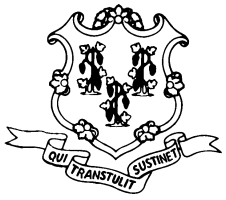
REPORT TO GOVERNOR RELL
AND
THE GENERAL ASSEMBLY

AN ACT PERMITTING STEM CELL RESEARCH AND BANNING THE CLONING OF HUMAN BEINGS

JUNE 30, 2009

Connecticut Stem Cell Research Advisory Committee
J. Robert Galvin, M.D., M.P.H., M.B.A., Commissioner; Chair



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Report to Governor Rell and the General Assembly

An Act Permitting Stem Cell Research and Banning the Cloning of Human Beings

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Public Act 05-149, "An Act Permitting Stem Cell Research and Banning the Cloning of Human Beings" (the Act), was approved by the Connecticut General Assembly and signed by Governor M. Jodi Rell on June 15, 2005. The Act appropriated the sum of twenty million dollars to the newly established Stem Cell Research Fund for the purpose of grants-in-aid for conducting embryonic or human adult stem cell research. For each of the fiscal years ending June 30, 2008 to June 30, 2015, inclusive, the Act specified that an additional ten million dollars should be disbursed from the State's Tobacco Settlement Fund to the Stem Cell Research Fund to support additional grants-in-aid.

The Act was subsequently codified at Section 19a-32 of the Connecticut General Statutes. In accordance with Section 19a-32f(g), the Stem Cell Research Advisory Committee shall report annually to the Governor and the General Assembly on: (1) the amount of grants-in-aid awarded to eligible institutions from the Stem Cell Research Fund pursuant to section 2 of the Act, (2) the recipients of such grants-in-aid, and (3) the current status of stem cell research in the State. This report covers the period from July 1, 2008 through June 30, 2009.

On March 31, 2009, the State allocated $9.8 million in grants-in-aid to researchers in Farmington, Storrs, New Haven, and Middletown. Since passage of the enabling legislation in 2005, the State of Connecticut has allocated a total of $39.42 million in support of stem cell researchers. To date, State funding has provided the resources to fully or partially support 78 positions at Yale University totaling approximately $2.2 million, and 115 scientists at the University of Connecticut totaling approximately $3.1 million. In addition, State funding supports the salaries of two graduate students and a technician at Wesleyan University. These highly skilled professionals represent a new breed of sophisticated work force in Connecticut, and are anticipated to have significant long-term impact on the State's economic development.

I. INTRODUCTION AND BACKGROUND

Public Act 05-149, "An Act Permitting Stem Cell Research and Banning the Cloning of Human Beings"[1] (the Act), was approved by the General Assembly of the State of Connecticut and signed into law by Governor M. Jodi Rell on June 15, 2005. The Act appropriated the sum of twenty million dollars to the newly established Stem Cell Research Fund for the purpose of grants-in-aid for conducting embryonic or human adult stem cell research. In addition, for each of the fiscal years ending June 30, 2008 to June 30, 2015, inclusive, the Act specified that an additional ten million dollars should be disbursed from the State's Tobacco Settlement Fund to the Stem Cell Research Fund to support additional grants-in-aid.

Passage of the Act positioned Connecticut as the third state in the nation, behind only California and New Jersey, to provide public funding in support of embryonic and human adult stem cell research. It mandated the establishment of the Connecticut Stem Cell Research (SCR) Advisory and Peer Review Committees[2] by October 1, 2005, and required the Commissioner of Public Health, as Chair of the Advisory Committee, to convene the first meeting by December 1, 2005. In accordance with Section 3(g)(3) of the Act, the Stem Cell Research Advisory Committee is required to report annually to the Governor and the General Assembly on (1) the amount of grants-in-aid awarded to eligible institutions from the Stem Cell Research Fund pursuant to section 2 of this act, (2) the recipients of such grants-in-aid, and (3) the current status of stem cell research in the State.

Within the Department of Public Health (DPH), the Office of Research and Development was tasked with implementation of the Act for the State of Connecticut, including identifying and recruiting members to the Connecticut Stem Cell Research (SCR) Advisory and Peer Review Committees. Additionally, the Act designated Connecticut Innovations, Inc. (CI) as administrative staff of the SCR Advisory Committee, responsible for assisting in the development of the application for grants-in-aid, reviewing such applications and preparing and executing assistance agreements in connection with awarding the grants-in-aid.

II. COMMITTEE ACTIVITIES

The primary focus of the SCR Advisory and Peer Review Committees from July 1, 2008 to June 30, 2009 was issuance of a third Request for Proposal, receipt and review of 77 applications for grant-in-aid, and the allocation of available dollars.

From December 2008 through March 2009, the 15 person Peer Review Committee completed the enormous task of rating and ranking each of the 77 applications for grants-in-aid from Connecticut's research community. During a teleconferenced meeting on March 17, 2009, the SCR Peer Review Committee agreed as a body on the ratings and rankings of the proposals.

[1] See Appendix A
[2] See Appendix B

SCR Advisory Committee meetings were held on July 23, September 16, October 21, November 18 and December 5, 2008, and on January 20, February 19, March 3, March 17, March 31, May 19, and June 16, 2009. The SCR Advisory Committee completed their review of applications and allocation of grants-in-aid during the meeting on March 31, 2009. All meetings are open to the public with notices and agendas on both the DPH and Secretary of State's websites. Minutes and transcripts of meetings are also posted on the DPH website.

III. RECIPIENTS OF GRANTS-IN-AID

During the current period, the State of Connecticut awarded the following 24 grants-in-aid totaling $9.8 million to researchers in Farmington, Storrs and New Haven:

Continuing and Enhancing the UCONN-Wesleyan Stem Cell Core, University of Connecticut Stem Cell Center, Farmington, Ren-He Xu, MD, PhD, Principal Investigator, $1,900,000.00.

Williams Syndrome Associated TFII-I Factor and Epigenetic Marking-Out in hES and Induced Pluripotent Stem Cells, University of Connecticut Health Center, Farmington, Dashzeveg Bayarsaihan, PhD, Principal Investigator, $500,000.00.

Cellular transplantation of neural progenitors derived from human embryonic stem cells to remyelinate the nonhuman primate spinal cord, Yale University, New Haven, Jeffrey Kocsis, PhD, Principal Investigator, $500,000.00.

Mechanisms of Stem Cell Homing to the Injured Heart, University of Connecticut Health Center, Linda Shapiro, PhD, Principal Investigator, $500,000.00.

Genome-wide screen to identify hESC-specific DNA transcription elements, Yale University, New Haven, Richard Sutton, MD, PhD, Principal Investigator, $500,000.00.

Molecular function of Lin28 in human embryonic stem cells, Yale University, New Haven, Yingqun Huang, MD, PhD, Principal Investigator, $500,000.00.

Therapeutic differentiation of regulatory T cells from iPS and hES for immune tolerance, University of Connecticut Health Center, Zihai Li, MD, PhD, Principal Investigator, $500,000.00.

Prevention of Spontaneous Differentiation and Epigenetic Compromise of Human ES and iPS Cells, University of Connecticut, Storrs, Theodore Rasmussen, PhD, Principal Investigator, $499,956.00.

Development of iPS cells to study craniometaphyseal dysplasia in humans, University of Connecticut Health Center, Farmington, Alex Lichtler, PhD, Principal Investigator, $500,000.00

piggyBac Transposon for Genetic Manipulation and Insertional Mutagenesis in Human Embryonic Stem Cells, Yale University, New Haven, Tian Xu, PhD, Principal Investigator, $500,000.00.

Brain Grafts of GABAergic Neuron Precursors Derived from Human and Mouse ES Cells for Treating Temporal Lobe Epilepsy, Wesleyan University, Middletown, Janice Naegele, PhD, Principal Investigator, $499,988.00.

MicroRNA regulation of hESC fates, Yale University, New Haven, Jun Lu, PhD, Principal Investigator, $500,000.00.

Molecular profiling and cell fate potential of hESC-derived early neural crest precursors, Yale University, New Haven, Martín I García-Castro, PhD, Principal Investigator, $200,000.00.

Neural Stem Cell Responses to Hypoxia, Yale University, New Haven, Qi Li, PhD, Principal Investigator, $200,000.00.

Induction and differentiation of beta cells from human embryonic stem cells, Yale University, New Haven, Kevan Herold, MD, $200,000.00.

Evaluation of homologous recombination in hESC and stimulation using viral proteins, University of Connecticut Health Center, Farmington, April Schumacher, PhD, Principal Investigator, $200,000.00.

Transcriptional control of keratinocyte differentiation in human ES cells, Yale University, New Haven, Valerie Horsley, Principal Investigator, PhD, $200,000.00.

Novel response to RNA editing in human embryonic stem cells, University of Connecticut Health Center, Farmington, Ling-Ling Chen, PhD, Principal Investigator, $200,000.00.

A human cell culture model of Angelman syndrome for drug screening, University of Connecticut Health Center, Farmington, Stormy Chamberlain, PhD, Principal Investigator, $200,000.00.

Can Natural Neuromodulators Improve the Generation of Nerve Cells From Human Embryonic Stem Cells?, University of Connecticut Health Center, Farmington, Srdjan Antic, MD, Principal Investigator, $200,000.00.

Investigating the role of nuclear RNA quality surveillance in embryonic stem cells, Yale University, New Haven, Sandra Wolin, MD, PhD, Principal Investigator, $200,000.00.

The Influence of Aberrant Notch Signaling on Rb Mediated Cell Cycle Regulation in Megakaryopoiesis & Acute Megakaryoblastic Leukemia, Yale University, New Haven, Stephanie Massaro, MD, Principal Investigator, $200,000.00.

Derivation and Functional Characterization of Heart Cells from Human Embryonic Stem Cells, Yale University, New Haven, Yibing Qyang, PhD, Principal Investigator, $200,000.00.

Hybrid Peptide/RNA Molecules for Safe and Efficient Gene Silencing in Human Embryonic Stem Cells, University of Connecticut, Storrs, Yong Wang, PhD, Principal Investigator, $200,000.00.

