. Even the composition of the liquid-phase or medium has yet to be optimized for stem cell

maintenance. Stem cell investigators often use a variety of components/additives including albumin and transferrin, insulin-like growth factors, fibroblast growth factor, and the differentiation inhibitor, noggin, to supplement their basal media of choice. The best combination of factors for supporting self-renewal remains to be determined, and it is still unclear whether different human ESC lines have different growth requirements.

Almost all tissues and organs are encased and surrounded by intricate sheaths of macromolecules known as the extracellular matrix. The extracellular matrix is made up of well-known structural proteins such as collagen and elastin, functionally specialized proteins such as fibronectin and laminin, and proteoglycans, which are hydrophilic molecules, composed of proteins attached to extensive chains of repeating disaccharides. Although the extracellular matrix is highly variable, it forms an important component of the microenvironment surrounding a cell. Scientists have used different matrix preparations in culture to simulate the extracellular matrix including Matrigel, a commercially available, solubilized, basement membrane preparation, heparin sulfate proteoglycans along with other proteoglycans, and a combination of collagen, fibronectin, and laminin. Researchers are also working to identify better characterized additives that replicate the *in vivo* function of the extracellular matrix.

Genomic stability is crucial for the maintenance of stem cell lines. The chromosomal makeup or karyotype of ESCs is stable when cells are maintained in serum and passaged by mechanical dissection. Pera's laboratory, as well as other research groups, has observed karyotypic abnormalities in serum-free cell cultures that have been passaged enzymatically. In fact, a genome-wide analysis of sub-microscopic DNA lesions revealed that genetic changes such as point mutations and the development of chromosomal abnormalities such as aneuploidy can occur in long-term cultures. The consequences of these changes must be examined as they could present complications for the creation and maintenance of clinical grade stem cell lines.

Discussion and Conclusions

Stem cells don't like to grow alone. – Peter Andrews

There are no currently available culture systems that support high-efficiency survival and proliferation of single human ESCs. Because these cells do not proliferate well at low densities and because karyotypic abnormalities develop, it is difficult to propagate clonal cultures – cultures that are derived from single human ESCs. Audience participants questioned the speakers in this session about whether any of the currently existing hematopoietic stem cell lines are maintained as clonal populations and, if not, whether it matters. Haifan Lin responded that "a certain level of variation [among cells in a population] should be tolerable," but too much variation makes it hard to define the effectors that mediate biological regulatory mechanisms. Session moderator Peter Andrews from the University of Sheffield in the United Kingdom added, "Yes, it matters. But once you've cloned a cell, you've selected for a particular type that is different from the bulk."

Pera predicted that within the next 12 months many defined systems for human ESC growth will be described in the literature, and called for an international collaborative effort to build and validate the most optimal system for standardized human ESC growth. Such a program should have the following components: (i) a meta-analysis of published culture methods including the patent literature; (ii) development of standardized assays that assess short-term stem cell attachment, survival, and maintenance (2 to 3 passages); (iii) the blind testing of multiple cell lines in two to three central laboratories; and (iv) more extensive evaluation, in many laboratories, of long-term support of pluripotency and genetic stability in the most promising culture systems.

Several audience members stressed the importance of cellular accounting - the assessment of genetic and phenotypic variability within a cell line or cell population. Pera remarked that one of the initiatives of the International Stem Cell Forum (see http://www.stemcellforum.org) is to characterize all available human ESC lines in terms of their gene expression patterns, antigen expression, and capacity to develop along a particular lineage. A number of other cellular parameters such as microbiological contamination and pluripotency will be assessed as well. He reminded us that the International Stem Cell Forum "team is a world team," and

that CIRM must determine how its efforts will fit in with the on-going research in other parts of the world.

Pera also described preliminary efforts to develop cell culture systems that support lineage-specific differentiation of human ESCs. This discussion flows into the topic of the next session – fate decisions (see *Session 3*). At present, the ability to control the differentiation of human ESCs in culture to provide cell types of interest is at a primitive stage. According to Pera, scale-up of culture systems to yield large numbers of progenitor or terminally-differentiated cells for research or therapy is a major challenge. This goal likely will not be met until basic research sheds more light on the properties that define specific progenitor cells and their differentiated cell phenotypes. This includes defining specific growth conditions, unique cell surface markers, and inducers of differentiation as well as characterizing the contribution of niche cells and extracellular components that regulate the fate of these cells.

Understanding progenitor cell biology is as important as understanding embryonic stem cell biology. – Martin Pera

An *in vitro* differentiation system is highly desirable if commitment and differentiation events occur in a predictable, reproducible, and stepwise fashion and if the majority of the cell population responds in a similar fashion to a given set of differentiation inducers. Scientists must have the ability to identify, propagate, and expand progenitor cells at various stages along the lineage. The effectors that promote differentiation need to be well defined, and the differentiated cells must display the appropriate functions and the expected patterns of marker expression if cells are to achieve their therapeutic potential.

Session 3: Fate Decisions – Good and Bad Choices

***Presenters*:**

Nissim Benvenisty, MD., Ph.D., Hebrew University of Jerusalem

Irving Weissman, M.D., Stanford University

Michael Clarke, M.D., University of Michigan

***Moderators*:**

Owen Witte, M.D., University of California at Los Angeles (UCLA)

Irving Weissman, M.D., Stanford University

Key Points of the Session:

- A detailed characterization of human embryonic development and lineage-specific cell differentiation is necessary to understand how fate decisions in human ESCs are made and how to control them.
- The assessment of stem cell function *in vivo*, including the generation of chimeras, is an essential step in the development of effective and safe therapies for humans.
- A new model of the how cancer originates proposes that only a small proportion of cells (5-10%) within the cancer actively divide to produce new metastasizing cells. Presumably these are endogenous adult stem cells that have lost the normal restraints on their proliferation to become cancer stem cells. The cancer stem cell concept constitutes a new paradigm for understanding oncogenesis and metastasis and predicts novel treatment strategies for cancer.

Introduction

The fundamental properties of ESCs – the ability to self-renew and the potential to differentiate into many cell types – make them highly desirable for broad application in regenerative medicine. Our limited understanding about the mechanisms that control these properties, however, presents a challenge in developing model therapies. Critical questions include: how is the fate of a stem cell determined; how does it become a specialized cells

and how do cancer stem cells arise. Scientists will need to address these questions before stem cell therapy can become a reality.

A common paradigm for stem cell therapy envisions that an ESC line will be grown in culture to expand its numbers (proliferation or self-renewal) and then directed down a pathway of differentiation to obtain the cell that is desired for transplantation – either a terminally differentiated cell or a late-stage progenitor cell that will undergo limited proliferation and terminal differentiation *in vivo* after transplantation. Each stage in the process of preparing cells must be carefully controlled and the fate and function of the transplanted cells followed. For preparation of the cells, markers must be developed so that progress from one step to the next can be followed and the specific biologic cues that trigger each step in the progression must be known. Finally, methods must be developed to assess the functional capability of the cells to be transplanted. It will be particularly important to exclude the presence of pluripotent stem cells, which can give rise to teratomas when transplanted, or of any stem cell that has mutated to become a cancer stem cell in the final preparation to be used.

This session highlights what is known about the control of stem cell fate and what further study is needed for the development of effective stem cell therapies. Nissim Benvenisty reviewed current progress on the development of culture conditions that promote the controlled differentiation of human ESCs *in vitro*. Once stem cells with specific traits are obtained in culture, the next step is to determine the effectiveness and fate of these cells *in vivo.* Irving Weissman stressed that animal models will be indispensable for testing the biological function of stem cells in an organism and for ensuring the safety of specific cell therapy protocols. Animal models of human injury or disease, such as spinal cord injury or diabetes, are two examples in which stem cell therapies can be tested in animals before their use in patients.

Ensuring the safety of such therapies also requires a better understanding of the molecular mechanisms that could lead a cell astray. Although adult stem and progenitor cells are normally present in our tissues, their proliferation is tightly regulated by genetic and environmental factors. Michael Clarke described experiments demonstrating that adult stem cells can escape these restraints through mutation, resulting in uncontrolled proliferation and cancer. Recent experiments demonstrate that tumors may be produced by a small

population of "cancer stem cells" which do not represent the bulk of the tumor. This insight forms the basis of a new model for oncogenesis and may provide useful clues about mechanisms that influence stem cell fate

Regulating Differentiation in Vitro

The first step in controlling stem cell differentiation and division is to define specific stages in the process. The biochemical, spatial, and temporal cues that so elegantly orchestrate the process of differentiation from a stem cell to a specific differentiated cell in the organism are not yet well-understood, but are being explored in several species including the mouse. As researchers begin to investigate the application of stem cell therapies in humans, they have learned that some of what has been learned in mouse models does not translate directly to man. Thus, it is appropriate to elucidate the basic mechanisms that control stem cell fate in humans. Nissim Benvenisty emphasized the need to study fundamental mechanisms of human development in order to develop specific protocols for human ESC differentiation in culture.

You can't separate basic research from therapy. — Nissim Benvenisty

Using recent technological advances to examine large numbers of genes, researchers have measured gene expression at various times during mouse embryo development to identify genes whose expression changes. By analyzing the spectrum of changes in gene expression, one can generate a specific profile, or "fingerprint", for each stage of embryonic development. Such a study cannot be performed with developing human embryos, but changes in gene expression can be tracked as human ESCs differentiate in culture. Gene expression profiles provide a starting point for understanding the differences between embryonic stem cells, progenitor cells, adult stem cells, and mature, specialized cells. The development of characteristic "fingerprints" for each stage of differentiation will allow the specific identification, selection and enrichment of cells at particular stages when grown in culture. Detailed profiling of changes in gene expression between human stem cells and their differentiated progeny could also allow scientists to identify early commitment steps in human embryology.

The possibility of tumor formation is a major concern if stem cell-derived tissue is to be used for transplantation. Benvenisty showed that it is possible to genetically engineer human ESC lines to express a "suicide gene" that renders the cells selectively sensitive to a specific drug. For example, human ESC lines modified to express the *Herpes simplex* virus *thymidine kinase* (*HSV-tk*) gene become sensitive to the antiviral drug ganciclovir. The modified cells can be destroyed at concentrations of ganciclovir that have no effect on cells that do not express *HSV-tk*. Proof of concept was demonstrated by injecting the engineered stem cells into mice. The tumor produced by these cells was eliminated when the mice were treated with ganciclovir. The prospect of eliminating the transplanted cells in a selective manner could provide an added measure of safety for cell replacement therapies.

The Fate of Transplanted Stem Cells In Vivo

The transplantation of human cells into animal models of disease and injury is critical to the successful development of stem cell-based therapies. More than a decade ago, human fetal blood-forming tissues were engrafted in immune-deficient mice to demonstrate their potential of cell-based therapies. This experiment was an essential step in the development of hematopoietic stem cell therapies to treat various leukemias. Animal models continue to be essential in testing the success of other cell-based therapies, such as the transplantation of nerve cells to treat Parkinson's disease or spinal cord injury, with hopes that efficacy in animal models will be predictive of similar results in patients. The transplantation of human cells into animal models not only allows scientists to evaluate the function and integration of transplanted cells at the site of interest but also to follow possible migration of cells to other locations. Such studies also allow scientists to test whether the transplanted cells may develop into undesirable cell types, such as tumor or cancer cells. Weissman asserted that the medical importance of understanding the fate of stem cells following transplantation into an intact organism cannot be understated.

To some, however, the introduction of human cells into animal models has raised fears of creating organisms that are part-human and part-animal (referred to as "chimeras"). Some members of the U.S. Congress have sponsored legislation to restrict the creation of chimeras, in particular, the engraftment of human neural cells into mouse or animal brains. In April of 2005, the National Academy of Sciences issued guidelines for human ESC research that allow the creation, but not the breeding, of chimeras, stating that the use of chimeras is

appropriate "under circumstances where no other experiment can provide the information needed."

If your goal is to advance medical knowledge, you have to push just as hard as you can at the edge … and that includes making chimeras. Pushing at the edge is what CIRM should be about. – Irving Weissman

In his presentation, Irving Weissman demonstrated the value of generating chimeras by describing several systems in which this approach has provided valuable information on the potential of cell-based therapies. Scientists at StemCells Inc. (a company founded by Weissman) have isolated human central nervous system (CNS) stem cells from brain tissue using monoclonal antibodies to antigens that are specific to these cells. To assess the ability of the human CNS stem cells to function *in vivo,* scientists expanded the cells in culture to form aggregates termed neurospheres which were then engrafted into the brains of mice. The engrafted human cells migrated to various locations in the mouse brain, proliferated, integrated and differentiated into neurons, astrocytes, or oligodendrocytes, demonstrating the ability of neural stem cells to respond to their local environments and behave appropriately.

Researchers then tested whether the transplantation of stem cells from the human CNS could restore the myelin sheath in two demyelinating syndromes in mice. The cells were transplanted into the spinal cords of immune-deficient mice suffering from spinal cord injuries, and into the brains of myelin-deficient *Shiverer* mice which harbor a defect in the gene that encodes myelin basic protein, a major component of myelin. Not only did the transplanted human cells and their progeny survive for long periods and migrate to various sites in the mouse nervous system, but particular cells differentiated to acquire markers for either oligodendrocytes or neurons which in some cases formed synapses with host neurons. Axons in both mouse models were shown to be myelinated by human oligodendrocytes formed from the transplanted stem cells. Most interestingly, mice with spinal cord injury displayed some recovery of motor function which was correlated with the quality of the human cell graft.