IV. INTERNATIONAL CENTER OF EXCELLENCE FOR STEM CELL RESEARCH

Connecticut continues to be recognized within the national and international stem cell research communities, both at a research and policy making level. The Connecticut DPH also continues its close relationship with the United Kingdom, Canada, and the International Society of Stem Cell Research (ISSCR).

The Interstate Alliance on Stem Cell Research (IASCR) met twice during the period covered by this report, on September 9–10, 2008 and May 5-6, 2009. On March 9, 2009, the co-chair of the IASCR attended the Presidential Signing of Executive Order 13505, Removing Barriers to Responsible Scientific Research Involving Human Stem Cells, at the White House. At the May meeting, senior officials from the National Institutes of Health provided an overview of the draft federal guidelines written in response to the Executive Order. Connecticut continues to serve as the chair of the IASCR, and additional information on the IASCR can be found at http://www.iascr.org/

On March 23 and 24, 2009, New Haven hosted StemCONN 09, Connecticut's second International Stem Cell Research Symposium. StemCONN 09 attendees included more than 600 stem cell researchers, policy makers, ethicists and students from around the world. The symposium covered the most recent discoveries surrounding stem cell research and associated policy, ethical and commercial challenges. Additionally, StemCONN 09 provided Connecticut's three stem cell research academic institutions an opportunity to showcase their successful programs, noted below. Additional information is available at http://stemconn.org/

V. CONNECTICUT'S STEM CELL RESEARCH COMMUNITY

Since passage of the enabling legislation in 2005, the State of Connecticut has allocated a total of $39.42 million in support of stem cell research at the University of Connecticut, Yale University, and Wesleyan University. The following describes the state of publicly funded stem cell research efforts at Yale University, Wesleyan University, and the University of Connecticut.

Yale University[3]

The $13.3 million in funding that Yale received from the State since 2007 has transformed stem cell research at Yale—it has allowed Yale, for the first time, to build an infrastructure and a vibrant community of investigators to conduct stem cell research. Prior to the passage of Public Act 05-149, there was only one investigator at Yale working on human embryonic stem cells. Today, there are 18 laboratories on Yale campus actively pursuing human embryonic stem cell research. Specifically, the initial funding of $7.3 million from the State in 2007 allowed Yale to build an infrastructure of core facilities, initiating new research projects, recruiting new faculty, and stimulating new collaborations both within the Yale community and throughout Connecticut. The additional $6 million in funding that Yale received from the State in 2008 has further generated a synergistic effect with the current funding to enhance stem cell research in Connecticut. Eleven new projects were awarded in 2008, eight to young investigators with interests in stem cell research and three to established investigators with interests in expanding their research into the stem cell research field. The access to human embryonic stem cell lines, imaging, and data analysis technology at Yale's core laboratories has paved the road for scientists to conduct their research.

A. Infrastructure of Core Facilities

The Yale Stem Cell Center (YSCC) moved into the new building on Amistad Street during the first week of August 2007. The YSCC established the following core laboratories, funded by a Core Facility grant from the Connecticut Stem Cell Research Fund (CSCRF). This Core grant allowed Yale to purchase major equipment and supplies, as well as salaries for some of the experts who manage the Cores.

a. Human Embryonic Stem Cell (hESC) Core. This Core, staffed by a Technical Director and a technician, serves as a storage, distribution, and training center for hESCs and develops new hESC technology for researchers in the State of Connecticut. In addition, it is an important research site for investigators who are extending their work to non-federally-approved hESC lines. The hESC Core staff has trained 40 investigators from 18 labs and is supplying hESC cell lines and is growing and differentiating cells (e.g., neurons and erythroid cells) for 16 labs.

b. Confocal Microscope Core. This Core provides state-of-the-art imaging for research on embryonic and adult stem cells. The equipment includes a Leica TCS SP5 AOBS Spectral Confocal Microscope equipped with a scanning stage. The Confocal Core lab was customized for this microscope and the equipment arrived in October 2007. This Core has been fully booked and provides service to 88 investigators from 26 labs.

[3] The progress report on Connecticut supported stem cell research was prepared by Haifin Lin, Ph.D., Director, Diane Krause, M.D., Ph.D., Associate Director, and Paula Wilson, M.B.A., Administrator

c. Fluorescence Analysis and Cell Sorting (FACS) Core. The Yale School of Medicine purchased a BD FACSAria cell sorter and a BDTM LSR II Cell Analyzer Special Order System. The FACS Core lab was customized for this equipment and the equipment arrived in September 2007. This Core complements the existing Core on the Medical School campus and between the two Cores they are providing service to over 800 investigators.

d. Genomics Core. This Core consists of a Cellomics High Throughput Cell Screen system and an Illumina Genome Analyzer. The Cellomics Cell Screen system was provided by the Yale School of Medicine and installed on the second floor of the Amistad building for analysis of stem cells including non-federally approved hESC lines. The Illumina Genome Analyzer, purchased in part with a Hybrid Grant from the CSCRF and with support from Yale, has been temporarily installed in the KBT building at Yale, where there is staff with the expertise to set up and train on the operation of the system. This analyzer has succeeded in trial runs and data has been obtained for the stem cell research projects of 6 laboratories and some of the results have been published in top tier journals such as *Science*. This service is expected to propel both academic and industrial stem cell researchers in the State to the forefront of the genomic and genetic research of stem cells. In addition, it will allow the Connecticut Stem Cell Initiative to dovetail with the Connecticut Genomics Initiative.

B. New Research Projects

1. *Dr. Eugene Redmond, Professor, Departments of Psychiatry and Neurosurgery, "Translational Studies in Monkeys of Human Embryonic Stem Cells for Treatment of Parkinson's Disease."* This project is advancing the field toward a clinical application of stem cells for treating Parkinson's disease. The State funding has allowed Dr. Redmond to use embryonic stem cells to treat Parkinsonian monkeys, by doing important final efficacy, toxicity, and side effect studies in monkeys that are a prerequisite to human clinical trials. The project will determine an optimal level of differentiation that maintains or increases the functional success of Dr. Redmond's prior studies, using fetal neural stem cells (PNAS, 104:12175-80, 2007). The cells will be characterized *in vitro* at several stages of development and differentiation, based upon state of the art biochemistry, pharmacology, histology, immunology, and genomics; and then studied in vivo using the best model of Parkinson's disease in monkeys. This project has the potential, and the investigators have the experience, to move to clinical trials in Parkinson's disease, if the experiments are successful by the end of the funding period. This project has also become a key component of a larger study that will compare the full profile and side effects of embryonic-derived cells with "reprogrammed" adult somatic cells derived from the skin call inducible pluripotent stem cells, which have received much recent attention. Determining side by side whether these cells are fully

equivalent will help to determine the most effective course for future cell replacement therapies for many other conditions in addition to Parkinson's disease. This project also helps to illustrate the economic advantages of the investment – because of this key project, other large federal projects have been funded for Dr. Redmond's studies in Connecticut.

2. *Dr. Flora Vaccarino, Professor, Child Study Center and Department of Neurobiology, "Effect of Hypoxia on Neural Stem Cells and Their Function in CNS Repair."* Neural stem cells (NSCs) can repair the brain after injury, but this repair, when present, is invariably incomplete. Dr. Vaccarino is in the process of identifying the changes in gene expression that enable NSCs to repair the injured brain and understand their role for human NSC development. Dr. Vaccarino has developed a mouse model of neonatal hypoxia which causes brain injury manifested by a loss of cortical neurons. Consistent with the observation that the outcome of neonatal injury is generally better than adult injury, this loss of cortical neurons is subsequently repaired. Dr. Vaccarino has established that repair occurs through the proliferation of astroglial NSCs (expressing Glial Fibrillary Acidic Protein, or GFAP) which then differentiate into new cortical neurons, astrocytes and oligodendrocytes 3-4 weeks after the insult. Dr. Vaccarino will target the enhanced green fluorescent protein (EGFP) gene to GFAP+ cells. The permanent EGFP expression in GFAP+ cells allows her to track their progeny following hypoxia. EGFP+ cells will be isolated after hypoxic insult to analyze their gene expression profile. By contrasting and comparing genes changes in GFAP+ cells isolated from different regions and ages, Dr. Vaccarino will select genes whose changes closely predict the therapeutic potential of the GFAP+ NSCs. The validated genes will be overexpressed or knocked-down in human NSCs (generated from both federally approved and non-approved human ES cell lines) using lentiviral shRNA. The induced changes in the human NSC phenotype will be characterized via a high-throughput cell-based imaging system. A selected number of genes that elicit changes in expression of differentiation markers in human NSCs will be identified. This project will elucidate key molecular mechanisms underlying the capability of NSCs to generate neuronal progenitors and differentiated neurons, and consequently foster recovery from brain injury. The study will allow the future development of genetically modified human NSC lines and mouse in vivo mouse models that will further consolidate the role of these NSC genes in fostering brain recovery after injury. Studies suggest that quiescent NSCs are present in the brain, but for unknown reasons their regenerative potential is only rarely revealed, and most adult brain injuries cannot be repaired. In contrast, in neonatal mice after chronic sublethal hypoxia, endogenous NSCs play an important role in brain repair (Fagel et al., Journal of Neuroscience, in press). In this project Dr. Vaccarino will take advantage of this model to isolate the genes that may be critical for empowering NSC with the capacity to generate cortical neurons and glia in the intact brain, and will study the role of these genes human NSC. Dr. Vaccarino's project is unique in that it seeks to understand the critical genes that NSC must express in order to overcome potent inhibitory tissue factors that obstruct brain repair.

3. *Dr. Joshua Breunig, Postdoctoral Associate, Department of Neurobiology, "Regulation of hESC-derived Neural Stem Cells by Notch Signaling."* The human brain is capable of very little regeneration following trauma or neurodegenerative disease. Thus, patients with such maladies require considerable long term care and have little hope of amelioration of their conditions. Neural stem cells derived from hESCs provide a potential avenue of treatment. Dr. Breunig seeks to determine the potential for repair of hESC-derived neural stem cells following molecular manipulation of the Notch pathway—one of the most powerful molecular mediators of cell fate (i.e. the cell's decision to remain a stem cell or generate neurons). The hope is that Notch signaling manipulations will provide neuron or stem cell enriched populations of cells for transplantation, depending on the desired result. Activating Notch should lead to increased numbers of stem cells, while blocking Notch will lead to enriched neuron populations. These populations will be transplanted into animal models of trauma with the hope of regenerating damaged circuits. Previous efforts have been hampered by a limited understanding of the factors leading to directed differentiation of stem cells, causing a lack of differentiated neurons. Dr. Breunig believes that the our new-found understanding of the Notch pathway will enhance transplantation therapies and lead to direct clinical applications for neural stem cells in the treatment of traumatic brain injury, spinal cord injury, and neurodegenerative disease.

4. *Dr. Natalia Ivanova, Assistant Professor, Department of Genetics, "Molecular Control of Pluripotency in Human ES Cells."* Embryonic stem (ES) cells promise to revolutionize medicine. If we determine how to control the expansion and differentiation of human ES cells, then we could produce cells of any human organ at will. In order to achieve this goal we need to gain a deep understanding of how cell fate decisions such as self-renewal, differentiation and cell death are controlled in these cells. Studies in the mouse have provided insights into the molecular regulation of ES cells. However, the biological equivalence of mouse and human ES cells remains unclear. While some regulatory components are functionally conserved, others appear to be species-specific. There are differences in morphology, patterns of embryonic antigen expression, cytokine dependence and cell cycle kinetics. These findings suggest that data accumulated in the mouse system cannot be extrapolated directly to hES cells. It is likely that additional genes are involved in the regulation of hES cells. Dr. Ivanova is identifying, in a comprehensive manner, molecular components and pathways that control pluripotency in hES cells using a direct shRNA-based functional screen. This approach was developed during her postdoctoral training and has been successfully applied to studies using mouse ES cells. These studies will provide data and reagents that should allow her to analyze hES cells at multiple molecular and biochemical levels. In addition to fundamental insights into cell fate control, her studies will extend our ability to develop therapeutic strategies for the treatment of various human diseases.

5. *Dr. Caihong Qiu, Associate Research Scientist, Department of Cell Biology, "Hematopoietic Differentiation of Human Embryonic Stem Cells Under Feeder-Free and Serum-Free Conditions."* hESCs represent an excellent tool for scientists to learn about how we develop in the womb. These cells are also very useful for applications in tissue engineering and drug screening. Much research is focused on differentiating hESCs into pure populations of different cell types. Dr. Qiu is developing approaches to efficiently induce hESCs into blood cells in a system that is free of any non-human products. This has never been done before, but it is important to remove animal serum and animal cells from the hESC growth conditions so that the cells can be used for humans in the future. Specifically, Dr. Qiu would like to be able to produce bone marrow cells and red blood cells that could be used for transplantations and transfusions, respectively. In addition to the important benefits to patients, Dr. Qiu's studies will also help us to better understand how blood cells form. There are many stem cell researchers throughout Connecticut who would like to be able to induce hESCs to form blood and related cell types, and Dr. Qiu will gladly share her findings with them in order to facilitate their research.

6. *Dr. Valerie Reinke, Associate Professor, Department of Genetics, "VRK-1-Mediated Regulation of p53 in the Human ES Cell Cycle."* The use of ES cells to investigate disease and develop therapies requires genetic manipulation of the ES cells in culture. Prolonged culture of undifferentiated ES cells can result in damage to the genome, which decreases the ability of ES cells to be used for therapeutic purposes. Another major concern of using ES cells in patient-specific therapy is the possibility of forming teratocarcinomas or other tumors if undifferentiated ES cells are inadvertently introduced into the body. Dr. Reinke believes that controlling the activity of a protein called p53 in human ES cells will help to prevent damage during culture, and limit the potential of tumor formation during stem cell therapy. The p53 tumor suppressor protein is known as "the guardian of the genome". Typically, in a wide variety of differentiated cell types, p53 senses damaged DNA and responds by inducing either apoptosis or cell cycle arrest. However, p53 does not respond to DNA damage in undifferentiated ES cells, despite being present at high levels. Dr. Reinke is trying to find a way to regulate and turn on p53 activity in ES cells, which may allow her to limit DNA damage during culture of ES cells, and decrease the likelihood of tumor formation during stem cell therapy. Dr. Reinke's early work in the mouse indicates that a second protein called Vrk1 modulates p53 activity specifically in stem cells. Vrk1 is a kinase, which is a type of protein that is easier to target with specific drugs than is p53. If she can use drugs to control Vrk1, which will in turn control p53, then she will be able to improve human ES cell culture and applicability in therapeutics. The CT Stem Cell Funds are critical for supporting this work, which is currently unfunded by any other source or agency.

7. *Dr. Masanori Sasaki, Associate Research Scientist, Department of Neurology, "Cortical Neuronal Protection in Spinal Cord Injury Following Transplantation of Dissociated Neurosphere Derived From Human Embryonic Stem Cells."* Spinal cord injury (SCI) results in dysfunction due to disruption of motor signals from

brain to spinal cord. Dr. Sasaki recently demonstrated that transplantation of gene-modified human mesenchymal stem cells to secrete the neurotrophic factor BDNF (BDNF-hMSC) from human bone marrow could inhibit apoptosis in the motor cortex after experimental SCI in rats, which could contribute to repair and functional recovery. Neural progenitor cells dissociated from neurosphere have an ability to differentiate into neurons and glia in vivo and vivo. Importantly, neurospheres have been prepared from hESCs. Although several mechanisms have been suggested including neurogenesis, regeneration, axonal sprouting, recruitment of endogenous Schwann cells and remyelination, there is considerable evidence suggesting that, under appropriate cell preparation and transplantation conditions, functional outcome in experimental SCI can be enhanced by cellular transplantation. Questions still remain with regard to cellular mechanisms responsible for improvement in functional outcome. This project will be to evaluate a potential additional role of neuroprotection by the supraspinal effects of transplantation of spinally transplanted neurosphere derived from hESCs to promote functional recovery after spinal cord injury (SCI). Dr. Sasaki will determine if transplantation of dissociated neurospheres derived from hESCs (hNSs) results in improved functional outcome and anti-apoptosis effects in M1 cortex in the brain. Success of this project using hMSCs would provide important preclinical work for the consideration of human clinical studies for SCI.

8. *Dr. Qiaoqiao Wang, Postdoctoral Associate, Department of Cell Biology, "The Role of the piRNA Pathway in epigenetic Regulation of hESCs."* The ultimate goal of this project is to understand some of the underlying mechanisms that control stem cell self-renewal, pluripotency and differentiation in humans. hESCs are derived from the human blastocysts, which are typically four or five days old and are a hollow microscopic ball of cells. hESCs possess two key properties: the self-renewing ability, i.e. the ability to remain in an undifferentiated state and to divide indefinitely, as well as pluripotency, i.e. the potential to produce every cell type in the human body. Therefore, hESCs represent the only experimental system available to explore the mechanisms of human development. Meanwhile hESCs are also recognized as an awesome candidate for regenerative medicine and tissue replacement after injury or disease, i.e, ES cell therapies. To fulfill these dreams, however, we need to discover the key molecular players that control the "stemness." Here we are proposing experiments to advance the stem cell research. Epigenetic remodeling is a hallmark of embryonic development. Dr. Wang's supervisor, Dr. Haifan Lin, has recently discovered an intriguing connection between piRNA/Piwi proteins and hetero-chromatin in fly germ-line stem cells, which may be the case in hESCs. The Piwi protein is highly conserved and is well characterized as a protein required for stem cell self-renewal. piRNAs have been recently discovered as a Piwi-interacting small non-coding RNAs. The Lin lab's latest study indicates that the Piwi/piRNA complex binds to piRNA-matching sequences in the genome to regulate their epigenetic state and such regulation is essential for stem cell self-renewal in fly. Dr. Wang hypothesizes that

this piRNA pathway is also required for hESC self-renewal. Her proposed research should allow her to learn how the piRNA pathway is involved in the regulation of chromatin in hESCs.

9. *Dr. Lloyd Cantley, Professor, Departments of Internal Medicine, Nephrology Section, and Physiology, "Functional Use of Embryonic Stem Cells for Kidney Repair."* The kidney functions to clear the blood of toxins and to maintain internal fluid and electrolyte homeostasis. These properties result in a marked sensitivity to reductions in blood flow, with resultant tubular cell necrosis and acute renal failure. Repair of this injury is dependent on surviving tubular cells. However, in older or severely ill patients, this repair process is often insufficient, leading to chronic kidney failure, dialysis and frequently death. Dr. Cantley is developing a strategy for priming of ES cells to become kidney progenitor cells that could be used in the treatment of patients with acute renal failure in whom endogenous tubule repair is either delayed or absent. While this work will transition to human ES cells in its later stages, the initial development of strategies to prime ES cells to adopt a renal epithelial cell fate, and the testing of methods of delivery of these cells to the injured kidney will be performed using mouse ES cells and mouse models of acute and chronic kidney injury currently performed in the laboratory. In the first specific aim, several approaches will be tested for the priming of ES cells towards adopting a kidney specific fate. These will include culture of ES cells in a sequential cocktail of growth factors and cytokines and co-culture of ES cells with explanted embryonic kidney. In the second aim, primed ES cells will be tested for their ability to home to and functionally incorporate into damaged kidneys by comparing intravenous injection, intra-arterial injection, intra-ureteral injection and direct injection into the kidney in mouse models of kidney injury. Thus these studies address a novel approach to the treatment of kidney disease by providing pathways to enhance kidney repair even after the acute injury has occurred.