In a third model, researchers transplanted human CNS stem cells into the hippocampus of mice afflicted with a model of Batten disease. Batten disease is an inherited, neurodegenerative disease that afflicts children causing seizures, motor skill deterioration, mental retardation, and early death. Preservation of host neurons was observed in Batten mice that received a graft of normal human CNS stem cells. In October 2005, the FDA

approved the first neural stem cell transplantation clinical protocol for six children with Batten disease.

Weissman stressed that preclinical research such as the examples he described could be advanced through increased availability of immune-deficient mice and access to large collections of cell surface markers for the isolation of pure stem cell populations. A key technical need is the development of high-speed, large-scale cell sorters that perform with the same accuracy as those now used for small numbers of cells.

Bad Fate Decisions: The Genesis of Cancer Stem Cells

The multi-step mutation model of cancer teaches that oncogenesis is the result of a series of mutations, "probably three to eight," according to Michael Clarke. For a cell to become tumorigenic, this collection of mutations must endow the cell with the capacity to divide uncontrollably. In order to accumulate multiple mutations, a cell must be long-lived; to amplify the accumulated mutations, the cell must also have the ability to proliferate. Most cells in the body are terminally differentiated and have finite life-spans. For these cells to contribute to tumor formation, they would have to acquire a series of mutations during their lifespan that interfere with programmed cell death, and promote uncontrolled, long-term proliferation.

In contrast, stem cells already possess two characteristics that make them particularly prone to tumorigenesis: they are long-lived, accumulating mutations over their lengthy lifetimes, and they self-renew throughout the life of the organism. Unlike cancer cells, stem cells do not proliferate uncontrollably. They do, however, undergo many rounds of cell division, generating daughter cells that bear the cumulative load of mutations accrued with time; some of these mutations may cause uncontrolled growth. In adult organisms, the biological stage appears to be set for resident stem cells to serve as a reservoir for oncogenic mutations, making these cells a prime candidate for the cell of origin for many cancers. Indeed, in 2003, Clarke and colleagues discovered a subset of cells in breast cancer tissue that may be cancer-forming stem cells.

Normal stem cells do not cause cancers because their proliferation is tightly regulated by genetic and environmental constraints. Multiple genes control stem cell self-renewal and proliferation. One such gene is *Bmi-1*, which encodes a transcriptional repressor that

regulates gene expression. Over-expression of the Bmi-1 protein has been observed in several leukemias suggesting a potential role in tumor formation. Further research has demonstrated that the Bmi-1 protein is functionally required for self-renewal of leukemic stem cells and also for normal hematopoietic stem cells. In the former case, cancer stem cells appear to have escaped the genetic constraints on proliferation exhibited by normal stem cells, including the controlled expression of the Bmi-1 protein. These findings imply that a detailed understanding of mechanisms of self-renewal in stem cells can provide insights into the genesis of cancer and possibly spur the discovery of novel therapeutic approaches.

As outlined by Clarke, the cancer stem cell model predicts that "cancer stem cells give rise to phenotypically diverse cells that recapitulate the complexity of the original tumor. Other tumor cells have limited proliferative capacity." This model contrasts with the stochastic model, which hypothesizes that all cells in the tumor can serve as sources for new tumors that comprise a collection of phenotypically heterogeneous cells. To distinguish between these two models, human breast cancer cells were sorted according to the presence or absence of a number of cell-surface markers, and the isolated cell populations were injected into immune-deficient mice. Only cells that were positive for the CD44 marker and negative for the CD24 marker ($CD44^+/CD24^-$) were able to form tumors in the mice. Similarly, when cells from a tumor of the colon were sorted and isolated, only ~10% appeared to be cancer stem cells and gave rise to tumors in immune-deficient mice. In addition, Clarke and colleagues found that tumors formed when the $CD44^+/CD24^-$ breast cancer stem cells were injected into mouse breast tissue, but not when injected into other tissues. Irving Weissman has also reported that when cells from different human tumors were engrafted into mice, they grew only in the tissues where they were found in the human. For example, human leukemia cells only grew in the mouse hematopoietic system and glioblastoma stem cells grew only in the mouse brain. These observations suggest that cancer stem cells may require specific environmental cues to support tumor formation and that the niche might therefore constitute a target for anti-cancer drug discovery [see Scadden's presentation in *Session 4*].

The cancer stem cell model has implications for cancer diagnosis, prognosis, and treatment. It follows from the model that the metastasis of cancer stem cells - and not the metastasis of non-tumorigenic cancer cells – leads to the formation of new tumors; this might explain why not all women with microscopic deposits of breast cancer cells in their bones develop new tumors. The finding also implies that the cancer stem cell composition of the original tumor

could predict the likelihood that a patient will suffer a relapse. Under this paradigm, effective cancer treatments must target the elusive cancer stem cells.

To influence cancer diagnosis and treatment, physicians and scientists must be able to identify cancer stem cells within the context of a tumor. This is a formidable task because cancer stem cells are rare and difficult to distinguish from other cells. One approach has been to subject cancer stem cells to gene expression profiling in the hope that the gene expression patterns – called gene signatures – of cancer stem cells differ from those of non-tumorigenic cancer cells. Clarke shared unpublished data collected by Oncomed Pharmaceuticals (a company founded by Clarke), that suggest that cancer stem cells can be identified by their gene signatures. In fact, in examining multiple types of cancer, patient survival was shown to correlate strongly with specific gene signatures. Gene signatures might also reveal the identity of cell-surface markers that appear selectively on cancer stem cells. With such markers in hand, the isolation of cancer stem cells could be streamlined and accelerated. Researchers believe that a detailed molecular characterization of cancer stem cells will lead to the discovery of drugs that kill cancer stem cells selectively and, thus, quell the recurrence of tumors.

Discussion and Conclusions

A stem cell can undergo self-renewal, progress on to a particular stage in a differentiation pathway, or even become a cancer stem cell. Understanding the mechanisms that regulate differentiation and self-renewal in human ESCs, normal human adult stem cells, and their malignant counterparts is a pivotal goal for stem cell researchers. Through such studies, scientists can discover how fate decisions in stem cells are influenced by their expression of specific factors and cell surface receptors and by their environments. Armed with this knowledge, scientists can develop the tools needed to steer stem cells along desired differentiation pathways, control certain (undesirable) fate decisions, and develop protocols to ensure that engineered stem cells will acquire and retain the expected functions *in vivo*.

To achieve a full understanding of how stem cells make fate decisions, enhanced technologies and novel reagents are required. Protocols specific for human cells must be formulated for the differentiation of human ESCs into desired cell populations for basic and clinical research purposes. These protocols will employ a variety of tools including tissue-

specific growth factors, genetic switches, and small molecules to inhibit self-renewal, stimulate specific differentiation, or even to “arrest” cells at a particular point in a given differentiation pathway. Sophisticated genetic screens, genetic fingerprinting, and purification schemes that exploit antibodies to novel cell surface markers are also means that make it possible to distinguish and isolate specific cell populations.

To understand and be able to control the fate decisions made by human ESCs and their derivatives *in vivo,* researchers must introduce them into animals – and analyze the behavior of the transplanted cells as well as the response of the host to them. The chimeric animals thereby created are absolutely crucial to the development of safe and effective stem cell transplantation protocols. Finally, all stem cell-based therapies for specific diseases, degenerative conditions and injuries must be tested in one or more animal models for safety and efficacy before moving on to clinical trials.

Session 4: Bridging the Gap between Bench and Bedside

Presenters:

David Scadden, M.D., Harvard Medical School

Jon Odorico, M.D., University of Wisconsin

Hans Keirstead, Ph.D., University of California, Irvine

Moderators:

Ian Duncan, Ph.D., University of Wisconsin

Arlene Chiu, Ph.D., CIRM

Key Points of the Session:

- For the stem cell field to advance, researchers require new cell-surface markers and other tools that permit the identification of different cell types at different stages of differentiation in humans and animal models.
- Novel and sophisticated imaging tools and techniques are needed to allow much more precise characterization and tracking of transplanted cells *in vivo* than is currently possible. Tools and techniques that allow non-invasive imaging in living animals and subjects are of particular value.
- Results of preclinical studies and clinical trials need to be brought back into the laboratory for reassessment and further testing.
- Quality assurance officers and the FDA can help guide academic laboratories when projects reach the preclinical stage (if not earlier, given complexities of derivation of clinical-grade stem cell lines), particularly in the early steps of producing a clinically compliant population of cells for transplantation.
- When possible, preclinical-stage assays should be standardized and should include an assessment of cell migration, distribution and the possibility of ectopic growth (imaging), tumorigenesis and genetic stability, inflammation, and other potential complications of therapy.
- The microenvironment that surrounds stem cells (the stem cell niche) might represent a target for drug discovery.

Introduction

As affirmed throughout the symposium, the goal of human ESC research will be treatments for human disease. Session moderator Ian Duncan stressed the importance of bridging the gap between the laboratory bench where basic research is done and the clinical bedside where the fruits of this research are applied. The 'bridge' is built from preclinical studies in which knowledge gained from basic research is used to design experiments that evaluate the promise of the treatment and its efficacy of action in animal models of human disease. Preclinical studies allow scientists to assess the premise of the treatment in animals and to eliminate or refine aspects that might otherwise go awry before embarking on clinical trials conducted on patients.

The symposium began with a review of the state-of-the-art use of cell-based therapies and clinical trials for hematopoietic disorders, neurological disorders, and type 1 diabetes. To illustrate how basic research progresses toward clinical trials, this session revisits these three clinical areas – diabetes, spinal cord injury and hematogenesis – and focuses on cutting-edge, preclinical studies of stem cell-based therapies that are in the process of translation.

All cell replacement therapies must meet certain basic requirements prior to applying them to patients. First, the composition of the material to be transplanted into the host must be well-defined. A strong understanding and management of the cell differentiation process are prerequisites to producing a well-defined preparation of the target cell population in a reproducible manner. Second, if the starting material consists of cells derived from human ESCs, it is critical that the transplanted material does not include undifferentiated ESCs that might generate tumors in the host. Third, once a differentiation protocol has been established, methods for large-scale production of the appropriate cell populations must be devised. Finally, an adequate level of immunological compatibility between the host and the transplanted material must be achieved. All gains would be lost if the transplant is rejected by the patient, although this complication might be managed through life-long use of immunosuppressive drugs. These issues need to be addressed in preclinical studies both *in vitro* and *in vivo*.

Much can change when cells move from the culture dish into an organism. Questions that loom large as one attempts to bridge the gap between bench and bedside are: (i) at what stage of differentiation do the cells achieve functionality in *in vitro* assays and in animal models; (ii) how does one guide differentiation of the cells to the appropriate stage and then select the desired cells from a mixed population; (iii) how stable are the cells after transplantation into an animal; and (iv) how should their function be evaluated?

As discussed in the first session of the symposium, medical indications that are most amenable to cell replacement therapy are those in which (i) the condition results from the loss of a single mature cell type; (ii) the anatomy of the replacement tissue is not complex; (iii) there already exists proof-of-principle in the form of successful cell replacement; and/or (iv) tissue transplants are effective, but cell source is the major limitation. Jon Odorico and Hans Keirstead described preclinical studies of human ESC-based treatments for diabetes and spinal cord injury, respectively - two disorders that meet many of these criteria. In their presentations, Odorico and Keirstead highlight both unique and shared issues that they face at the preclinical stage of project development.

An alternate approach for exploiting or extending the use of stem cells for therapy was presented by David Scadden. He and his associates dissected the hematopoietic stem cell niche in bone marrow and found that they could regulate the numbers, fate, and function of stem cells by targeting the niche rather than the stem cells directly.

Cell Replacement Therapy for Human Diabetes – Bridging the Gap

Type 1 diabetes displays many characteristics that make it an amenable candidate for cell therapy. First, the disease is caused by the destruction of a single type of cell: the insulin-producing islet cells of the pancreas. Furthermore, recent clinical trials involving the transplantation of human islet cell preparations demonstrate a proof-of-principle for cell therapy. Cadaveric tissue, however, is far too limited a source of cells to meet the needs of the growing population of diabetic patients (see *Session 1*). An essentially inexhaustible source of transplant material, such as that derived from human ESCs, offers a possible solution to the dilemma posed by current islet cell transplantation therapy with cadaveric tissue.

Human islet-based transplantation has provided the bridge for embryonic stem cell transplantation in diabetes.

– Jon Odorico

In order to use human ESCs for the treatment of diabetes, significant challenges must be overcome before clinical trials can begin. First and foremost, we need to generate well-characterized populations of beta islet cells or their progenitors and demonstrate robust function in *in vitro* assays as well as in at least one animal model of the disease. According to Odorico, the cells in the transplanted preparation need not be 100% viable. Islet preparations from human cadaver donors, which are generally 80% to 90% viable, still display acceptable *in vitro* and *in vivo* function in small animal models such as mice; the same may be true for human ESC-derived islet cell preparations. Ensuring the safety of the transplanted material, including removal of any tumorigenic cells will be crucial. Although not required before initiating clinical trials, an assessment of the immunogenicity of the cell preparation will help researchers decide how much immunosuppression is needed in the transplant recipient. Finally, the protocol must be tested in a large animal model of diabetes to determine the appropriate dosage and to uncover unexpected side effects before starting clinical trials.

The development of conditions that permit the generation of functional beta islet cells from human ESCs has proven to be a challenge. Cells originally thought to produce insulin were later found to be taking up and concentrating insulin from the medium. This artifact of culture was a disappointment because, to function as islet cells, differentiated stem cells must be able to synthesize insulin on their own. In more recent studies, investigators have used expression of a marker gene, the pancreatic-duodenal homeobox protein (PDX1) as an early indicator of differentiation along the islet cell pathway. Although cells expressing PDX1 thus far have been rare in culture, Odorico reported that the conditions for their growth and differentiation are being developed and refined.

Identifying the optimal cell type and stage of differentiation for transplantation are fundamental for developing cell-replacement therapies. For example, a terminally differentiated islet cell may have all the requisite functional capabilities, but may not survive or engraft with host tissue as well as an islet progenitor cell. On the other hand, while the latter cell type may have some capacity to proliferate as a progenitor, it may not demonstrate glucose-driven secretion of insulin, the defining function of islet cells.

Decisions on what constitutes a favorable site for transplantation will undoubtedly require understanding the requirements for a "niche" in which progenitors or terminally differentiated cells can flourish and function. It may be important to co-transplant other pancreatic "support" cells in order to reconstitute the niche. These essential elements of cell transplantation therapy can be assessed in preclinical studies to identify the cell preparation and delivery approach most appropriate for successful transplantation into humans.

Finally, because type 1 diabetes is an autoimmune condition, some level of immunosuppression will always be required, even if somatic cell nuclear transfer is used to generate genetically matched material for cell replacement. As previously discussed, the tumorigenic capacity of an ESC-derived cell preparation, particularly in immune-suppressed animals and patients, is a serious safety issue. Odorico suggested sophisticated approaches to prevent the proliferation of unwanted cells such as magnetic cell sorting or the use of suicide genes to control tumorigenic potential from the transplant population.

Bridging the Conduction Gap in Acute Spinal Cord Injury

At first glance, it may not be obvious that spinal cord injury is particularly amenable to cell therapy. "Spinal cord injury" is an umbrella term that covers a range of conditions. The medical consequences of injury vary with the nature of the damage (complete vs. incomplete; transection vs. contusion), location of the lesion along the spinal column, and time following injury (acute vs. chronic). As a result of these variables and the complexity of the condition, a variety of cells and circuits may be damaged or dysfunctional; it is therefore possible to identify multiple therapeutic opportunities.

When stem cells are directed to treat spinal cord injury, repair and healing can take place via a number of routes. Cells might promote neuron survival through neuroprotective mechanisms, such as the secretion of agents that: (i) limit secondary damage or scar formation; (ii) stimulate vascularization; or (iii) promote axonal growth and regeneration. Or, stem/progenitor cells might generate new neurons that integrate into local circuits and restore connections across the damaged areas of the spinal cord. Finally, because myelination is lost following spinal cord injury, the transplanted cells might restore this insulating sheath on axons that have been denuded by the damage and thus reinstate the ability to communicate electrical signals rapidly up and down the injured cord.

Oligodendrocytes are the sole source of myelin in the central nervous system. Keirstead believes that stem cells are particularly well suited for restoring lost myelin because these cells can be readily coaxed to differentiate into oligodendrocytes. Unlike the stringent requirements for specialization, migration, and integration faced by neuronal replacement, myelin replacement merely asks the transplanted cells to locate and enwrap exposed segments of axons. Toward this goal, Keirstead and colleagues have developed protocols to generate highly purified preparations of human oligodendrocyte progenitors from human ESCs.

As a first proof-of-principle, the human oligodendrocyte progenitors increased myelin formation when transplanted into the *Shiverer* animal model, a myelin-deficient line of mutant mice. The transplanted cells differentiated into mature oligodendrocytes and produced swaths of compact myelin sheaths. Next, using a moderate contusion model of spinal cord injury in rats, Keirstead introduced human ESC-derived oligodendrocyte progenitors at the site of the lesion and, two months after the surgery, he and his colleagues assessed the fate of the cells and the functional recovery of the animals. In this type of injury, many axons are not severed, but become demyelinated around the site of the lesion. Keirstead found that the rats showed definite improvements in locomotion and other assays of behavioral recovery if oligodendrocyte progenitors were introduced 7 days after injury. In contrast, no improvements were observed in rats that underwent cell transplantation surgery 10 months after injury, suggesting that this protocol may have benefits for acutely injured patients, but not for chronic patients of spinal cord injury.

The medical literature varies, but, in general, an acute spinal cord injury is one that has existed for a few days to weeks. Keirstead hypothesized that the formation of scar tissue was one of the factors responsible for the failure of transplanted cells to remyelinate the chronic lesion. If scarring can be reduced, the transplanted cells might then be able to remyelinate the compromised axons. Keirstead speculated that pharmacological interventions to diminish the scarring, in combination with other approaches may result in treatment for patients suffering from chronic spinal cord injury in the future.

Alternative Strategies to Target Stem Cells

The therapeutic approaches proposed by Odorico and Keirstead are only possible if researchers can deliver well-defined cell preparations into the hands of surgeons for transplantation into patients. However, an alternative strategy was outlined by David Scadden of Harvard Medical School. This approach stimulates endogenous tissue regeneration through the pharmacological modulation of the hematopoietic stem cell niche. Scadden showed that manipulation of the niche components enhances hematopoietic stem cell number and controls cell fate.

He began by characterizing key components that constitute the hematopoietic stem cell niche in bone marrow. The niche consists of a variety of cell types, including mesenchymal progenitor and stromal cells, myeloid progenitors, and bone-producing osteoblasts. Using mouse genetic models to dissect the regulatory functions of these components, Scadden discovered that osteoblasts and the bone matrix glycoprotein osteopontin are key players in determining the number and function of hematopoietic stem cells. He also noticed that transgenic mice that carry a constitutively active form of the receptor for parathyroid hormone in osteoblasts produce twice as many hematopoietic stem cells as mice that lack the modified receptor. Furthermore, treatment of wild-type (normal, non-transgenic) mice with parathyroid hormone results in an increase in hematopoietic stem cell number. Scadden and his associates also found that mice treated with parathyroid hormone after bone marrow transplantation enjoyed a significant improvement in survival and higher numbers of hematopoietic stem cells compared with mice that underwent bone marrow transplantation in the absence of parathyroid hormone treatment. These studies reinforce the idea that the endogenous stem cell niche constitutes a therapeutic target.

Patients with Hodgkin’s disease, non-Hodgkin's lymphoma, and multiple myeloma undergo a grueling treatment regimen that includes leukopheresis and high-dose chemotherapy followed by autologous stem cell rescue. This treatment protocol sometimes fails because patients end up with too few white blood cells to fight off infections. Scadden reported that an experimental regimen, in which parathyroid hormone is administered prior to the return of autologous stem cells, is currently being tested in patients with the hope that parathyroid hormone will help to speed repopulation of the bone marrow with the infused hematopoietic stem cells.

Scadden speculated that other currently existing drugs may be found, like parathyroid hormone, to modulate stem cell niches. Indeed, he and his associates have shown that hematopoietic stem cells respond to changes in calcium concentrations in the niche via a calcium-sensing receptor and the calcium-binding protein, calmodulin. Agents that modulate the activity of the calcium/calmodulin system are known and might represent starting points for the discovery of drugs that control the biology of hematopoietic stem cells by altering the niche.

Discussion and Conclusions

"Bench-to-bedside is a two-way street." – Arlene Chiu

To bring reliable and standardized protocols for transplantation of stem cells or their derivatives into the clinic, significant research efforts directed at the generation of high-purity, well-defined, and clinically useful cell preparations are required. This effort includes basic research on lineage-specific differentiation, which, in turn, requires the discovery of stage-specific markers for the various cell lineages and the development of assays to assess the functions of cell populations both *in vitro* and *in vivo*. For example, Odorico felt that a better understanding of pancreatic development would help researchers identify specific genetic and cell-surface markers for definitive endoderm and for islet progenitor cells. With these markers in hand, lineage- and stage-specific selection tools can be produced; such reagents are essential for acquiring and/or sorting out cell populations that are optimal for transplantation purposes. *In vitro* and *in vivo* assays are needed to assess the purity of cell preparations and to test the ability of a progenitor to proliferate and yield the appropriate mature cell types. Finally, investigators must define clinically compliant growth conditions for generating the cells of interest. Lack of planning during these formative steps, Keirstead stressed, could lead to delays in moving therapies to the clinic.

Keirstead reiterated the following points made in earlier sessions: (i) modulation of the immune response to transplanted material is an important area of research; and (ii) the development of improved and novel imaging techniques will allow clinicians and scientists to follow the migration, proliferation, fate, and function of transplanted cells in living patients and animal models with greater fidelity and resolution.

To target the stem cell niche as a means of stimulating repair by selected endogenous stem cell populations, Scadden stressed that we first need to understand how various niches control the functions of their resident stem cells. It is possible that niches in different mammalian tissues act via distinct mechanisms. Fred Gage commented that astrocytes and endothelial cells are crucial components of the neural stem cell niche and asked whether hematopoietic stem cells, like neural stem cells, prefer to be closely associated with blood vessels. Scadden replied that hematopoietic stem cells reside in two niches, one at the endosteal surface and another in less well-defined settings around blood vessels. The notion that blood vessels can serve as a niche for hematopoietic stem cells is in agreement with developmental studies, which show that primitive blood elements emerge from the ventral floor of the embryonic dorsal aorta. Whether blood vessels serve as a niche for multiple stem cell types is a provocative hypothesis given the fact that neural, hematopoietic, and mesenchymal stem cells all reside therein. It was also suggested that further illumination of niche mechanisms might come from studies on the role of the niche in salamander limb regeneration and fin regeneration in the zebra fish, a model organism highly amenable to genetic manipulation.

If preclinical studies represent a bridge between the bench and bedside, then it is important to remember that the bridge runs in both directions. Although clinical trials often fail on the first try, new knowledge from both successful and unsuccessful trials can be brought back to the laboratory and used to design experiments that re-examine and extend basic premises. This process will lead to refined hypotheses and improved protocols for subsequent preclinical and clinical testing. In this way, basic, preclinical, and clinical research form a partnership that facilitates the development of well-designed, successful clinical trials and, ultimately, efficacious treatments for patients.

Session 5: Stem Cells and Therapies – Lessons from the FDA and Industry

Presenters:

Jane Lebkowski, Ph.D., Geron Corporation
Alan Smith, Ph.D., Cognate Therapeutics
Donald Fink, Ph.D., Food and Drug Administration

Moderators:

Edward Penhoet, Ph.D., Vice Chair, ICOC,
Susan Bryant, Ph.D., ICOC

Highlights of the Session:

- For projects with potential for therapeutic applications, stem cell researchers need to consider the scope and requirements for preclinical testing early in the research process.
- The FDA and scientists in the stem cell field should work together to clearly define and streamline preclinical efforts.
- Although some preclinical quality assurance measurements will be project specific, certain standard assays will likely be required for many cell replacement projects. Stem cell scientists should work to identify common assays to enable therapeutic applications.
- The development of stem cell-based therapies may give rise to new approaches to data-sharing (bioinformatics tools), as well as novel model systems, reagents, cell lines, and bioengineering facilities.
- Cell preparations intended for clinical use will require Good Manufacturing Practice (GMP) facilities for their derivation and subsequent handling.
- A central resource for learning about GMP quality control, and quality assurance for stem cell-based treatments will be helpful to basic scientists.

Introduction

"It is not like the early days of recombinant DNA technology, when there were more than 2,000 biotechnology companies focused on some aspect of recombinant DNA technology," said session moderator and vice chair of CIRM's Independent Citizens' Oversight Committee, Edward Penhoet. Today, there are only about 20 companies devoted to stem cell science. Commercial research organizations have long borne responsibility for and maintained expertise in, performance of preclinical studies and execution of Investigational New Drug (IND) application filings. With fewer companies dedicated to stem cell science, academic researchers will need to take on some or all of these roles if stem cell therapies are to be rapidly translated to clinical settings. Traditionally, academic scientists have been neither adequately trained nor appropriately funded to assume these roles. In this session, two representatives from industry – Jane Lebkowski and Alan Smith – and one spokesperson from the FDA – Donald Fink – described various aspects of the road-less-traveled that awaits basic scientists whose ultimate goal is to develop stem cell-based therapies. One message was echoed by all the speakers: if they are to move their results efficiently into the clinic, academic scientists must understand preclinical and clinical regulations and requirements long before their work reaches these stages.

Preparing for Preclinical Studies

While academic research often provides first proof-of-principle for a particular treatment, basic researchers generally lack the knowledge and training required to advance an early discovery through to clinical evaluation. Industry, on the other hand, is well-versed in product development but not always willing to take on projects without compelling evidence of therapeutic potential in humans. This gap can be bridged by strong preclinical studies. According to Jane Lebkowski of Geron Corporation, preclinical studies focus on issues of safety, efficacy, dose exploration, and route of administration. An important consideration is the economics of therapy development – the ultimate treatment must be affordable for the patient and for the company hoping to develop it.

In designing preclinical studies for new stem cell-based therapies, researchers can draw on past experience with other cell-based therapies. Among several issues that investigators need to consider are: (i) the source of the cells (different sources require different controls);

(ii) what the cells are supposed to do *in vitro* and *in vivo*; (iii) which model systems are most appropriate (more than one system may be necessary); (iv) what dose is required for the desired effect (an estimate that draws from animal studies with theoretical calculations for human equivalence); (v) where the cells need to go *in vivo* (how they will be delivered and tracked *in vivo*); (vi) what impurities exist in the cell preparation and whether they influence observed outcomes; and (vii) what the target patient population is.