10. *Dr. Laura Niklason, Associate Professor, Departments of Anesthesiology and Biomedical Engineering, "Human Embryonic and Adult Stem Cells for Vascular Regeneration."* The general goal of Dr. Niklason's project is to find clinically viable means of creating arterial replacements optimal cell source. Dr. Niklason's lab is going to evaluate two types of stem cells: human bone marrow-derived mesenchymal stem cells (hMSCs) and hESCs-derived biopotent mesenchymal stem cells. Dr. Niklason's lab has shown the feasibility of directing mesenchymal stem cells that are derived from adult human bone marrow down a vascular smooth muscle lineage. In addition, they have gone on to show the feasibility of using such cells to culture entire human arteries. However, the differentiation from MSC to SMC seems incomplete which lacks the expression of late contractile SMC markers, implying an intrinsic limitation of adult MSCs. The application of adult marrow-derived MSC in vascular regeneration is further hindered by their paucity in the marrow, unclear impacts of aging, and their limited passage number in vitro. Furthermore, the pathways that are involved in this differentiation process are not well understood. hESC-derived MSCs may be an attractive alternative due to their

unlimited proliferative and differentiation capacity although it is not known whether mesenchymal stem cells that are derived from human embryos have the same vascular smooth muscle differentiation potential. Dr. Niklason's lab is utilizing soluble factors, physical stimuli, and substrate matrix cues that are known to induce smooth muscle differentiation, and test their impact on the differentiation of mesenchymal stem cells derived from human embryonic stem cells. In addition, they will probe the signal transduction pathways of both adult and embryonic-derived cells, in order to determine their differences. Lastly, Dr. Niklason's lab will document the utility of vascular smooth muscle cells that are derived from hESCs for vascular tissue engineering. The results from these studies will not only inform the field whether hESC-derived MSCs share the same ability to undergo SMC differentiation as those from adults, but will also elucidate the signaling pathway responsible for SMC differentiation from mesenchymal stem cells (adult and embryonic). In addition, the implantation of the engineered vascular grafts in an immunodeficiency rodent model will represent the first in vivo assessment of human vessels engineered from embryonic and adult mesenchymal stem cells. The results could pave the way for development of a novel therapy for vascular disease.

11. *Dr. Dianqing Wu, Professor, Department of Pharmacology, "Wnt Signaling and Cardiomyocyte Differentiation from hESC."* Studies have strongly implicated Wnt signaling in cardiogenesis. Although the precise involvement of Wnt signaling in each step of the differentiation from embryonic stem cells (ESCs) to cardiomyocytes is not clear, studies from a number of organisms suggest that canonical Wnt signaling promotes the differentiation to the mesoderm, while non-canonical or inhibition of canonical Wnt signaling promotes cardiomyocyte specification and differentiation. Preliminary studies of hESCs in Dr. Wu's lab as well as other labs suggest that Wnt regulation of cardiomyocyte differentiation may be highly conserved between mouse and human ESCs. Dr. Wu's lab hypothesizes that differential manipulation of Wnt signaling at different stages of hESC-cardiomyocyte differentiation can be exploited to enhance production of cardiogenic cells from hESCs and that cardiogenic precursor cells may be more suitable for cardiac implantation and repair in vivo. Dr. Wu's lab is systemically dissecting the involvement of Wnt signaling in hESC-to-cardiomyocyte differentiation in culture by investigating the role of Wnt signaling in hESC differentiation to the mesoderm, to cardiogenic precursor cells, and to mature cardiomyocytes. Cells are treated with reagents that stimulate and inhibit canonical and non-canonical Wnt signaling, and specific marker gene expression will be examined by QRT-PCR. Functional and structural phenotypes of terminally differentiated cells will also be characterized. Furthermore, Dr. Wu's lab is testing if cardiogenic progenitor and cardiomyocyte precursor cells, when transplanted, improve cardiac function recovery in a myocardiac infarction (MI) mouse model. A novel biodegradable synthetic scaffold, which has been successfully used in generating artificial blood vessels, will also be tested for facilitating the engraftment. The fate of transplanted cells will be characterized by general pathology and immunohistochemistry. Cardiac functional improvement will be evaluated by electrocardiography, measurements of cardiac pressure, and

echocardiography. By taking advantage of their extensive expertise in Wnt signaling, the possession of unique small molecule compounds that regulate Wnt activity, and a team of cross-discipline co-investigators who have expertise in biomaterial sciences and cardiac surgery, Dr. Wu's lab will contribute to a better understanding of the role of Wnt signaling in hESC-to-cardiomyocyte differentiation and establish a method for efficient cardiogenic cell production and engraftment, which lead to cardiac function recovery in a heart disease model.

C. Recruitment of Leading Stem Cell Researchers

The State stem cell funding has aided Yale's ability to recruit seven faculty members to the Yale Stem Cell Center. These include: Caihong Qiu, PhD, Albert Einstein College of Medicine; Natalia Ivanova, PhD, Princeton University; Jun Lu, PhD, Broad Institute of MIT and Harvard; Shangqin Guo, PhD, Harvard University; Yibing Qyang, PhD, MGH, Harvard Stem Cell Institute; Valerie Horsley, PhD, Rockefeller University; and Matthew Rodeheffer, PhD, Rockefeller University. The State stem cell funding played a critical role in Yale's winning these faculty members over other competitive institutions such as Harvard University and prominent universities in California, New York, and New Jersey. These faculty members represent some of the best stem cell researchers in the world.

A new faculty search is underway to recruit one to two additional faculty members in 2009. Yale received 72 applications from stem cell experts from around the world, including 13 applications from California and 15 from Massachusetts. Other applications came from major stem cell research states such as Maryland, New York, and New Jersey. The Search Committee selected seven candidates with exceptional credentials to interview for the positions. The individuals have been received with overwhelming enthusiasm by the current faculty at Yale. Of the seven, four candidates will return to Yale for a second visit and to begin negotiations for their recruitment packages. The support from the State has significantly strengthened Yale's position in competing with major stem cell research centers in the world for these candidates.

D. Collaborations within Yale and throughout Connecticut

The funding from the State to develop the Yale hESC Core Facility has given the YSCC the ability to provide hESC lines to investigators. This has stimulated a number of collaborative stem cell research projects. The majority of the investigators who received funding from the 2008 and 2009 CSCRF will use hESC lines from the hESC Core Facility and rely on the expertise of Dr. Qiu, who was recruited to the YSCC in 2007 as the director of the Facility, for guidance on the use of these lines.

Yale's relationship with UConn, Wesleyan, the Department of Public Health, and CURE has flourished as they work together to build the stem cell research base in the State. Examples of these forums and collaborations include the following:

1. **June 18, 2008:** Yale Stem Cell Center Presentation, "STEM CELLS: Everything You Wanted to Know But Were Afraid to Ask." Dr. Haifan Lin and Mr. Robert Mandelkern, Connecticut State Coordinator of the Parkinson's Action Network presented the basics of stem cell biology and research focused on a non-scientific audience. It was attended by staff from Connecticut Innovation, the Connecticut Department of Public Health, Yale faculty, students, staff, and members of the community. It was also videoconferenced to the University of Connecticut and the University of Connecticut Health Center.

2. **October 10, 2008:** Yale Stem Cell Center First Annual Retreat. Keynote Speaker: Dr. David Scadden, Co-director of Harvard Stem Cell Institute. This Retreat was attended by over 215 Yale faculty, staff, and students. In addition to oral presentations by Yale faculty members, it also included a poster session with 25 posters from students and postdoctoral fellows. All of these activities are crucial for fostering a new generation of scientists who are enthusiastic about stem cell research.

3. **October 16, 2008:** Connecticut Department of Public Health, 2008 Northeast Epidemiology Conference. Dr. Haifan Lin participated in a Plenary Session, "Looking into the Future" to introduce stem cell research to epidemiologists throughout the State.

4. **October 31, 2008:** Yale Stem Cell Center Inaugural Distinguished Lecture. Dr. Irving Weissman, Director of the Stanford Institute of Stem Cell and Regenerative Medicine and Director of Stanford Cancer Center, presented a talk on "Normal and Neoplastic Stem Cells" at Yale.

5. **March 23-24, 2009:** StemCONN 09, Connecticut's International Stem Cell Research Symposium, was an overwhelming success and a premier expression of the stem cell research collaborations within the State. Over 600 participants from US and UK attended the conference, among whom 81% were from the State of Connecticut and 50% were students (pre and post doctoral).

Additional collaborations with pharmaceutical and biotech industries are also evolving. The senior management of corporations such as BD Biosciences, Medtronic, Pfeifer, Novartis, Alnylam Pharmaceuticals, Polaris Ventures, RainDance Technologies Inc., etc. have visited and/or contacted the YSCC to express their interest in collaborating with the YSCC. These partnerships will potentially create many opportunities for Yale to help establish a stem cell industry in the State. Yale Stem Cell Center is also a lead participant of the Northeast Stem Cell Consortium—a rapidly expanding organization that promotes interaction and collaborations among seven universities in the Northeastern US (Harvard, UConn, Univ. of Maine, UMass, Univ. of Vermont, Wesleyan and Yale), affirming the leading position of Connecticut in stem cell research.

E. Economic Impact on the State of Connecticut

To date, the State funding has provided the resources to fully or partially support 78 positions totaling approximately $2.2 million. These highly skilled professionals

represent a new breed of sophisticated work force in Connecticut, and are anticipated to have long-term impact on the economic development in the State.

The University of Connecticut[4]

The University of Connecticut is dedicated to establishing an internationally recognized program focused on human embryonic stem cells and regenerative medicine. In collaboration with scientists at Yale and Wesleyan Universities, we have developed state-of-the-art research programs aimed at bringing human stem cell therapies to patients. With support from our citizens and legislators, the grant awards from the State of Connecticut Stem Cell Fund support research in over 30 laboratories at the University of Connecticut. We are also training the next generation of clinical and basic research scientists who will lead this new field of investigation into areas of medical practice and launch new Connecticut-based biotechnology companies.