One of the most important goals is to determine the range of cell dosage that is likely to be safe and efficacious. Other parameters also need to be defined, including route of administration, transplantation site, the final product formulation, and the dose(s) of the transplanted material. Once the cells are transplanted, it is critical to understand whether or not they leave the transplant site and if so where they go and what happens to them. Polymerase chain reaction-based assays and sophisticated imaging techniques may be used to investigate cell fate after transplantation. From this information, researchers may be able to determine whether the final *in vivo* location of the cells affects safety or efficacy. Do the transplanted cells survive, and, if not, who not? Tumorigenic, infectious, and inflammatory properties of the cell preparation also must be assessed. Lebkowski pointed out that developing and validating potency assays for stem cell therapies may be more challenging than for small molecule drug discovery efforts. According to Alan Smith, the efficacy of specific treatments in clinical trials may require the evaluation of multiple parameters.

Geron is at the preclinical stage, and it has been very expensive. – Jane Lebkowski

Translation from academic laboratories to preclinical testing can be costly and time consuming, warned Lebkowski. Among others, review of a protocol may require approval from an institutional review board, an animal care committee, and a biosafety committee; the FDA's Center for Biologics Evaluation and Research, and, when appropriate, the Secretary's Advisory Committee on Xenotransplantation. A comprehensive list of FDA guidance documents for researchers attempting to make the bench-to-preclinical testing transition is available at: http://www.fda.gov/cder/guidelines.htm

We will require a new model of sharing … of data, reagents, and bioengineering facilities. — Alan Smith

Lebkowski believes that for maximum effectiveness, the preclinical process must be clearly defined and streamlined. Although some preclinical assays will be project specific, a number of standard methods and reagents could be applied to many cell-replacement projects. All the speakers in this session urged the stem cell research community to identify these common needs and share efforts to create commonly useful tools and technologies in order to improve the efficiency of the preclinical process. Reagents such as antibodies to cell surface marker antigens, animal and cellular models, novel bioinformatics and imaging techniques, and specialized assays – for example, assays for tumorigenic capacity, pathogen detection, and clonogenic assessments – could be used by many groups and would lower the overall costs of the research if pursued in a collaborative manner.

Developing Good Manufacturing Practices

The production of all cell-based therapeutics must follow a set of standard operating procedures (SOPs) that includes detailed documentation at each step of the process. SOPs are devised by a research team preparing to bring a therapy to clinical application and are critical elements of GMP used in translational research. Stem cell-based therapeutics must follow SOPs beginning with the derivation of stem cells, and must include all manipulations, scale-up operations, characterization procedures, storage, shipping and utilization, to name a few of the steps involved. The use of SOPs provides standardized documentation for the FDA throughout process development and also limits the variables that contribute to reliability, reproducibility and consistency of cellular products. For example, archiving of clinical samples is necessary to deal with questions that may arise; if a problem occurs in a preclinical experiment or during a clinical trial, one should be able to trace the samples and the personnel who handled them to the early stages of derivation to determine causes of the problems and possible solutions to them. SOPs for documentation are also useful. Documentation requires numerous and detailed data entries, as well as systematic methods for updating documents to remove old versions from circulation.

Smith emphasized that the key to commercialization is the ability to manufacture, store, and ship high-quality material reliably, reproducibly, and consistently. It is crucial, therefore, for stem cell scientists to have access to cell processing facilities that follow GMP guidelines. If CIRM decides to provide such facilities for manufacturing to California scientists, planning for this capital-intensive endeavor (estimates start at $3 – 5 M) should begin immediately.

Comprehensive facilities are expensive to build, operate and maintain. They require experienced and specialized staff and state-of-the-art cell culture machinery that uses controlled manufacturing processes; a highly-trained team that is well-versed in quality assurance protocols; substantial cell and reagent storage capabilities; emergency power capabilities; laboratories for assay development and product testing; and a layout in which waste and in-coming manufacturing materials and reagents never cross paths. Furthermore, GMP facilities must meet the requirements of the California State Department of Health Services Food and Drug Branch, which inspects such facilities and issues licenses to compliant institutions. Finally, Smith urged academic researchers to seek advice from the FDA on preclinical and clinical protocols in advance of requesting a pre-IND meeting, and to take advantage of the expertise of the FDA early in the process.

Lost in Translation

Despite great strides in fields such as genome sequencing, proteomics, cellomics, systems biology and molecular characterization of diseases, the number of novel, cell-based therapy applications submitted to the FDA remains small. Many scientific advances thus remain to be translated into innovative approaches for treating patients. Often, investigational cell therapies are held up in the process of clinical evaluation. Because the development and approval processes for novel, cell-based medical products are quite different from those required to develop drugs, the FDA has initiated efforts to develop and provide an appropriate set of tools that will facilitate clinical application of stem cell-based discoveries.

Fink reported that the FDA developed its Critical Path Initiative to help generate "new ideas that make sense in this new frontier." The mission of the Critical Path Initiative is to focus on "the need for employing scientific efforts to modernize techniques and methods used to evaluate the safety, efficacy, and quality of medical products during translation from candidate selection and design to market distribution." Scientific advances that might be applied to the medical product development process include computer simulation models, newly validated biomarkers, and novel clinical trial designs. The FDA aims to stimulate the development of applied research programs in critical-path scientific areas. These programs will minimize obstacles encountered during product development and facilitate FDA involvement in the preclinical process.

To obtain a biologics license for a stem cell-based product, the Code of Federal Regulations for Food and Drugs requires that sterility, purity, potency, identity, stability, safety, and efficacy be demonstrated through analytical and clinical testing. Fink outlined what the FDA deems necessary to move a product into early clinical trials that assess safety. He summarized the many steps on the path to development of a stem cell-based product. Standardized criteria must be developed for accepting donated biological materials for the production of a stem cell-based investigational product. In addition, investigators need to evaluate human cell applications for safety. Manufacturing process controls, which include standardization and optimization of reagents and processing procedures, as well as product characterization and development of acceptance criteria, must also be developed. Detailed characterization of stem cell-based therapeutics will necessitate multi-parametric analytical testing for verification and characterization of products intended for clinical testing. Parameters to be developed and assessed in preclinical evaluation include the use of phenotype-specific cell surface antigens, biochemical markers, gene and protein expression analysis, the amounts and nature of cellular impurities, biological activity (a reflection of potency), and major histocompatibility complex (MHC)/human leukocyte antigen (HLA) expression. Such preclinical evaluation is required before Phase 1 clinical trials can begin.

It is expected that additional tests must be developed to facilitate the success of stem cell-based treatments in clinical trials. For example, techniques to compare cell preparations of differing origins (such as those derived from different donor samples or using variant protocols for differentiation) will be needed. Tests that can confirm the genetic stability of cells that have been maintained in culture for long periods of time will also be of critical importance. Toxicity, dose, potency, and clinical parameters that will be used to monitor patients during the trials must be developed and evaluated in preclinical animal-based or *in vitro* model systems. Novel methods for delivery of the cells need to be tested for their ability to reliably deliver the appropriate number of cells to the correct physiological environment. Finally, non-invasive imaging technologies with the necessary resolution will be invaluable to the assessment of the *in vivo* fate of cellular products at different times after transplantation.

A clear message underscoring the collaborative intent on the FDA was a theme of the session. Researchers were encouraged to contact the FDA's Center for Biologics Evaluation and Research for help early and often in negotiating the critical path to Phase 1 clinical safety trials. Said Fink, "We're anxious to be in touch with you."

Discussion and Conclusions

With few studies approaching the preclinical stage, some participants were less enthusiastic than Smith in recommending immediate investment of CIRM funds in GMP facilities. Paul Berg and Irving Weissman both argued for what they called "just-in-time" facilities, to be developed when a particular line of cells from basic research enters the preclinical domain. Others voiced agreement with this assessment and added that only small-scale facilities are needed to support early preclinical experiments. According to this idea, large pharmaceutical companies would be likely to partner with researchers with clinically-viable projects and would provide for large-scale GMP facilities. For the benefit of academic investigators, Fink pointed out that a GMP facilities license is not required prior to submission of a Biologics License Application for FDA approval of their product. California institutions with GMP facilities include UCLA, Stanford, UCSF, and City of Hope. Owen Witte and others remarked that before investing large amounts of money in the construction of new facilities, CIRM should assess resources already available in California. If and when more manufacturing capacity is needed, CIRM should consider supporting an expansion of existing facilities, but that should not happen immediately.

The complex process of producing a clinically compliant cell preparation with required documentation is new territory for many academic researchers. The challenge of progressing from promising cell preparations with desired activities in laboratory assays to GMP products that show efficacy in patients is significant, and resources to help academic researchers along the way will be of critical importance. Owen Witte pointed out that preclinical consulting services are already available at several major California institutions. If CIRM finds that lack of preclinical consulting is a barrier to progress, the Institute might consider supporting the development of such a service for investigators in California. CIRM could look to the International Society for Cellular Therapy (ISCT) for help; established in 1992, the goals of ISCT are to "provide communication, discussion, education, and training regarding recent developments in the basic science, processing, translational, and regulatory aspects of cell, tissue, and gene therapies."

Session 6: Stem Cells as Tools for Disease Research and Therapy

Presenters:

Rudolf Jaenisch, M.D., The Whitehead Institute, MIT

Peter Schultz, Ph.D., The Scripps Research Institute

Inder Verma, Ph.D., The Salk Institute

Moderators:

Rusty Gage, Ph.D., The Salk Institute,

Mary Maxon, Ph.D., CIRM

Key Points of the Session:

- An increased number of human ESC lines of known genetic background is necessary to advance basic and disease research.
- Using somatic cell nuclear transfer, researchers may create ESC lines that represent specific models of human diseases. These cellular reagents will permit the molecular characterization of a variety of diseases leading to the identification of new drug targets, and can serve as cell-based assay systems for drug discovery.
- The basic biology of nuclear reprogramming should be investigated, using first animal then human models. A key goal is to decipher the mechanisms by which the oocyte accomplishes somatic nuclear reprogramming.
- Methods for the introduction and stable expression of genes in human ESCs are limited; new viral vectors and methods for homologous recombination need to be developed.
- High-throughput screening of small molecule libraries on human ESCs can hasten the discovery of therapeutic molecules and tools for manipulating stem cell growth and differentiation.

Introduction

Embryonic stem cells are very useful artifacts. – Rudolf Jaenisch

Besides their leading roles on the therapeutic stage, stem cells play other key roles in basic research. The best-known and most widely publicized use of stem cells is for cell replacement therapy, in which stem cells or their derivatives are implanted into patients to replace damaged or dead cells. Many scientists, however, are equally excited about the use of stem cells as research tools for scientific discovery, possibly leading to novel therapeutic opportunities. In fact, many believe that the earliest pay-off of stem cell research will come from an improved understanding of the mechanisms of disease.

Much of the excitement arises from research on somatic cell nuclear transfer, a technique that may allow the creation of cell lines with genetic predispositions to particular diseases. These "disease-specific" cell lines can potentially be used to create human cellular models of many inherited disorders. These models can be used to explore disease mechanisms and to screen compounds for therapeutic efficacy or toxicity. Rudolph Jaenisch discussed issues related to nuclear transfer technology, including new directions that this research might take.

To make full use of human stem cell lines both for research and therapy, scientists must be able to introduce genes and nucleic acid sequences into cells and to control and maintain their expression. Inder Verma described recent progress in identifying viral vectors for human stem cell lines.

High-throughput chemistry also has emerged as a key technology for the discovery of biologically functional molecules. Peter Schultz reviewed how this technology has evolved and how it can be used to identify small molecules for therapy or for controlling stem cell fate and self-renewal.

Embryonic Stem Cells as Tools

Nuclear transfer experiments in animals demonstrate its importance for studies of disease. In somatic cell nuclear transfer, the nucleus from a mature, differentiated cell is transferred into an unfertilized, enucleated oocyte. Mild electrical stimulation then induces the nuclear

transfer construct to undergo several rounds of cell division and differentiation, resulting in the formation of a blastocyst. In animals, implantation of a blastocyst derived in this way into the uterus of a pseudo-pregnant female can give rise to a cloned animal ("reproductive cloning"), although the success rate is extremely low. This milestone, first achieved by creation of a sheep named "Dolly", demonstrated for the first time in mammals that the oocyte can successfully reprogram an adult nucleus – which normally displays the gene expression pattern of a differentiated cell – so that it functions as an embryonic nucleus with the full potential for producing a complete organism.

Several observations suggest, however, that nuclear reprogramming by the oocyte is incomplete or highly inefficient, resulting in an abnormal blastocyst. Successful implantation and birth, for example, occurs at a much lower frequency with blastocysts from somatic cell nuclear transfer than with blastocysts derived from *in vitro* fertilization. In addition, the cloned animals that do result often exhibit physiological abnormalities. In fact, Rudolph Jaenisch asserted that "a normal animal has yet to be produced by reproductive cloning," a view shared by some, but not all in the field. The defect in the nuclear transfer blastocyst presumably arises because the adult cell nucleus has undergone epigenetic changes during development, and these changes are not completely reversed following transfer of the nucleus into an oocyte. Epigenetic changes are defined as those that lead to a change in the pattern of gene expression without a change in the underlying DNA sequence. Gene expression array experiments confirm this in that a significant fraction of imprinted genes (i.e., genes that are selectively expressed depending on whether they are inherited from the mother or father) are not properly expressed in nuclear transfer blastocysts. Jaenisch also pointed out that nuclei from different adult somatic cells in mice vary in their ability to be reprogrammed, as measured by the ability to form blastocysts: neurons are difficult, adult stem cells are easy, and breast cells are somewhere in-between. Because all cells from a single animal have the same DNA, this heterogeneity presumably reflects differences in epigenetic DNA modifications among the various cell types. Taken together these results suggest that there may be a fundamental difference between blastocysts produced by *in vitro* fertilization and those produced by somatic cell nuclear transfer.