A. Major accomplishments for 2008-2009

a. The University of Connecticut Stem Cell core facility generated the first two human embryonic stem cell lines, CT1 and CT2, in the State of Connecticut, through the efforts of Drs. Ge Lin and Ren-He Xu.

b. UConn scientists reported the results of their exciting research in many prestigious scientific and medical journals and at international stem cell conferences. Among the outstanding research publications of work funded by the State Stem Cell Program include articles on tracking transplanted stem cells in live animals using sophisticated fluorescent visual markers by Dr. David Rowe and colleagues from the Harvard Stem Cell Institute in ***Nature***, the world's most prestigious scientific journal; and discovering chemical signals that instruct human embryonic stem cells to differentiate into tissues by Dr. Ren-He Xu and colleagues at WiCell in ***Cell Stem Cell***, the world's top stem cell journal.

c. The University of Connecticut Stem Cell Institute continues to enhance its mission of maximizing the benefits of the stem cell research awards made to the University. The Institute now includes 40 faculty members from both the Farmington and Storrs campuses. Dr. David Goldhamer has assumed the role Associate Director. Dr. Goldhamer is also Director of the Center for Regenerative Biology and Associate Professor of Molecular and Cellular Biology.

B. Economic impact of State Stem Cell funding for UConn

The University of Connecticut's investment in creating the Core facility and its subsequent funding by the State of Connecticut led to the recruitment of Dr. Ren-He Xu (Director) and Ms. Leann Crandall (Manager) from WiCell Research Institute.

[4] The progress report on Connecticut supported stem cell research was provided by Marc Lalande, Ph.D., Director, University of Connecticut Stem Cell Institute

Two research assistants and four postdoctoral fellows have since been added to the core staff. The University of Connecticut was also successful in recruiting Dr. Xue-Jun Li to the faculty from WiCell as well Dr. Steven Crocker from the Scripps Institute and Dr. Dashzeveg Bayarsaihan from the University of Louisville.

In the past two years, over 115 UConn scientists have received partial or full salary support. This represents a payroll expenditure of approximately $3.1 million.

C. New Research Projects

1. *Human ES Cell Core at Univerity of Connecticut and Wesleyan University, Investigator: Ren-He-Xu, Ph.D.* The University of Connecticut Stem Cell Core was established in April 2006 and first awarded a Core Facility grant (shared with Wesleyan University) in November 2006 by the State Stem Cell Research Program. The overall objective of the UConn Stem Cell Core is to meet the ever-increasing demand by Connecticut scientists for human embryonic stem cells and stem cell-related training and services, and help advance stem cell-based therapies to treat human diseases.

   In the past year, the Core has expanded its expertise in culturing, quality control and banking of nine human embryonic stem cell (hESC) lines; developing a Memorandum of Understanding with WiCell to distribute, with minimal paperwork, hESCs to over 30 laboratories at University of Connecticut, Wesleyan University, and Yale University; holding 15 training sessions on hESC culture for 100 researchers and graduate students statewide and beyond; producing patient-specific induced pluripotent stem cells using cutting-edge techniques that do not require any manipulation of human embryos. The core also obtained approvals by the Internal Review Board (IRB) and Embryonic Stem Cell Research Oversight (ESCRO) of University of Connecticut to derive human embryonic stem cell lines from donated frozen embryos. Two new hESC lines CT1 and CT2 have been derived and registered with the UConn ESCRO and are approved for distribution to eligible researchers.

   The Core also provided post-training services and technical support to stem cell researchers to assure the quality of the stem cells. To facilitate neural regenerative research by using stem cells, its first Neural Differentiation Workshop was held on September 19, 2008. To promote stem cell education, the Core continued cross-campus stem cell seminars by inviting world-renowned scientists. These seminars were teleconferenced to University of Connecticut-Storrs, Wesleyan University, and Yale University. The core also organized seven outreach stem cell seminars at other colleges in the state.

2. *Flow Cytometry Core for the study of human Embryonic Stem Cells Investigator: Hector Leonardo Aguila, PhD.* Flow cytometry is a powerful technique that permits the identification of rare cell types within complex populations of cells to isolate them to homogeneity, and to evaluate their characteristics as cell division, cell death and metabolic functions. The implementation of this technology is dependent on highly specialized and costly instrumentation. The University of

Connecticut Health Center established a Flow Cytometry Facility about 20 years ago to assist immunologists. At the present time, the facility is directed by Dr. Hector L. Aguila and Dr. Leo Lefrancois and provides services to scientists with research interests spanning most of the disciplines represented in the institution plus researchers from other institutions in the State. An increasing number of scientists with interests in Stem Cell Biology are active users of the facility creating the need for analysis and isolation. During the past year, the institution committed funds to purchase an Aria cell sorter instrument (Becton Dickinson, San Jose, CA). This state of the art instrument is custom designed to perform applications especially suited for hESC research and it is currently dedicated mostly to the analysis and isolation of hESCs and their derivatives. The acquisition of a new instrument allowed us to establish a core to provide advice, training and services on flow cytometry to stem cell researchers. Beyond services, priority is given to create active collaborations with multiple investigators to develop novel flow cytometry applications for studying properties of hESCs and their derivatives. These include: profiling and selection of undifferentiated hESCs, new detection techniques to evaluate expression of endogenous fluorochromes (i.e. green fluorescent proteins expressed in the context of developmentally regulated promoters) and cells surface markers with antibodies coupled to multiple fluorochromes. The consolidation of these knowledge will be important to the design of more efficient ways to isolate stem cell sources to be used in regenerative protocols in the clinic. This core also interacts closely with the institutional hESC Core in screening existing and newly generated cell lines to design quality control parameters, and enhancing the educational mission of the core.

3. *Group Grant Group Leader: David Rowe MD*

   a. *Project 1: Skeletal Mesenchymal Progenitor Cells, Investigator: Alexander Lichtler, PhD.* Diseases of muscle, skeleton and skin together represent a tremendous medical burden on the US population. Bone defects, which range from those caused by trauma such as war injuries or accidents to osteoporosis, affect millions of people, as do cartilage defects caused by arthritis or sports injuries. Muscle and skin injuries are also ma jor problems. We believe that the ability of doctors to provide the best treatment would benefit if it was possible to produce the types of cells that are found in the damaged tissues. Human embryonic stem cells are a promising source for all of the types of cells that are in the human body, however, methods need to be developed to cause them to changes into the specific cell types that are affected by specific diseases. The basic goal of our project is to develop improved methods to get human embryonic stem cells to change into cell types that can be used to repair defects in bone, cartilage, skin or muscle. One of the first steps in becoming a bone, cartilage, skin or muscle cell is to become a mesoderm cell. Mesoderm is one of the three basic cell types in the body, and one of our main goals is to develop ways to easily detect when an hESC has changed into a mesoderm cell. To do this we have produced hESC that contain genes that we hope will cause the cells to glow green when they become mesoderm; it is much easier to detect the

green color than it is to tell if the cells have become mesoderm by traditional methods. We plan to use these cells to screen many proteins or chemicals to find a combination that can induce mesoderm formation from hESC. We hope to use a similar approach to develop methods for getting the cells to then become more specialized kinds of mesoderm. We expect that these cells will be a good source of cells to be further changed into bone, cartilage, skin or muscle cells.

b. *Project 2: Phenotyping and Isolation of hES Derived Cells of the Musculoskeletal Lineage Investigator: Hector Aguila, PhD*. One of the requirements to use defined stem cells in regenerative therapies is the development of methods to identify them and isolate them from other cells that would not contribute to the repair process or that in some instances could turn into potential cancer cells. One of the best ways to identify differences between types of cells is using antibodies that differentially recognize molecules in their surface. These antibodies can be tagged with fluorescent molecules making them ideal reagents to visualize, dissect and isolate live cell populations. The main purpose of this project is to generate new antibodies that would allow the identification of stem cells capable to contribute to the regeneration of the cartilage, bone, and muscle. Human embryonic stem cells are directed to differentiate into these tissues and as they progress into differentiation, cells are harvested and injected into mice that will generate specific antibodies against cell surface molecules specific for different stages of human development including the desired type of stem cells. At this moment we have already generated a battery of over 30 antibodies that bind hESCs and other cells representing very early stages of human development. Using differential screening techniques that include the cross examination with commercial antibodies of known specificities we are now analyzing the binding patterns of these new antibodies. We will use these antibodies to study the diversity of early stem cells, and as a reference to antibodies recognizing cells more committed to form bone, cartilage and muscle. We are actively working on the generation of additional antibodies against later stages of differentiation. We expect these antibodies will be critical tools to isolate stem cells for clinical use and if so they will have the potential to be commercialized.

c. *Project 3: Microarray and Genetics Networks Investigator: Dong-Guk Shin, PhD.* Bioinformatics has become an essential component of modern life science research because the amount of data being generated with the advanced life science research instrumentations far exceeds the limits of manual handling of produced data. Particularly, the use of microarray (a.k.a. DNA chip) technology generates multiple data points for each of the 40,000 human genes from one chip experiment. Scientists repeat experiments with varying experimental conditions. They even repeat each experiment multiple times in order to gain statistical confidence in their findings. Without use of computer programs, stem cell researchers will not be able to analyze the experimental data. Our computerized methods for analyzing stem cell data will drastically shorten the time needed to evaluate the efficacy of stem cell compounds in treating patients with various injuries.

d. *Project 4: Biometric Surfaces for Efficient and Stable Stem Cell Differentiation,*

Investigator: Liisa Kuhn, PhD. There is an increasing prevalence of degenerative diseases of the bones and joints among our aging population, along with battlefield trauma and other extensive injuries. These conditions cannot heal on their own and current treatments are often unsatisfactory. Stem cells could potentially be used to regenerate these diseased or damaged tissues, but directing the stem cells to convert to the needed tissue is still a technical challenge. Since all cells respond to their immediate physical environment, the goal of Project 4 is to create biomaterial surfaces that will help direct stem cells to regenerate the needed bone, cartilage, tendon or muscle tissue. This work is done in close collaboration with the other projects which study the biology of stem cells. During the past year flat (2-dimensional) collagen and hydroxyapatite surfaces were formed. These mimic the environment in our target tissue, so we expect cells contacting these biomaterial surfaces to more rapidly transform into bone, muscle, etc. The experimental surfaces were thoroughly characterized to insure the proper chemistry, purity and structure. Initial experiments with mouse calvarial cells showed successful attachment, growth and differentiation of the cells. Future studies will use human embryonic stem cells provided by the other projects. Additionally, 3-dimensional, porous biomaterials carrying cells will be used to repair tissues in animal experiments. Results from these experiments will be used to improve the design of the biomaterials.

e. *Project 5: Optimizing Mesoderm derived bone cell differentiation from hES cells Investigator: David Rowe, MD*. We established an animal model for testing the ability of the bone stem cells repair was established using mouse stem cells carrying a gene that makes the cells emit a color that can be detected by a microscope. By having the mice that contribute the stem cells emit one color and the host that receives the donor transplant be another color, it is possible to distinguish the host and donor contribution to the bone repair. Having demonstrated that this model is very effective in interpreting the cellular events within a mouse based stem cell bone repair, we are adapting it to human stem cells. A colony of genetically engineered mice capable of accepting and sustaining a human transplant was established and the process to place the color identification genes into these mice was begun. Before the mice become useful, a specialized breeding protocol is necessary that requires a gene chip study to direct which mice are best for mating. Establishing this breeding technology will be undertaken in the second year. The steps for placing the color identification genes into human cells require a very different process than that used for mice. Specialized viruses were constructed to contain the color identification genes that previously have been shown to be functional in human adult stem cells directed to form bone in a mouse repair model. Now we are in the process of placing these genes into hES cells so that an hES cell line containing this vector can be used to test ways to make the hES cells progress to become a bone stem cell. We believe we are on track to begin a systematic process to optimize the conditions for directing hES cell to become bone progenitor cells that will be useful in bone repair.

f. *Project 6: Optimizing mesoderm derived bone cell differentiation from hES cell Investigator: Mei Wei, PhD.* Over 10 million Americans are currently carrying at

least one major implanted medical device in their body. Among these implants, those for repair of bone fracture and other damage constitute a large proportion, and play an essential role in more than 1.3 million bone-repair procedures per year in the USA. Bone tissue engineering is a new emerging field, which has major potential to improve human health by repairing and maintaining existing bone or generating new bone. Three-dimensional biodegradable tissue-engineering scaffolds have become a promising alternative approach for bone repair. The scaffold provides a framework for cell attachment, proliferation and differentiation; formation of extracellular matrix; and templating new bones into various shapes. During the process of new bone tissue formation, the scaffolds gradually degrade and are replaced by regenerated host tissue. Thus, scaffolds have the advantages of autografts - the "gold standard" for grafting materials, but are not restricted by supply. With this approach, however, the success of bone repair is heavily dependent on the design of the scaffold. Despite many early successes, there are few bone tissue-engineering scaffolds available on the market for clinical use, and significant challenges still remain in the success of long-term bone repair. In this study, a series of tissue engineering scaffolds have been prepared. By adjusting the processing parameters, we have been successful in controlling the pore size, porosity, degradation rate and mechanical properties of these scaffolds. Currently, we are in the process of conducting in vivo tests and using the obtained results to carefully tune our scaffold fabrication parameters. It is our goal to produce tissue engineering scaffolds with properties comparable to autografts.

g. <u>*Project 7*</u>*: Craniofacial Sciences Optimizing neural crest derived bone cell differentiation from hES cells, Investigator: Mina Mina, PhD.* There is substantial need for the replacement of tissues in the craniofacial complex that are lost due to congenital defects, disease, and injury. Virtually all craniofacial structures are derived from a special population of embryonic cells called cranial neural crest cell that are different from cells that give rise to the skeletal elements in the appendicular or axial skeleton. Thus, effective cell-based therapies for skeletal tissues in the craniofacial complex are dependent on isolation and identification of stem/progenitor cells capable of regeneration of skeletal tissues with structural, morphological and mechanical properties similar to craniofacial skeleton. To address these issues we are optimizing conditions for generation and identification of neural crest progenitors from mouse embryonic stem cells that can be applied to human embryonic stem cells. Our hope is to use these cells towards regeneration of loss tissues in the craniofacial skeleton including the teeth.

h. <u>*Project 8*</u>*: Cartilage Differentiation from hES derived progenitor cells, Investigator: Robert A. Kosher,* Degenerative diseases of cartilage particularly osteoarthritis are among the most prevalent and debilitating chronic health problems in the United States. About 90% of the population over the age of 40 exhibits some form of cartilage degeneration in their joints resulting in pain and immobility. Treatment of degenerative cartilage diseases is a clinical challenge because of the limited capacity of the tissue for self-repair. Because of their ability to differentiate into multiple cell types and their unlimited capacity for self-renewal, human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPSCs), which are adult human

cells that have been reprogrammed to a pluripotent state, are potentially powerful tools for the repair of cartilage defects. To this end, we have successfully developed culture systems and conditions that promote the progressive and uniform differentiation of hESCs and iPSCs into cartilage cells and their precursors. We are now poised to test the ability of these cells to repair damaged and diseased human cartilage from osteoarthritic patients. Thus, we are on the verge of developing cell based therapeutic approaches for the treatment of very common and destructive joint diseases such as osteoarthritis.

i. *Project 9: A mouse model to study the myogenic potential of human embryonic stem cells, Investigator: David Goldhamer, PhD.* Cell therapies for muscular dystrophy have shown some promise in animal models. Muscle precursor cells (myoblasts) injected into leg muscles of the mdx mouse (a mouse model for Duchenne muscular dystrophy) will fuse with damaged host muscle and with each other, resulting in muscle fibers that now produce Dystrophin, the protein missing in Duchenne muscular dystrophy. Injected myoblasts, however, are inefficient in muscle repair and are unable to efficiently produce muscle stem cells (satellite cells)—an essential requirement for long-term therapeutic benefit. Injected myoblasts also show poor survival, which will limit their treatment effectiveness for the large muscle masses encountered in a clinical setting. A major objective of the present proposal is to evaluate and optimize the ability of hESC-derived progenitor cells to repair skeletal muscle and to produce muscle stem cells. Optimization of hESC differentiation and isolation of cells with myogenic capacity will be aided by the development of reporter constructs to monitor the production of cells with myogenic capacity. hESC-derived myogenic cells will be tested for their ability to repair muscle in new and existing models of muscular dystrophy.

j. *Project 10: Use of hES cell derived dermal fibroblasts for therapy of cutaneous wounds, Investigator: Stephen Clark, PhD.* The skin is the largest organ of the body. It serves as the first line of defense for unwanted pathogens and participates in regulation of body temperature. It is frequently subjected to environmental insults to which in most settings it can readily repair. However, in a variety of disease states, the skin cannot complete the repair process leading to the presence of chronic wounds. The failure of skin injuries to heal successfully is particularly problematic in the elderly and diabetic patients. The objective of this research program is to identify cell based approaches to improve the healing of cutaneous wounds. We have been utilizing animal models to determine if the application of specific cells to a cutaneous wound will improve the wound healing process. The progress to date indicate that the application of a combination of cells of the immune system and cells with the potential of producing proteins that form new tissue at the wound site may be important for improving wound repair. Thus the use human embryonic stem (hES) cells or reprogrammed patient specific somatic cells as a source to produce large numbers of specially selected immune cells as well as cells that create new skin tissue could be used in a clinical setting in the treatment of chronic skin lesions.

4. *Mechanisms of Stem Cell Homing to the Injured Heart, Investigator: Linda Shapiro, Ph.D.,* Stem cells have the amazing capacity to contribute to the growth and healing of many different types of tissues. This ability is critically dependent on the cells successfully finding the damaged tissue and effectively incorporating into the site. Currently, stem cells are generally injected into the site of an injury to increase the chances of correct cell delivery, but injection into the heart is quite invasive and carries a certain degree of risk. Stem cell therapy would be greatly simplified if the cells could be injected into the bloodstream and allowed to "home," or find their way to the damaged tissue. It is known that both the blood vessels of injured tissues and the traveling stem cells display a number of unique molecules on their surfaces that allow them to recognize and attach to each other to begin the process of integrating the stem cells into the damaged tissue. Interestingly, stem cells will bypass healthy blood vessels that lack these special molecules as they search for vessels with the correct "address." This prevents incorrect positioning. A few of these special molecules have been identified, but stem cell homing is so complex that more of them must exist in order to regulate this intricate process. The researchers have identified a molecule – CD13 – that is found in damaged heart vessels following myocardial infarction (a heart attack) as well as on stem cells of many lineages. CD13 could serve as a recognition molecule and, indeed, the researchers have observed that it participates in the attachment of other types of circulating cells to blood vessels. The researchers have devised a method to improve CD13's ability to influence circulating cells to recognize and attach to injured blood vessel walls. Using that method, the researchers will investigate the role CD13 plays in stem cell homing to the injured heart and their capacity to enhance homing.

5. *Williams Syndrome Associated TFII-I Factor and Epigenetic Marking-out in Human ES Cells and Induced Pluripotent Stem (iPS) Cells, Investigator: Dashzeveg Bayarsaihan PhD.* Williams syndrome (WS) is a complex disorder with distinctive features that include craniofacial defects, mental retardation, microcephaly and short stature. Recent findings have pointed to the gene GTF21 as the prime candidate gene responsible for WS. The TFII-I factor is a product of GTF21. It regulates a set of enzymes and it is thought that TFII-I deficiency might disturb embryonic development. The researchers hypothesize that TFII-I is required for maintaining the correct special and temporal expression of a specific subgroup of epigenetic marker genes. The purpose of the project is to investigate epigenetic – that is, changes in gene expression – marking-out in the WS-derived iPS cells.