Surprisingly, there is no evidence of molecular or biological differences between ESCs generated from mouse blastocysts that were created by nuclear transfer and those derived from blastocysts produced by *in vitro* fertilization of mouse oocytes. Jaenisch suggests that

the "harsh" conditions of generating and adapting ESCs to culture completely removes the epigenetic modifications characteristic of adult nuclei. In fact, ESCs may be an artifact of culture that selects for a particular type of cell that is not naturally present in the animal (see the *Session 2* summary on stem cell self-renewal).

Although somatic cell nuclear transfer (SCNT) has yet to be successfully performed using human cells, the technique has the potential to create cell lines that have the same genetic background as that of a patient. This could be accomplished by transferring the nucleus of a mature cell from a patient into an enucleated oocyte; the human ESCs derived from the resulting blastocyst would be genetically identical to the patient, and are therefore “patient-specific.” One potential benefit is that cells and tissues produced from patient-specific ESCs would be less likely to be rejected by the immune system when transplanted into that patient. Scientists hope to develop these techniques for patient-specific tissue engineering. A second benefit is the ability to produce “disease-specific” cell lines since the resulting stem cell lines would bear the genetic mutation(s) leading to the patient’s medical condition.

Jaenisch cited several experiments in which nuclear transfer constructs have been used to study fundamental issues of great clinical significance - epigenetic versus genetic causes of disease and the feasibility of cell replacement therapies. In the first study, nuclei from cancer cells in adult mice were transferred into unfertilized mouse oocytes and then used to produce mice by reproductive cloning. The resulting progeny were found to have few cancers, suggesting that epigenetic rather than genetic changes are a major factor in oncogenesis. Jaenisch also described an important proof-of-principle experiment illustrating that cell replacement therapy can be used to treat an incurable genetic disease in mice. First, the nucleus of an adult somatic cell was isolated from an immune-deficient strain of mice (*Rag2*$^{-/-}$, a mouse model analogous to human "bubble-babies") and transferred into an enucleated mouse oocyte. The resulting blastocyst was used to produce an immune-deficient ESC line, which was then genetically engineered by introducing a normal or wild-type version of the *Rag2* gene into the genome through homologous recombination. The *HoxB4* gene was also inserted into the genome to stimulate blood cell proliferation. Following successful transfer of these two genes, the ESCs, now *Rag2*$^{+}$, *HoxB4*$^{+}$ were grown in culture under conditions that caused them to differentiate into hematopoietic stem cells. When these genetically engineered cells were transplanted into the *Rag2*$^{-/-}$, immune-deficient mice, partial correction of the immune defect was achieved.

Because of their importance to disease research, every effort must be made to derive genetically-defined human stem cell lines. Although the procedure has been demonstrated in animals, no human stem cell lines have been derived by SCNT. One barrier to widespread use of this technique in humans is the requirement for oocytes which must be obtained only under stringent ethical standards.

Alternative methods that would avoid the use of human oocytes are being developed. One approach involves the use of blastocysts following testing for disease-specific genes in pre-implantation genetic diagnosis (PGD). This form of genetic diagnosis is used by couples where one or both members are at high risk for conferring a genetic disease to their children. A single cell is isolated from an embryo derived by *in vitro* fertilization for genetic analysis. Embryos that are determined to carry a disease-specific mutation are not implanted. These embryos can be used to create disease-specific human ESCs. Today, dozens of genetic diseases including Tay Sachs disease, Huntington's disease, cystic fibrosis, and retinoblastoma can be diagnosed in this manner, so presumably dozens of genetically-defined, disease-specific ESC lines could be generated from PGD-tested embryos.

Jaenisch strongly urged support of research on another approach: developing cell-free methods of reprogramming adult nuclei. By studying the molecular machinery that governs early embryonic development, it should be possible to devise methods to accomplish this feat. Such experiments would not only yield valuable information about the molecular underpinnings of embryonic pluripotency – or "stemness" – but would also allow human ESC lines to be made without oocytes and the ethical and practical challenges that currently attend somatic cell nuclear transfer.

Tools to Manipulate Embryonic Stem Cells

Altering genes with viral vectors. The experiment described above in which a genetic defect in mice was corrected by introducing a gene into an autologous stem cell line that was subsequently used in cell therapy illustrates the utility and importance of methods that allow precise genetic manipulation of stem cells. Such methods could, in principle, be used to regulate stem cell self-renewal, direct the differentiation of ESC lines (see later sessions), investigate the biology of individual ESC lines, and correct genetic defects.

Unfortunately, the genetic methods that have proven to be so powerful in experiments with mouse cells are much less reliable when applied to human cells. For example, procedures that allow homologous recombination in human ESCs are not well-developed (only one example has been reported). In addition, the introduction of genes into human cells with the use of viral vectors has been problematic. The retroviral vectors currently used to manipulate human ESCs have three major drawbacks: they integrate into the genome in a nonspecific manner, they are unable to infect non-dividing cells, and they fail to sustain prolonged gene expression after infection and integration.

Recent experiments by Inder Verma and colleagues suggest the possible utility of lentiviral (LV) vectors in the genetic manipulation of human ESCs. Verma presented data on a novel HIV-based LV vector that has several advantages over other viral vectors now in use. This LV vector is able to transduce many types of dividing and non-dividing cells, and is non-infectious.. When the LV vector was used to introduce a marker gene (green fluorescent protein) into one of the federally-approved human ESC lines, gene expression was maintained over a period of 22 weeks after engraftment of the transduced cells into immune-deficient mice. In addition, the cells remained "stem-like" in that they continued to express the stem cell marker, Oct 4 and retained the ability to form teratomas in immune-deficient mice when tested for pluripotency.

LV vectors are also being used to express siRNAs as a means of silencing the expression of specific genes in stem cells. This technique has significant clinical implications because it could be used to shut off defective or disease-causing genes. Verma and colleagues have created an inducible LV vector that can be used to turn the silencing siRNA on and off as needed. On-going studies between the Gage and Verma laboratories suggest that the gene control elements from a fly gene, the *Drosophila* ecdysone receptor, might be a useful tool for achieving differential regulation of multiple genes in stem cells simultaneously. Although these findings are promising, much more research is needed to refine the methods and confirm the results in other human ESC lines. Because the work of developing this methodology is costly and time-consuming, Verma suggested that one or more core facilities that would produce, test, and store viral vectors for shared use could be of major benefit to California researchers.

A screening approach to stem cell biology. Peter Schultz made a compelling argument for the importance of high-throughput screening of small molecule libraries in stem cell research. The impressive technology, which also can be applied to growth factors, antibodies, siRNAs, or cDNAs, uses ordered arrays of compounds created with automated plating methods, sophisticated robotics for automated cell screening, and intricate data analysis software to test up to 2 million molecules per day. Using these techniques, Schultz's team has identified molecules that hold stem cells in a self-renewal state as well as others that can direct differentiation along specific pathways. Two examples of this avenue of research are summarized below.

First, Schultz described an assay for identifying agents that induce stem cells to form cardiac muscle cells. A reporter gene (luciferase under the control of the promoter for atrial natriuretic factor, a heart-specific protein) expressed only in differentiated cardiac myocytes was introduced into mouse embryonic carcinoma cells, which were then used to screen a library of compounds. By scoring for molecules that caused expression of the reporter gene, Schultz and colleagues were able to identify a novel compound, cardiogenol C that induces cardiac muscle cell differentiation. Addition of cardiogenol to cultures of mouse ESCs produced spontaneously contracting cardiac muscle cells.

Schultz also described a cell-based screen to discover molecules that maintain pluripotency in mouse ESCs. Researchers first engineered a mouse ESC line that expresses the Oct 4 protein fused to green fluorescent protein (GFP) as a marker for pluripotency. Oct 4 is a transcription factor that is characteristic of ESCs; GFP is a fluorescent marker protein that can be visualized by fluorescence microscopy. By transfecting cells with an expression vector that contains the gene for the Oct 4-GFP fusion protein, visualization of GFP can be used to detect Oct 4 expression and, thus, maintenance of the stem cell state. When these mouse cells are grown without the use of feeder cells or leukemia inhibitory factor, they begin to differentiate and no longer synthesize Oct 4-GFP. Using this cell line to screen for agents that maintain Oct 4-GFP expression under conditions where cells would normally begin to differentiate, a potent compound was identified that allows the self-renewal of mouse ESCs in simple, chemically defined media. This compound also shows activity with human ESCs, and biochemical and genomics studies have begun to elucidate a novel mechanism of action.

Perhaps an even more promising use of screening technologies in stem cell research will involve studies with genetically-defined, “disease-specific” human ESC lines and cell types derived from them. Such studies may not only lead to insights into the fundamental mechanisms of disease, but may also provide new molecules with therapeutic potential to prevent, treat or cure disease.

> If there is a central screening resource, "efficiency and the quality of people you can hire go up and cost goes down."
>
> – Peter Schultz

Schultz stressed that a central high-throughput screening facility with appropriate infrastructure – including a highly-trained staff, capability for library generation and curation, automated screening systems and data analysis, and an extensive database linking chemical structure with functional activity – could serve investigators throughout the state, and urged CIRM to consider playing a pioneering role in such an effort.

Discussion and Conclusions

The session revealed that stem cell research will benefit greatly from the development of critical tools, reagents and techniques. Few individual laboratories or institutions however are able to bear the financial burden of creating these tools alone. One recurrent suggestion is that CIRM could create central facilities or cores where new technology and tools for stem cell research would be developed and/or made available to researchers in California.

Because genetic manipulation of human ESCs is necessary for a broad spectrum of experiments, there is a need for more tools such as viral-based vectors, inducible gene systems, and siRNAs. The development of vector cores would provide much-needed support for these costly and time-consuming efforts. Another recommended central facility is one designed to conduct high-throughput screening of small molecule libraries using novel stem cell-based assays. The ability to carry out such screens can lead to powerful pharmaceuticals as well as requisite research tools.

If homologous recombination can be conducted in human ESCs efficiently, researchers will be able to integrate genes into specific regions of the chromosome where these genes can be continuously expressed with no adverse side effects. Once the mutation and gene that

cause a specific disorder are known, investigators will be able to apply homologous recombination to create cell lines bearing the gene defect.

Somatic cell nuclear transfer is an important technique that may allow researchers to develop disease-specific human ESC lines even when the gene(s) responsible have not been identified. The availability of such lines, which are essentially model systems for human disease, will permit characterization of these genetic syndromes at the molecular level. Equally important, they will provide the basis for drug discovery using cell-based high-throughput assay systems. At present, the requirement for oocytes is a significant hurdle that prevents broad use of this technique. It is not yet understood how an oocyte is able to reprogram a nucleus taken from differentiated or mature cells. If reprogramming can be accomplished in a test tube, however, this will eliminate the need for human oocytes. Thus, more research is needed to understand the mechanisms involved in the reprogramming of nuclei from adult somatic cells.

Inder Verma formerly served on the NIH Recombinant DNA Advisory Committee (RAC) which reviews the state of gene transfer research and therapy in the United States. When asked what lessons stem cell researchers might learn from clinical trials designed to assess therapeutic uses of recombinant DNA, he replied that his experience was consistent with the report issued by the Panel to Assess the NIH Investment in Research on Gene Therapy. This panel, established by then NIH Director Harold Varmus and chaired by Arno Motulsky and Stuart Orkin, reported that the participating basic scientists were often too eager to move quickly into the clinic and did not spend enough time developing the optimal vectors for clinical use. Creating a vector core for Californian scientists may be a wise and timely investment for stem cell research.

Finally, Theo Palmer of Stanford University pointed out that many of the biological problems studied by basic researchers were first defined clinically. He stressed the importance of maintaining a strong reciprocal connection between basic and clinical research as stem cell research progresses.

RECOMMENDATIONS FROM THE SYMPOSIUM

The field of stem cell research "needs more transformative thinking. …Think of Eric Lander and the genome project. … California is in a position to do something transformative." – George Daley

The goal of CIRM is to fund research on stem cells to develop therapies and treatments that will help prevent, detect, diagnose and treat disease and disability. Proposition 71 also mandates that priority be given to research that:

(i) Is not funded or underfunded by the NIH
(ii) Is risky and innovative
(iii) Is of the highest quality
(iv) Enables young researchers to enter the stem cell field

The recommendations presented at the symposium largely fall into four categories: Basic and Clinical Research Areas, Tools, Core Facilities, and Strategic Approaches. The proposed priority for implementing each recommendation is described as near-term (**NT**) if it is to be initiated in the first 3 years; intermediate term (**MT**) to begin in 3-6 years; and long-term (**LT**) to begin in 5 years or more.

I. Basic and Clinical Research Areas

Because the field of stem cell research is new, scientists lack a comprehensive understanding of the basic biology of ESCs. The molecular bases of many fundamental properties of stem cells are still unknown. The following 6 research projects are recommended to provide fundamental knowledge that is required for the design of meaningful preclinical and clinical applications of stem cells.

1) Creation of new embryonic stem cell lines **(NT)**
   The creation of new and novel cell lines is a high priority for the whole field. Because federal funds can only be used for work on a limited number of existing cell lines, U.S. scientists have extremely limited resources to create new lines such as those described below. Proposition 71 provides one of the few avenues for this effort in the U.S.

a. More “normal” or wild-type lines are needed for genetic diversity and to obtain clinical grade lines free of exposure to animal cells or products.
b. Disease-specific human ESC lines, produced either by homologous recombination, or with embryos from pre-implantation genetic diagnosis (PGD) or via somatic cell nuclear transfer (SCNT) are critical for studying disease mechanisms in familial disorders, for drug discovery and for the development of treatments and diagnostics.
c. Development of robust cryopreservation techniques will enable stem cell researchers to ensure quality, viability and reliability of frozen stem cell lines and preparations for research and clinical applications.