6. *Therapeutic Differentiation of Regulatory T Cells from iPS for Immune Tolerance, Investigator: Zihai Li MD, PhD.* One of the main challenges of the body's immune system is to maintain a fine balance between the simultaneous tasks of fighting against germs, but not damaging healthy tissues. Scientists have found that this balance is managed, in part, by a key regulatory T cells called Tregs. Bearing a unique gene, Foxp3, Tregs can suppress self-reactive immune responses and they have emerged as a promising therapeutic tool for autoimmune diseases such as diabetes, lupus, arthritis and inflammatory bowel diseases. The purpose of this research is to generate regulatory T cells from stem cells for treatment of autoimmune diseases. The researchers will derive Tregs from both human

embryonic stem cells (hESCs) and induced pluripotent stem cells (iPS cells). To date, no study has specifically addressed the issue of Treg development from stem cells. Moreover, this is the first in-depth study to compare Tregs generated from different sources of stem cells.

7. *Prevention of Spontaneous Differentiation and Epigenetic Compromise in Human ES Cells and iPS Cells, Investigator: Theodore Rasmussen PhD.* Human embryonic stem cells (hESCs) and induced pluripotent stem cells (iPS cells) are of great promise for medicine because they can be coaxed to differentiate into all cell types in the human body. However, both hESCs and iPS cells frequently undergo spontaneous and irreversible alterations during their culture in vitro. Those alterations are often epigenetic in nature, meaning that though the DNA sequence may remain unaltered, gene expression becomes mis-regulated. This compromises the quality of the cells and their usefulness for clinical applications. The object of this research is to identify methods and chemical compounds that can prevent the spontaneous loss of quality of hESCs and iPS cells.

8. *Targeting Lineage Committed Stem Cells to Damaged Intestinal Mucosa, Investigators: Daniel W. Rosenberg, PhD and Charles Giardina, PhD.* Damage to the intestinal mucosa can occur as a result of inflammatory bowel disease, or even as a result of radiation therapy-induced damage. Fortunately, the intestinal mucosa provides an excellent experimental system for studying tissue renewal and repair. Our work requires the development of new methodologies for working with human embryonic stem cells (hESCs) to induce their commitment into multi-potent intestinal stem cells. We hypothesize that these lineage-committed stem cells will migrate to the damaged gut wall, undergo engraftment, and ultimately form a fully repaired and functionally reconstituted epithelium, thus providing enormous therapeutic potential.

9. *Directed Differentiation of ESCs into Cochlear Precursors for Transplantation as Treatment of Deafness, Investigator: Kent Morest, MD*. This grant will lay the foundation for stem cell therapy for human hearing disorders, including deafness, partial hearing loss and ringing in the ears (tinnitus) due to noise, drugs, infections, and aging. Current therapies are not adequate in most cases, since they do not deal with the brain degeneration which may progress over time. Replacing the receptors in the ear does not correct the central problem. New sensory neurons and their central connections are necessary. One promising approach is to replace the sensory nerve cells with healthy new ones. We are developing methods to produce the sensory neurons from human embryonic stem cells. Our preliminary results suggest that we can transplant such stem cells into postnatal and adult mice so that they survive and form new connections within the damaged parts of the brain. We will test the hearing of the host mice. Our clinical collaborators are ear surgeons and aging experts who will explore possible applications to human patients. There is a rising need for this therapy due to veterans, factory workers, and youngsters exposed to noise, children exposed to infections, cancer patients exposed to drugs, and an aging population.

10. *Optimizing axonal regeneration using a polymer implant containing human embryonic stem cell-derived glia, Investigator: Akiko Nishiyama, PhD.* Cell replacement is an attractive approach to minimize brain damage after injury and promote recovery. In the proposed project, we are investigating the ability of glial cells generated from hESCs to promote axonal regeneration in vitro and in vivo. This proposal differs from most other studies that have used stem cells for cell replacement therapy in that it seeks to generate glial cells that will be used as a supportive cellular substrate to promote endogenous neuronal regeneration, rather than attempting to develop neurons or oligodendrocytes from hESCs to replace lost cells. Optimization of methods to utilize supporting cells derived from hESCs to promote regeneration of damaged neurons will have a wide range of clinical applications ranging from treatment of acute spinal cord injury to chronic neurodegenerative diseases.

11. *Human embryonic stem cells (hESC) as a source of radial glia, neurons and oligodendrocytes, Investigator: Nada Zecevic, PhD.* To understand human brain in health and in disease we need to better characterize cortical neural progenitor cells and factors that determine their differentiation in neurons, oligodendrocytes or astrocytes. We previously established method of isolating and differentiating radial glia cells (RG) from human fetal brains (fetal RG) and characterized these cells as multipotent neural progenitors. Numerous ethical and practical problems, however, exist in harvesting these cells from human fetal brains. In contrast, hESC are readily available and can be developed to a precise level of maturation in well controlled conditions. We will investigate whether hESCsc could be used as a source of human radial glia (RG) cells by comparing their cellular characteristics (molecular, morphological, electrophysiological), proliferation and differentiation potentials to the fetal RG. Furthermore, we will examine factors that determine their differentiation into either neurons or oligodendrocytes (OLs). OLs produce myelin sheaths damaged in multiple sclerosis, and thus are of particular clinical interest. By studying how to manipulate hESC *in vitro*, we can better understand conditions necessary to generate a larger number of progenitors for future cell therapies, and determine the exact stage of development for grafting to avoid risk of tumor formation. This knowledge is necessary for better understanding development of the human brain and for creating novel cell therapies for neurodegenerative and demyelinating diseases, such as multiple sclerosis.

12. *Synaptic replenishment through embryonic stem cell-derived neurons in a transgenic mouse model of Alzheimer's disease, Investigator: Ben Bahr, PhD.* While there is great interest in the medical application of human stem cells in regards to treating age-related diseases like Alzheimer's, little is known about their ability to survive once transplanted into the aged brain. Since earlier studies indicate significant cell death within a few days of implantation, our challenge is to address age-related processes that contribute to the poor transplant survival rate in the aged brain. Over the last 15 years, we have helped to identify novel drug classes that can block pathogenic pathways that are most pronounced in the aged brain. This project will test such drug classes and combinations there of to enhance the survival of transplanted stem cells. These efforts may increase the effectiveness of stem cells to offset the loss of connections between nerve cells

that occurs in Alzheimer's disease. Reducing the loss of nerve connections and the associated memory deficits will be tested in a mouse model of Alzheimer-type pathology. The proposed study will provide important insights into how to slow the cellular compromise and associated cognitive deficits of human dementias.

13. *dsRNA and Epigenetic Regulation in Embryonic Stem Cells, Investigator:Gordon Carmichael, PhD.* The goal of this project is to elucidate some of the fundamental molecular mechanisms that govern stem cell self-renewal, pluripotency and differentiation in humans. Of particular interest to us are the pathways by which embryonic stem cells respond to double-stranded RNA molecules (dsRNA). In work supported by this grant we have recently discovered that hESCs respond in unique ways to dsRNAs, and that this may be essential for the maintenance of pluripotency. This novel response involves how cells deal with messenger RNA molecules (mRNAs) that contain regions of dsRNA within them. In differentiated cells many such mRNAs are stored in the nucleus and not exported to the cytoplasm until needed. One mRNA of this type is that for Lin28, an important regulator of pluripotency. In hESCs, however, mRNAs with dsRNA structures in them (including that for Lin28) are never sequestered in the nucleus but are rapidly sent to the cytoplasm for translation. Moreover, our stem cell work has helped us to understand the underlying basis of this phenomenon. This involves the function of a special RNA molecule in the nucleus called NEAT1, which is expressed in every cell ever examined so far, but is lacking in hESCs. NEAT1 is induced immediately upon differentiation. Thus, our recent studies on NEAT1 RNA suggest that NEAT1 and other components of our dsRNA response system might serve as important new markers for stem cells. In addition, this work allows us the opportunity to examine the exciting possibility that manipulation of NEAT1 RNA levels might help us to more easily convert somatic cells to pluripotency, as well as to fine tune stem cell growth and differentiation.

14. *SMAD4-Based Chip-Chip Analysis to Screen Target Genes Of BMP and TGF in Signaling in Human ES cells, Investigator: Ren-He Xu, PhD.* Our goal is to elucidate fundamental mechanisms that control the self-renewal and differentiation of human embryonic stem cells (hESCs), which is important for understanding how to use hESCs as a powerful tool for basic and clinic research. We are particularly interested in identifying target genes regulated by the signaling pathways stimulated by the transforming growth factor beta (TGFb, which sustains hESC self-renewal) and bone morphogenetic protein (BMP, which induces hESC differentiation). We examined in detail the expression of pluripotency genes in hESCs treated with molecules that stimulate or inhibit the TGFb and BMP pathways. By inhibiting the TGFb pathway, we observed that the expression of the key pluripotency gene *NANOG* appears to rapidly decrease. These important findings suggest that *NANOG* is a direct target for the molecules called SMAD2 and -3 that are activated in the TGFb pathway. By using multiple approaches, we confirmed that these SMADs control the expression of *NANOG*. This is the first evidence that links a signaling pathway such as TGFb to a pluripotency gene in hESCs. This finding is of great significance in advancing our understanding of the mechanisms for hESC self-renewal and differentiation. It was published in the prestigious journal *Cell Stem Cell* in August 2008.

15. *Tyrosine phosphorylation profiles associated with self-renewal and differentiation of human embryonic stem cells, Investigator: Bruce Mayer, PhD.* The aim of this project is to understand the mechanistic details of how the switch between self-renewal and differentiation is controlled in human embryonic stem cells. This knowledge will allow us to better control the behavior of these cells in order to generate specialized cells that can be used to treat patients. It is known that one of the mechanisms controlling this process is tyrosine phosphorylation, which is the addition of a phosphate group to the amino acid tyrosine in proteins. Unfortunately, despite their importance, tyrosine phosphorylated proteins are present in the cell at vanishingly low levels, making it very difficult to study and characterize them. The investigator's group has recently developed a new and highly sensitive method to profile the entire spectrum of tyrosine phosphorylated proteins in a cell sample, termed SH2 profiling. This new method will be used to profile the phosphorylation patterns in federally approved human embryonic stem cells under conditions that affect self-renewal, survival, and differentiation. Based on these results, the investigators will go on to identify specific phosphorylated proteins that play a key role in these cell fate decisions. By applying a new, cutting-edge proteomic method to human embryonic stem cells, important new insights into how to maintain human stem cells in culture and manipulate them for therapeutic purposes will be gained.