2) Controlling self- renewal of human embryonic stem cells **(NT- MT)**
A fundamental understanding of the processes that regulate ESC self-renewal is essential for basic and clinical research.
a. Developing standard culture conditions that minimize or eliminate the use of animal cells and products while optimizing self-renewal, homogeneity and genetic stability will provide a basis for the development of clinical-grade cell lines.
b. Understanding the mechanisms and regulation of self-renewal will allow large scale production of stem cell populations - a prerequisite for therapeutic treatments and cell transplantation.
c. Determining methods for reversible blocking of differentiation by human ESCs may facilitate the maintenance of stem cells in a self-renewing state.

3) Lineage-specific differentiation by stem and progenitor cells **(NT-MT)**
A detailed molecular characterization of stem cell differentiation, especially human ESCs is required to generate appropriate and functional target cell preparations for cell replacement.
a. An understanding of, and control of, lineage commitment and maturation pathways pf different target populations will enable preclinical research and provide a basis for the reliable production of therapy-grade cells.
b. Development of methods to control self-renewal may allow the prevention of teratoma formation by transplanted cells.

4) Understanding of stem cell fate decisions **(MT-LT)**

The discovery that cancer stem cells may be the etiological cause of several cancers is a recent and potentially revolutionary finding with tremendous potential for drug discovery and novel treatment approaches. It also highlights the fact that stem cells may make fate decisions that lead them astray. Understanding the molecular mechanisms that allow such transformations of a stem cell is vitally important for preventing tumor formation when stem cells are used for therapy. Recommended areas of research are:

   a. Identification of cancer stem cells and distinction from normal stem cells or tumor cells.
   b. Identification of oncogenic changes as early biomarkers of disease and targets for study of basic mechanisms of renewal, differentiation, and proliferation.

5) Somatic cell nuclear reprogramming **(MT-LT)**

Understanding of mechanisms employed by oocytes to reprogram the nucleus from a somatic cell is needed for the long-term goal of reprogramming somatic cells for tissue repair. These studies include:

   a. Mechanisms of somatic cell nuclear reprogramming using oocytes
   b. Developing methods for reprogramming nuclear function without oocytes

6) Modulation of the immune system for recipients of cell transplants **(MT-LT)**

Graft rejection and graft-versus-host disease are significant obstacles to successful cell replacement. Research on inducing tolerance or reducing rejection of transplanted cells will address these obstacles. Recommended areas of research are:

   a. Development of techniques to cross MHC barriers
   b. Methods to re-educate the immune system to accept alloantigens on transplanted cells.

II. Tools

Numerous calls were made throughout the meeting for the development of a variety of research tools and essential reagents that are too labor-intensive and expensive for any single laboratory to shoulder. These include:

1) Bioinformatics tools for collecting, sharing, and analyzing large datasets **(NT-MT)**

2) Library of antibodies against stage-specific cell surface markers for identifying, sorting and purifying cells at different stages of differentiation **(NT-MT)**

3) Collection of DNA microarray profiles for stem cells and their differentiated progeny to "fingerprint" cells at various stages of differentiation **(NT-MT)**

4) Standardized and quality-controlled reagents and growth factors for cell culture to reproduce experimental conditions more effectively across different laboratories **(NT-MT)**

5) Imaging approaches and reagents to track the fate and function of transplanted cells, especially using non-invasive technologies **(MT)**

6) *In vivo* cell delivery methods and techniques that minimize immune rejection or reaction and promote therapeutic function such as encapsulation and scaffolds **(MT)**

7) Guidance and consulting on the application of federal regulations, including those of the FDA to assist investigators in the planning and implementation of preclinical research **(MT)**

III. Core Facilities

A consistent theme throughout the conference was a recommendation for the development of state-wide research core facilities that would decrease financial and labor burdens imposed on individual laboratories, and greatly facilitate stem cell progress through the provision of sophisticated tools and technologies. The availability of such shared regional facilities will be a tremendous advantage for Californian stem cell investigators, particularly those working in small institutions. These include:

1) Vector core **(NT-MT)** to develop molecular tools for:
    a. Conditional gene expression in stem cells
    b. Bioimaging markers to track cells and cell components

   c. RNAi vectors for regulating protein expression
   d. Facilitating homologous recombination in human cells

2) Small animal core **(NT-MT)** to assess cell preparations that are intended for transplants. Such a core will develop and run standardized assays to evaluate:
   a. Tumorigenicity (using immune-deficient mice and other models)
   b. Cellular function and stability
   c. Efficacy
   d. Toxicology

3) Pre-clinical core **(NT-MT)** to train and guide researchers on regulatory issues, good manufacturing practice and quality assurance in preparation for preclinical projects.

4) Embryonic stem cell bank **(MT)** for:
   a. Derivation of new ESC lines
   b. Analysis and characterization of ESC lines
   c. Maintenance/distribution of well-characterized human ESC lines to researchers in California

5) High-throughput screening core **(MT)** for:
   a. Basic studies such as screening for small molecule or other regulators of:
      i. Self-renewal
      ii. Differentiation
   b. Drug discovery studies using:
      i. "Normal" or wild-type cells for studying toxicology
      ii. Disease-specific cells for drug discovery

6) Large animal facility **(MT-LT)** for the use of large mammals to assess cell preparations that are being planned for application in clinical trials as transplants. The following will be assessed:
   a. Tumorigenicity
   b. Cellular function and stability
   c. Efficacy
   d. Toxicology

7) Good Manufacturing Practice (GMP) cores GMP facilities currently exist in California at UCLA, UCSF, Stanford University and the City of Hope Medical Center. The type of GMP facility needed however varies with the stage of development of a treatment. Recommendations for new GMP facilities include:
    a. Small GMP facilities **(NT)** — used in early stages of cell derivation in anticipation of obtaining clinical grade cells in the future
    b. Medium GMP facilities **(MT)** — to produce sufficient quantities of clinical grade cells for Phase I trials
    c. Large GMP facilities **(LT)** — for manufacturing quantities of cells for late phase clinical trials involving large numbers of patients.

   At present, few projects have yet to reach the preclinical or clinical stage; therefore, the requirement for large GMP facilities is not seen as an immediate need.

IV. Strategic Approaches

Four recommendations describe strategic approaches that will promote the growth of stem cell research in California and the integration of this field with other disciplines.

1) Organize a Strategic Planning Committee to provide the CIRM with advice on scientific and clinical matters ranging from basic research approaches to designing effective clinical trial networks. In order for the CIRM to benefit from a broad range of ideas and points of view, this group should not include members of the ICOC or of any of the CIRM Working Groups.

2) Encourage multidisciplinary and interdisciplinary collaborations between clinicians, engineers, physicists, computer scientists, chemists as well as biologists from other disciplines to work with stem cell scientists on complex problems and technologies.

3) Encourage partnerships between academic scientists and industry to accelerate the process of developing promising preclinical studies into actual treatments for patients.

4) Support a sabbatical program to encourage a global exchange of ideas, tools and reagents between Californian stem cell scientists and scientists in other parts of the country and the world.

GLOSSARY

This is an alphabetized glossary of essential concepts to grasp the ideas presented in this report.

Adult stem cells. These undifferentiated cells have been isolated from a number of differentiated tissues and are distinct from ESCs isolated from embryos. Adult stem cells undergo self-renewal and can differentiate into specialized cell types, but to a more limited extent and do not appear to be as plastic as ESCs. A well-studied source of adult stem cells is the bone marrow which contains hematopoietic stem cells (which form the mature cells found in blood), endothelial stem cells (which form blood vessels), and mesenchymal stem cells (which form bone, cartilage, and fat). Neural stem cells from the CNS are capable of forming cells (neurons, astrocytes and oligodendrocytes) of the nervous system.

Aneuploidy. An abnormality involving changes in chromosome number where one or more chromosomes of a normal set of chromosomes are missing or present in more than their usual number of copies. Such abnormalities can have drastic effects on phenotypic expression.

Autoantibody. An antibody that reacts with antigens found on the cells of a person's own body. Autoantibodies can cause autoimmune diseases.

Autoimmune disease. A condition in which a person's immune system attacks that person's own tissues, leading to inflammation and tissue destruction. Autoimmune disease occurs when the process of immune tolerance goes awry. Examples of autoimmune diseases are rheumatoid arthritis, lupus and type 1 diabetes.

Blastocyst. An early stage mammalian pre-implantation embryo that consists of a hollow ball of several hundred cells. If implanted into a uterus, the outer layer of cells forms the placenta and the interior cluster of cells (the inner cell mass) forms the embryo. ESCs are produced by culturing the cells of the inner cell mass. ESCs from mice, monkeys, dogs and humans have been generated by this method. Human ESCs are produced from unused embryos that resulted from *in vitro* fertilizations for reproductive purposes.

B lymphocytes. A type of white blood cell that produces a single, specific antibody. When stimulated by antigen, B lymphocytes mature into plasma cells that produce large quantities of the specific antibody.

Bone marrow*.* Tissue that fills bone cavities and contains hematopoietic stem cells (which form the mature cells found in blood), endothelial stem cells (which form blood vessels), and mesenchymal stem cells (which form bone, cartilage, and muscle).

Clonal. A clonal population of cells arises when a single cell, through symmetrical cell divisions, produces a colony of "clones" -- cells that are identical to the parental cell.

Data mining*.* The use of sophisticated statistical algorithms in the analysis of large amounts of data. Mining allows researchers to identify patterns in large databanks and thus to discover novel insights from existing data.

Embryoid bodies. Aggregates of cells that self-assemble in cultures of ESCs. This type of aggregation is the first sign of differentiation. Embryoid bodies will begin to differentiate spontaneously into ectoderm, mesoderm, and endoderm.

Embryonic carcinoma cells. Cell lines produced from stem cells isolated from germ cell tumors.

Embryonic stem cells (ESCs)*.* Isolated from the inner cell mass of a blastocyst-stage embryo, ESCs are pluripotent and have the capacity for long-term self-renewal.

Feeder cells. The layer of non-dividing cells on which ESCs are grown in culture. Mouse embryonic fibroblasts are often employed as feeder cells to nurture the growth of ESCs in culture. ESCs can also be grown without feeder cells if certain additives are present in the culture medium.

Immune rejection. A condition that occurs in organ transplants and cell replacement therapies. Rejection of the transplanted material is caused by the differences between the major histocompatibility proteins on the donor's cells and the transplant recipient's cells. The

rejection process is spurred by the donor's T lymphocytes. A condition known as graft-versus-host disease results from such an immune response-mediated injury to the engrafted tissues.

Immunosuppression. Treatment of transplant patients with drugs that blindfold the immune system to prevent it from responding to the transplanted antigens and rejecting the graft. Immunosuppression protocols are adjusted in a variety of ways in hopes that the alloantigen response is preferentially affected.

***Major histocompatibility complex* (MHC).** A collection of genes that encode cell-surface histocompatibility antigens. These antigens are the chief cause of rejection of allogeneic tissue transplants.

***Markers**.* Proteins and sugars often uniquely expressed by a specific cell type that provide information about the cell. For example, human ESCs express Oct 4, and Nanog intracellularly; these proteins appear to be associated with a pluripotent phenotype. Cells can be sorted on the basis of the markers they possess. Antibodies to the marker proteins are generated and can be detected with a fluorescent probe. If the markers are on the cell surface, investigators can selectively retrieve cells using a fluorescence-activated cell sorter, which separates individual cells on the basis of the amount of fluorescence it emits.

Niche. The microenvironment inhabited by adult stem cells *in vivo*. Studies are in progress to determine whether the *in vivo* behavior of adult stem cells can be directed by manipulating the environs of the cells.

Pre-implantation Genetic Diagnosis (PGD). This procedure is useful for couples with a known history of genetic disease and involves screening the early stage embryos formed by IVF before they are implanted into the uterus. A single cell is removed from the embryo and the genetic material is examined to screen for genetic abnormalities. Only embryos without known genetic defects are implanted into the uterus. Some of the diseases which may be screened out with this procedure include Downs Syndrome, Tay Sachs disease, Huntington’s disease and cystic fibrosis as well as many X-linked diseases.

Pluripotent. Having the ability to differentiate into cells that form all three embryonic germ layers — the ectoderm, mesoderm and endoderm,.

Progenitor cells. Early descendants of stem cells that may have a limited ability to replicate and may display a more limited repertoire of cell types that it can become. Progenitor cells are further along in a cell type-specific differentiation pathway than ESCs. Investigators hope to use endogenous progenitor cells and their progeny to repair adult organs.

Somatic cell nuclear transfer (SCNT). A technique by which the nucleus from an adult somatic cell is transferred into an egg whose nucleus has been removed. The nuclear-engineered egg is then encouraged to develop into a blastocyst from which ESCs are generated. Scientists hope to use this technique for producing disease-specific stem cells for drug discovery or patient-specific stem cells for tissue engineering. To do so, the nucleus from a somatic cell isolated from a patient is introduced into an enucleated human oocyte, thus creating a blastocyst with the same genetic background as the donor who contributed the nucleus. ESCs made from such an engineered blastocyst will also have this genetic background and can serve as cellular models of the disease for research. If they are directed to produce differentiated cells of interest, the resulting tissue also would be genetically identical to that of the patient who donated the nucleus. When cells and tissue generated from these ESCs are transplanted into the patient for treatment, the graft should not be recognized as foreign and therefore will not be rejected by the patient's immune system. This process is referred to as therapeutic cloning. This technique has been successfully used in animals for reproductive cloning – the production of an animal that has a genetic background identical to the animal who donated the somatic cell nucleus for transfer. This process was used to create Dolly the sheep.

Teratoma. When ESCs are injected into immune-deficient mice, they develop into benign tumors called teratomas. This process is used to test for the pluripotency of ESCs because teratomas comprise cell types from all three primary germ layers of the embryo. The ability to generate teratomas *in vivo* is a defining feature of ESCs.

T lymphocytes. White blood cells that constitute an important part of the immune system. Helper T cells express the CD4 protein on their surface and initiate an immune response by producing mediators such as cytokines. In contrast, cytotoxic or killer T cells have the CD8

protein on their surface. Once activated by helper T cells, cytotoxic T cells destroy infected cells in the body.

Tolerance. Immature T cells have the ability to recognize a wide array of antigens, including some antigens that exist on the surface of cells in the body of the host (self-antigens). In order to prevent T lymphocytes from attacking the body's own cells, T cells are “educated” in the thymus, a tissue that resides in the upper chest cavity where T cells that interact with self-antigens are eliminated. This education process is referred to as induction of tolerance. For cell replacement therapies, one goal is to use short-term or intermittent therapies that harness the basic immune regulatory systems and cause the re-education of the immune response so that it treats alloantigens as self.

Biographical Sketches: Presenters and Moderators

Peter W. Andrews, D.Phil.
University of Sheffield, United Kingdom
Dr. Andrews is the Arthur Jackson Professor of Biomedical Science and former chairman of the Department of Biomedical Science at the University of Sheffield. In addition, he co-founded and serves as director of Axordia Ltd., a company established to develop commercial applications of stem cell biology. Dr. Andrews received a B.S. from the University of Leeds, a D.Phil. from Oxford University, and an M.B.A. from the University of Pennsylvania's Wharton School. A leader in developing infrastructure to support collaborative stem cell research, he coordinates the International Stem Cell Initiative and has organized an annual U.K. human embryonic stem cell training course. He is also a member of the User Liaison and Management Committees of U.K.'s Stem Cells Bank. Dr. Andrews' own research interests focus upon the biology of human embryonic carcinoma cells and human embryonic stem cells.

Nissim Benvenisty, M.D., Ph.D.
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Dr. Benvenisty received his M.D. (1983) and Ph.D. (1986) in developmental biochemistry from Hebrew University of Jerusalem, Israel, and is currently a professor in the Department of Genetics and the Herbert Cohn Chair in Cancer Research at the Hebrew University. In 1999, he was a visiting professor in the Department of Genetics at Harvard University. Among the numerous distinctions that he has received are the Teva Prize for excellent research on stem cells in 2003 and the Hestrin Prize in Biochemistry and Molecular Biology. Dr. Benvenisty is a member of the International Stem Cells Initiative Steering Committee.

Paul Berg, Ph.D.
Stanford University, Palo Alto, CA
Dr. Berg, the Robert W. and Vivian K. Cahill Professor in Biochemistry and Cancer Research and professor emeritus at Stanford University, is a renowned pioneer of the gene-splicing field. He received his B.S. from Pennsylvania State University (1948) and his Ph.D. in biochemistry from Case Western Reserve University (1952). After postdoctoral work in Copenhagen, Denmark he joined the faculty of Washington University Medical School (1954). In 1959, he moved to Stanford University where he served as executive head of the Department of Biochemistry from 1969 to 1974. Professor Berg has received international recognition and many prestigious awards for his work on the genetic mechanisms through which cells form proteins. Most notably, Dr. Berg was awarded the Lasker Basic Science Award and the Nobel Prize in Chemistry for developing methods to map the structure and function of DNA.

Jeffrey Bluestone, Ph.D.

University of California, San Francisco, CA

Dr. Bluestone is the A.W. and Mary Margaret Clausen Distinguished Professor and Director of the Diabetes Center at the University of California, San Francisco (UCSF). He currently serves as director of the Juvenile Diabetes Research Foundation Collaborative Center for Cellular Therapy and was interim director (2002-2004) of UCSF's Developmental and Stem Cell Biology Program. A graduate of Rutgers University (B.S., M.S.) and Cornell University (Ph.D.), Dr. Bluestone has received numerous awards for his research and is internationally recognized as an expert on diabetes and immune system research, in particular for his contributions toward clarifying the biological basis of immune tolerance. He is perhaps best known for studies on molecular-level approaches to control the immune activity of antibodies and efforts to boost the beneficial effects of tolerance-inducing drugs. This research has stimulated recent progress in the use of islet cell transplantation to treat type 1 diabetes.

Susan V. Bryant, Ph.D.

University of California, Irvine

Dr. Bryant is Professor of Developmental and Cell Biology and Dean of the School of Biological Sciences at the University of California, Irvine. She obtained her undergraduate degree at King's College and her Ph.D. degree at St. Mary's Hospital Medical School, University of London in the United Kingdom. Dr. Bryant then studied as a postdoctoral fellow at Case Western Reserve University, after which she joined the faculty at UC Irvine. In 2005, she was elected a Fellow, the highest honor bestowed by the Association for Women in Science. Her research includes wound healing and pattern development during the processes of development and regeneration of the limb. She has served on national committees, including Advisory Boards for the VA Office of Regeneration Programs, and for the Indiana University Axolotl Colony. Dr. Bryant is a member of the Independent Citizens' Oversight Committee, the governing body of the California Institute for Regenerative Medicine. She also serves on the editorial boards of *Developmental Biology*, *Regenerative Medicine* and the *Journal of Experimental Zoology*.

Arlene Y. Chiu, Ph.D.

California Institute for Regenerative Medicine

Dr. Chiu, director of scientific activities at CIRM, served as associate director of the Office of Research Administration of the National Institute of Biomedical Imaging and
Bioengineering at the National Institutes of Health (NIH). Prior to that, she was the program director for Stem Cell Research and for research on Spinal Cord Injury at the National Institute of Neurological Disorders and Stroke. Dr. Chiu served on the NIH Stem Cell Task Force and the NIH Stem Cell Implementation Committee, organized workshops on stem cells and led efforts to promote cooperation with the U.S. Food and Drug Administration in expediting the use of stem cells in therapies. In 2004, she received the NIH Director's Award for her outstanding contributions to the development of stem cell research. Dr. Chiu graduated *summa cum laude* from Stanford University and received pre-doctoral training at the California Institute of Technology and postdoctoral training at Washington University, St.

Louis. As an independent investigator, her research focused on mammalian motor neurons and their responses to injury and disease.

Michael Clarke, M.D.
Stanford University, Palo Alto, CA
Dr. Clarke is deputy director of the Cancer Stem Cell Institute and Professor of Internal Medicine at Stanford University. After receiving his medical degree from Indiana University he specialized in general oncology with a main focus on blood and marrow transplantation. Dr. Clarke's research focuses on the molecular regulation of hematopoiesis and hematopoietic transformation, gene therapy and the molecular mechanism of malignant transformation. His laboratory was the first to identify a molecular pathway that regulates self-renewal of adult stem cells, and has developed methods to prospectively identify a "cancer stem cell" population in breast cancer. These findings link the process of self-renewal in normal stem cells to cancer and offers implications for the treatment and diagnosis of human cancers.

George Q. Daley, M.D., Ph.D.
Harvard Medical School, Boston, MA
Dr. Daley is an associate professor of Biological Chemistry and Pediatrics at Harvard Medical School. He received his bachelor's degree *magna cum laude* from Harvard University (1982), and a Ph.D. in biology from MIT (1989), working with Nobel laureate David Baltimore. Dr. Daley also earned an M.D. from Harvard Medical School and the Harvard-MIT Division of Health Sciences and Technology. Among his many awards, Dr. Daley is a recipient of the NIH Director's Pioneer Award, which provides a five-year unrestricted grant to pursue highly innovative research. Dr. Daley's laboratory (together with Dr. Rudolf Jaenisch) reported the first successful application of therapeutic cloning of embryonic stem cells to treat a genetic disease in a mouse model of immune deficiency, and the first creation of functional sperm cells from embryonic stem cells, work that was cited by *Science* magazine as a "Top Ten" breakthrough for 2003. Dr. Daley's research is aimed at translating insights in stem cell biology into cellular therapies for degenerative, malignant, and genetic diseases.

Ian D. Duncan, Ph.D.
University of Wisconsin, Madison, WI
Dr. Duncan is professor of neurology in the Department of Medical Sciences at the University of Wisconsin, Madison, School of Veterinary Medicine. He received his doctorate in neuropathology from Glasgow University in the United Kingdom and completed a postdoctoral fellowship in experimental medicine at McGill University in Montreal, Canada. Dr. Duncan has received significant recognition for his research contributions, including election as a fellow of the Royal College of Veterinary Surgeons and of the Royal College of Pathologists for meritorious contributions to the literature. His research focuses on the use of embryonic stem cell-derived progenitors in brain repair. Dr. Duncan's main interest is to investigate the potential of glial cell transplantation as a therapeutic approach to repair the demyelinated or nonmyelinated areas of the central nervous system.

Donald W. Fink, Jr., Ph.D.
Food and Drug Administration, Center for Biologics Evaluation and Research, Rockville, MD
Dr. Fink is a regulatory review scientist at the FDA, in CBER's Office of Cellular, Tissue and Gene Therapies, where he coordinates CBER's human embryonic stem cell review team and serves as the FDA liaison to the NIH Stem Cell Task Force. Dr. Fink received his B.A. from Northwestern University and his Ph.D. in pharmacology from the University of Minnesota. With over 10 years of experience evaluating Investigative New Drug applications and compiling a review portfolio of biologic products that includes recombinant therapeutic proteins, monoclonal antibodies, therapeutic vaccines, and cellular therapies, Dr. Fink recently has been actively involved in regulatory matters pertaining to biologic products comprised of stem cells. He has organized an FDA advisory committee meeting about the use of stem cells in cellular replacement therapies for neurological disorders, and co-founded and co-chaired (with Dr. Arlene Chiu) an FDA-NIH interagency cell and gene therapy working group. Dr. Fink recently completed a detail appointment in the FDA's Office of Combination Products and is a member of the Tissue Reference Group.

Fred H. Gage, Ph.D.
Salk Institute, La Jolla, CA
Dr. Gage is a professor of Laboratory Genetics at the Salk Institute for Biological Studies. Known for his discovery of structural and functional plasticity in the adult mammalian brain, Dr. Gage has shown that adults continue to generate new neurons throughout life, and that birth and survival of the neurons is regulated by behavior. He also demonstrated that neurotrophic factors can induce functional repair of the damaged and aged brain. Dr. Gage earned his B.S. from the University of Florida and his Ph.D. from Johns Hopkins University. He has received broad recognition for his research, including the Charles A. Dana Award for Pioneering Achievements in Health and Education, the Christopher Reeve Research Medal, and the Max Planck Research Prize. He is past president of the Society for Neuroscience and a member of the National Academy of Sciences.

Zach W. Hall, Ph.D.
California Institute for Regenerative Medicine
Dr. Hall is president of the CIRM, established by Proposition 71 to promote stem cell research in California. A former director of the National Institute of Neurological Disorders and Stroke, Dr. Hall recently served as the senior associate dean at the Keck School of Medicine of the University of Southern California, and as executive vice-chancellor of the University of California, San Francisco, where he led planning for the new Mission Bay campus. He has published over 100 original articles and was a founding editor of *Neuron*, a leading journal in the field of neuroscience. In 2003, he received the Purkynje Medal for Scientific Achievement from the Czech Academy of Science. An English major at Yale University, Dr. Hall received his Ph.D. from Harvard University followed by a postdoctoral

fellowship at Stanford University. He was a faculty member at Harvard Medical School and later at the University of California San Francisco.

Rudolph Jaenisch, M.D.
Massachusetts Institute of Technology, Cambridge, MA
Dr. Jaenisch is a professor of biology at the Massachusetts Institute of Technology and founding member of the Whitehead Institute for Biomedical Research in Cambridge, MA. After completing his thesis in molecular biology at the Max Planck Institute for Biochemistry in Munich, he received postdoctoral training at Princeton University and at the Salk Institute in molecular virology before moving to his current position at the Whitehead Institute. Dr. Jaenisch is a pioneer in the use of transgenic mice; his work has led to important advances in understanding cancer, neurological and connective tissue diseases, and developmental abnormalities. Mice from his laboratory have been used to explore basic questions such as the role of DNA modification, genomic imprinting, X chromosome inactivation, and most recently, the nature of stem cells, epigenetic reprogramming and somatic cell nuclear transfer. The long-range goals of his laboratory are to understand epigenetic regulation of gene expression in mammalian development and disease. Dr. Jaenisch has coauthored more than 300 research papers and has received numerous prizes and recognition, including an appointment to the National Academy of Sciences in 2003.

Hans Keirstead, Ph.D.
University of California, Irvine, CA
Dr. Keirstead is an associate professor at the Reeve-Irvine Research Center, a leading center for spinal cord injury research, at the University of California at Irvine. His thesis research, conducted at the University of British Columbia in Vancouver, Canada, led to a novel method for regeneration in the damaged spinal cord and formed the basis of several patents and the formation of a company in 1999 to bring this treatment toward clinical trials. For these achievements, he received the Cameron Award for outstanding Ph.D. thesis in Canada. As a postdoctoral fellow at the University of Cambridge, he was elected to two senior academic posts: Fellow of the Governing Body of Downing College, and Senate Member of the University of Cambridge. Dr. Keirstead was recently awarded the Distinguished Assistant Professor Award, the UCI Academic Senate’s highest honor. The focus of his research is the development of strategies to limit degeneration and enhance regeneration after spinal cord injury, and his laboratory has developed human reagents necessary for clinical trials.

Jane S. Lebkowski, Ph.D.
Geron Corporation, Menlo Park, CA
Jane Lebkowski received her Ph.D. in biochemistry from Princeton University in 1982. Following a postdoctoral fellowship in the Department of Genetics, Stanford University, she joined Applied Immune Sciences in 1986, where she served as vice-president of Research and Development. When Applied Immune Sciences was acquired by Rhone Poulenc Rorer (RPR), Dr. Lebkowski remained at RPR (currently Sanofi-Aventis) as the vice-president of Discovery Research. In that position she coordinated preclinical investigations of gene therapy approaches for the treatment of cancer, cardiovascular disease and disorders of the nervous system, and directed the vector development, formulation, and delivery programs. In 1998, Dr. Lebkowski joined Geron Corporation as the senior director of Cell and Gene

Therapies, and is currently senior vice-president of Regenerative Medicine. Dr. Lebkowski heads the human embryonic stem cell program at Geron, and coordinates both preclinical research and product development activities.

Haifan Lin, Ph.D.
Duke University, Durham, NC
Dr. Lin is an associate professor in the Department of Cell Biology and the founding co-director of the Duke University Stem Cell Research Program. He received his B.S. in biochemistry from Fudan University in Shanghai, China (1982), and his Ph.D. from Cornell University (1990), where he studied maternal control of embryonic mitosis in *Drosophila*. For his thesis research, Dr. Lin received Honorary Mention (second place) for the Larry Sandler Award by the Genetics Society of America (1990). He was awarded a Jane Coffin Child Fellowship for his postdoctoral training at the Carnegie Institution of Washington, where he studied oocyte determination mechanism in *Drosophila*, and pioneered the use of the *Drosophila* germline stem cell as a model for stem cell research. Dr. Lin's accomplishments in stem cell research include the discovery of the *piwi/argonaute* gene family, presently the only known gene family that regulates stem cell self-renewal – a function that is highly conserved during evolution in both animal and plant kingdoms. Dr. Lin has received several prestigious awards for his scientific contributions, is a founding officer of the International Society for Stem Cell Research and a member of the editorial board of the journal *Stem Cells*.

Olle Lindvall, M.D., Ph.D.
University of Lund, Sweden
Dr. Lindvall is the chairman of the Department of Clinical Neuroscience, professor of neurology, and head of the Section of Restorative Neurology and the Clinical Neurotransplantation Program at the University of Lund in Sweden. He received his Ph.D. (1974) and M.D. (1978) from the University of Lund and has distinguished himself as a world-renowned neurologist and researcher in gene and cell therapy for Parkinson's disease. Among the many awards that Dr. Lindvall has received are the Jubilee Prize from the Swedish Society of Medicine, an honorary medal from the Swedish Parkinson Association, and the Soderberg Prize from the Swedish Society of Medicine. Dr. Lindvall is an elected member of the board of the Swedish Research Council's medical division and served for four years as the chairman of the Swedish Movement Disorder Society.

Mary E. Maxon, Ph.D.
Independent Citizens' Oversight Committee, CIRM
Dr. Maxon is the deputy vice chair to Vice Chair Edward Penhoet of the ICOC. She received a Ph.D. from the University of California, Berkeley in molecular cell biology, where she studied human gene expression. Dr. Maxon studied genetics as a postdoctoral researcher at the University of California, San Francisco as a Helen Hay Whitney Fellow and began her career in biotechnology at Microbia, Inc. in Cambridge, MA. Recently, she was associate director and program leader at Cytokinetics, Inc. where she led the infectious diseases drug discovery program. Earlier this year, Dr. Maxon organized a conference in India in

conjunction with the National Academy of Sciences on drug discovery opportunities for neglected diseases of the developing world and remains active in the global health community.

Andras Nagy, Ph.D.

Mount Sinai Hospital, Toronto, Canada

Dr. Nagy is a professor in the Department of Medical Genetics and Microbiology at the University of Toronto and a senior investigator in the Program of Development and Fetal Health at the Samuel Lunenfeld Institute. He completed a B.A. and an M.A. in mathematics and received a Ph.D. in genetics from the Lorand Eotvos University in Budapest, Hungary. Dr. Nagy currently holds the Canadian Institute of Health Records Senior Scientist Award, and, in partnership with Bristol-Myers Squibb, he was awarded the Medical Research Council of Canada/Pharmaceutical Manufacturers Association of Canada Scientist Award. His research interest is focused on the use of mouse genetics to study mammalian development and to apply this knowledge to human disease.

Robert S. Negrin, M.D.

Stanford University, Palo Alto, CA

Dr. Negrin is a professor of medicine at Stanford University, chief of the Division of Blood and Marrow Transplantation, and medical director of the Clinical Translational Laboratory. He received his undergraduate degree from the University of California, Berkeley and an M.D. from Harvard University. He then trained in internal medicine and hematology at Stanford University and joined the Stanford faculty in 1990. Dr. Negrin has authored over 125 manuscripts and 35 book chapters on basic and clinical transplantation immunology and hematopoietic cell transplantation. He has served as the president of the International Society of Cellular Therapy and is the president-elect of the American Society of Blood and Marrow Transplantation.

Jon S. Odorico, M.D.

University of Wisconsin, Madison, WI

Dr. Odorico is director of the Islet Cell Transplantation Program and associate professor in the Department of Surgery, Division of Organ Transplantation, at the University of Wisconsin. He also is a research associate at the WiCell Institute in Wisconsin. Dr. Odorico received his B.S. in Chemistry from Duke University, an M.D. from New York University, and completed his residency in general surgery as well as a postdoctoral research fellowship studying islet transplantation and thymic tolerance at the University of Pennsylvania in Philadelphia. Dr. Odorico has an active research laboratory that focuses on islet differentiation from embryonic stem cells.

Edward E. Penhoet, Ph.D.
Independent Citizens' Oversight Committee, CIRM
Dr. Penhoet earned his A.B. in biology from Stanford University in 1963, and his Ph.D. in biochemistry from the University of Washington in 1968. He is currently the president of the Gordon and Betty Moore Foundation in San Francisco and has served as dean and professor of the School of Public Health and professor of Molecular and Cell Biology at the University of California, Berkeley. Since 2000, he has been a director at Alta Partners, a pioneering venture capital firm that focuses on early-stage investing in life sciences, information technology and communications. He is also a co-founder and director of Chiron Corporation, one of the world's leading biotechnology companies. From the company's founding in 1981 until April 30, 1998, Dr. Penhoet served as Chiron's president and chief executive officer. Dr. Penhoet is vice chairman of the Independent Citizens' Oversight Committee, the governing body of the California Institute for Regenerative Medicine.

Martin Pera, Ph.D.
Monash Institute of Medical Research, Clayton, Australia
Dr. Pera is a research professor at the Monash Institute of Medical Research at Monash University and the director of Embryonic Stem Cell Research at the Australian Stem Cell Centre. He received a B.A. in English language and literature from the College of William and Mary, and a Ph.D. in pharmacology from George Washington University. Dr. Pera trained as a postdoctoral fellow at the Institute of Cancer Research and the Imperial Cancer Research Fund in London, and was a research fellow at the Department of Zoology at Oxford University. His research interests include the cell biology of human pluripotent stem cells, early human development, and germ cell tumors. Dr. Pera was among a small number of researchers who pioneered the isolation and characterization of pluripotent stem cells from human germ cell tumors of the testis, which provided an important framework for the development of human embryonic stem cells. His laboratory at Monash University was the second in the world to isolate embryonic stem cells from the human blastocyst, and the first to describe their differentiation into somatic cells *in vitro*. He has provided extensive advice to state, national, and international regulatory authorities on the scientific background to human embryonic stem cell research, and is a member of the Steering Group of the International Stem Cell Initiative.

David Scadden, M.D.
Harvard Medical School, Boston, MA
Dr. Scadden, a professor of medicine at Harvard Medical School, is director of the MGH Center for Regenerative Medicine and Technology. He is also co-director of the Harvard Stem Cell Institute and chief of the Hematologic Malignancies Program at the MGH Cancer Center. Dr. Scadden is a board certified hematologist/oncologist having received his training at the Brigham and Women's Hospital and the Dana-Farber Cancer Institute. His research interest is in adult stem cell biology, focusing on reconstituting immune function using the hematopoietic stem cell in settings of cancer and AIDS. He directs both a basic research laboratory and a translational clinical research program. His basic research has defined molecular regulators constraining hematopoietic stem cell proliferation and identifying critical components of the microenvironment in which stem cells reside. Dr. Scadden serves on the executive committee of the Harvard Medical School Division of AIDS and is a member of the National Cancer Institute Board of Scientific Counselors at the National Institutes of Health.

Peter G. Schultz, Ph.D.
The Scripps Research Institute, La Jolla, CA
Dr. Schultz is currently the Scripps Professor of Chemistry at the Scripps Research Institute and director of the Genomics Institute of the Novartis Research Foundation in La Jolla, CA. He received both his bachelor and doctoral degrees from the California Institute of Technology. His work, which spans the interface of chemistry, biology, and materials science, has brought him distinction and recognition through numerous awards. Most notably, Dr. Schultz was elected to the National Academy of Sciences, USA (1993) and the Institute of Medicine of the National Academy of Sciences (1998). He has also founded several biotechnology companies including Affymax Research Institute, Symyx Technologies, Syrrx, Kalypsys, Phenomix, Ilypsa, and Ambrx.

Alan K. Smith, Ph.D.
Cognate Therapeutics, Inc., Baltimore, MD
Dr. Smith earned a Ph.D. in biochemistry from Utah State University and is currently president and chief operating officer for Cognate Therapeutics, Inc. where he oversees general management, research and development, preclinical, regulatory, manufacturing, quality control and clinical functions. From 2000 to 2002, prior to joining Cognate, Dr. Smith served as chief operating officer and senior vice-president of Research and Development at Osiris Therapeutics, a company with three stem cell products for oncology, cardiology, and orthopedics currently in clinical trials. Dr. Smith has also held senior management roles in several other cell therapy companies, including Aastrom Biosciences, Inc. and Geneic Sciences. Dr. Smith also serves on the board of directors of Chata Biosystems of Fort Collins, CO and MGP Biotechnologies of Irvine, CA.

Inder M. Verma, Ph.D.
The Salk Institute, La Jolla, CA
Dr. Verma is one of the world's leading authorities on the development of viruses for gene therapy vectors. He received his Ph.D. in biochemistry at the Weizmann Institute of Science in Israel and completed his postdoctoral work at MIT. He is a professor in the Laboratory of Genetics at the Salk Institute and was named the American Cancer Society Professor of Molecular Biology. His laboratory has two principal aims: 1) understanding the molecular mechanism of the function of protooncogenes and suppressor genes, and 2) gene therapy. Dr. Verma has received broad recognition for his research contributions including election to the National Academy of Sciences, the Institute of Medicine of the National Academy of Sciences, and American Academy of Arts and Sciences.

Irving L. Weissman, M.D.
Stanford University, Palo Alto, CA
Dr. Weissman is a professor in the departments of Pathology, Developmental Biology, and Neurosurgery at Stanford University. He is also the director of the Institute for Cancer and Stem Cell Biology and Medicine. He has co-founded several companies, including SyStemix in 1988, StemCells in 1996, and Celtrans (now Cellerant), the successor to SyStemix, in 2001. Dr. Weissman's research focuses on the biology and evolution of stem cells and progenitor cells, mainly from blood and brain. His laboratory was the first to identify and isolate blood-forming stem cells from mice. Dr. Weissman pioneered the study of the genes and proteins involved in cell adhesion events required for lymphocyte homing to lymphoid organs *in vivo*, either as a normal function or as events involved in malignant leukemic metastases. He is the recipient of numerous awards for his research contributions, and is a member of the National Academy of Sciences, the Institute of Medicine at the National Academy, and the American Association of Arts and Sciences.

Owen N. Witte, M.D.
University of California, Los Angeles, CA
Dr. Witte received his undergraduate degree from Cornell University and his M.D. from Stanford University. He completed postdoctoral research at MIT then joined the faculty at UCLA, where he is presently an investigator of the Howard Hughes Medical Institute. He is also a professor in the department of Microbiology, Immunology and Molecular Genetics and the department of Molecular and Medical Pharmacology at the David Geffen School of Medicine at UCLA. In 2005, he was named the Founding Director of the Institute of Stem Cell Biology and Medicine. Dr. Witte has made significant contributions to the understanding of human leukemias, immune disorders, and epithelial cancer stem cells. He is a member of the National Academy of Sciences, the American Academy of Arts and Sciences, and was recently elected to the Institute of Medicine.

Janet S. Wright, M.D., F.A.C.C.
Independent Citizens' Oversight Committee, CIRM
Dr. Wright, a member of the Independent Citizens' Oversight Committee of the CIRM, currently practices invasive cardiology with Northstate Cardiology Consultants in Chico, California. She received her M.D. from the University of Tennessee and completed her residency in internal medicine at Children's Hospital and Adult Medical Center in San Francisco. She also completed a cardiology fellowship at San Francisco General Hospital and the University of California at San Francisco. Dr. Wright is a former chair of the Cardiology Department at Enloe Medical Center in Chico and served as its first Medical Director of Cardiology. She currently is medical director of the Cardiac Rehabilitation Program.

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