16. *Modeling Motor Neuron Degeneration in Spinal Muscular Atrophy Using Human Embryonic Stem Cells, Investigator: Xue-Jun Li, PhD.* Spinal muscular atrophy (SMA), the leading genetic cause of death in infants and toddlers, is caused by by an abnormal or missing gene known as the survival motor neuron gene (SMN1). This gene is responsible for the production of survival motor neuron (SMN) protein. Without sufficient SMN protein, lower motor neurons in the spinal cord degenerate and die. The loss of motor neurons leads to weakness and shrinkage of muscles. Currently there is no cure for this disorder, primarily due to the lack of an experimental system for understanding why human motor neurons are specifically susceptible to diminished levels of SMN protein and for screening effective therapeutic agents. This project, built upon our successful generation of spinal motor neurons from human embryonic stem cells (hESCs), aims to model the motor neuron degeneration that occurs in spinal muscular atrophy through modifying hESCs. First, we intend to establish stable hESC lines with a deficiency in SMN protein levels. Spinal motor neurons will then be differentiated from these hESC lines and a control line and assayed for a variety of functional changes including survival, neurite outgrowth, ability to form synaptic connections with muscle cells, apoptosis and cell death. By comparing the responses of motor neurons and other neurons to reduced levels of SMN protein, we will be able to understand why motor neurons are affected in this disorder. The successful establishment of such a human cell model has the potential to greatly advance research and treatment of spinal muscular atrophy. Our system will also provide a unique platform with which high-throughput drug screening may pinpoint compounds to treat this debilitating and fatal genetic disorder.

17. *Alternative Splicing in Human Embryonic Stem Cells, Investigator: Brenton Graveley, PhD.* Cells and organisms function based on the expression patterns, actions, and interactions of thousands of genes and their products. To fully understand how stem cells work and to develop the power to differentiate (hES) cells into specific cell types for therapeutic use, it is essential to determine their complete gene expression program. Many studies have been conducted to identify the genes that endow hES cells with their amazing potential to generate all possible cell types. However, these studies have overlooked an important aspect of the gene expression program of these cells - alternative splicing. Alternative splicing is a process by which the quality and function of a gene can be altered thereby increasing the complexity of how our genes work. This process can, for instance, allows a given gene to function one way in one cell type, but in a completely opposite way in another cell type. Alternative splicing is one of the most important mechanisms by which gene expression is regulated and at least 90% of human genes use this process of gene regulation. The main goal of this project is to identify the alternative splicing events that occur in undifferentiated hES cells and in hES cells undergoing differentiation into different cell types as well as the roles of specific RNA binding proteins such as Musashi1, in controlling these alternative-splicing events. This research project will allow us to obtain a more thorough understanding of the gene expression program of hES cells which is essential knowledge for the long term goal of directing the differentiation of hES cells into specific cell types. During the second year of this project we have begun using high throughoput DNA sequencing technology that allows us to examine all human genes at the same time and to determine which genes are on and off, and which alternative spliced versions are made in each cell. Using this technology, in collaboration with Dr. Ren-He Xu, we have analyzed these characteristics in undifferentiated hES cells grown under three different culturing conditions. We have also begun analyzing the gene expression and alternative splicing patterns in the CT1 and CT2 cell lines that were recently derived in Dr. Xu's laboratory. In collaboration with Dr. Xue-Jun Li, we are also studying how the gene expression and splicing patterns change in hES cells as they differentiate into motor neurons. The results of these experiments will significantly increase our understanding of the gene expression program in hES cells which will be useful in directing hES cell differentiation for therapeutic purposes.

18. *Migration and integration of embryonic stem cell derived neurons into cerebral Cortex, Investigator: Joseph LoTurco, PhD.* The cerebral cortex of the brain is the major target of several currently untreatable degenerative and traumatic brain disorders including Alzheimer's disease and stroke. If neural cell transplantation therapies are to be successful it will be necessary to direct the migration and positioning of transplanted neurons. Many of the mechanisms that operate during normal development to ensure the appropriate migration and positioning of neurons in the developing brain have been discovered over the past twenty years, and we hypothesize that by manipulating some of these same mechanisms it will become possible to direct the patterns of migration of hECS-derived neurons transplanted into the damaged or diseased brain. Towards this effort in the first year of the funding period we have established culture of H9 human embryonic stem cells and grown them into neuronal progenitor cells. We have also

constructed DNA vectors that can now be used to alter the stem cell derived neurons in ways that may improve their migration once transplanted.

19. *Human Cell Culture Model of Angelman Syndrome for Drug Screening, Investigator: Stormy Chamberlain PhD*. Angelman syndrome (AS) is a human neurodevelopmental disorder that causes mental retardation, lack of speech, ataxia (the inability to coordinate muscle movements) and seizures. The purpose of the research is to develop a human neuronal cell culture model to study and develop therapies for this devastating disorder. Researchers will use two different pluripotent stem cell lines generated to model AS. The cells will be differentiated into neurons in order to determine how the AS gene is regulated during human neuronal development. With a cell culture to study, researchers will screen for drugs that may be useful in treating Angelman syndrome.

20. *Hybrid Peptide/RNA Molecules for Safe and Efficient Gene Silencing in Human Embryonic Stem (ES) Cell, Investigator: Yong Wang PhD.* Small interfering RNA (siRNA), which is also known as "short interfering RNA" or "silencing RNA," is a class of RNA molecules that interfere with the expression of specific genes. They are of critical importance in human embryonic stem cell (hESC) research, but current methods used for their delivery into hESCs have many limitations. The purpose of the research is to develop novel hybrid molecules that address those limitations, facilitating efficient siRNA delivery.

21. *Can Natural Neuromodulators Improve the Generation of Nerve Cells from Human Embryonic Stem Cells? Investigator: Srdjan Antic PhD.* Parkinson's disease is caused by the degeneration and death of a small group of neurons called dopaminergic neurons that release an important substance dopamine. It is a neurotransmitter that plays an important role in behavior, cognition, motor skills and many other brain functions. Human embryonic stem (hESC) cells may serve as a renewable source of neurons. Indeed, several research groups have been able to grow dopamine-releasing neurons in laboratories and then transplant them into an animal model of Parkinson's disease. Several obstacles, however, must be surmounted before this can become a treatment for Parkinson's. The research will explore a novel method for improving the procedures for nerve cell generation from hESC using natural neuromodulators, whose positive effects on neuron proliferation, migration, differtiation and maturation are well known.

22. *Novel Aspects of RNA Editing in Human ES Cells. Investigator: Ling-Ling Chen PhD.* RNA editing is the process through which the genetic information in molecule of RNA is chemically changed. It is known that RNA editing plays a role in inhibiting gene expression in some instances. The purpose of this project is to understand how this complex cellular process impacts human embryonic stem cells and to clarify the role of RNA editing in the maintenance of self-renewal and pluripotency, stem cells' capacity to become many different kinds of tissue.

23. *Evaluation of Homologous Recombination in Human Embryonic Stem Cells and Stimulation Using Viral Proteins. Investigator: April Schumacher PhD.* Gene targeting is a process that alters genes through recombination, the introduction of

replacement genetic material. A number of targeting methods have been developed, but knowledge of cellular pathways – the chemical sequences that change cell behavior, and can result in disease – is still quite limited. In order to use stem cells therapeutically, researchers will have to target specific genes to generate the modified cells that will be required. The purpose of this project is to improve gene targeting in human embryonic stem cells in order to capitalize on the potential of embryonic stem cells to treat human injury and disease.

24. *Pragmatic Assessment of Epigenetic Drift in Human Embryonic Stem Cell Lines. Investigator: Theodore Rasmussen, PhD.* The overall purpose of this grant is to develop unambiguous assays that allow us to determine and monitor the developmental quality of human embryonic stem cell (hES) lines grown in vitro. All human cells contain identical content of DNA, yet cells come in many different varieties. The variety or "type", of a cell is therefore determined largely by the set of genes that are expressed in that cell (out of the total set of genes present within the human genome). Each cell's characteristic set of genes are expressed and maintained semi-stably by an epigenetic system that operates within each cell. This system functions by mechanisms that operates through the action of proteins that physically bind to DNA in sequence-specific ways that are characteristic for each cell type. The involved proteins are called histones, and these can be modified by small molecular additions such as methylation and acetylation, which in turn can affect the status of expression of associated genes. We have completed optimization and validation of a new molecular biology method that can quantitatively monitor epigenetic states in ES cells. Our experiments indicate that epigenetic status of human ES cells in quite stable over protracted growth of these cells in the laboratory. This bodes well for their future use in therapies. However, sometime epigenetic alterations do occur in human ES cells. The methods being developed in my lab will allow us to monitor and confirm the quality of specific isolates of ES cells to ensure their utility and safety prior to their use in future cell-based therapies. We continue to expand these epigenetic analyses to cultured ES cells, and to improve the sensitivity and accuracy of our epigenetic validation methods.

25. *Cytokine-induced production of transplantable hematopoietic stem cells from human ES cells. Investigator: Laijun Lai, PhD.* Hematopoietic stem cell transplantation (HSCT), the most common cell-based therapy applied today, is widely used in the treatment of a variety type of cancer, aplastic anemia, complications of irradiation and chemotherapy, primary (hereditary) and secondary (acquired) immunodeficiency disorders, organ transplantation and autoimmunity. Bone marrow, umbilical cord blood, and mobilized peripheral blood are the major sources of hematopoietic stem cells (HSCs). However, especially in adult patients, HSCT is frequently limited by the unavailability of sufficient freshly harvested HSCs and by the inability to reliably expand the number of transplantable HSCs from these sources *in vitro*. Therefore, the evaluation of alternative sources of cells for HSCT remains an important goal. Given that embryonic stem cells have dual ability to propagate indefinitely *in vitro* in an undifferentiated state and to differentiate into different cells, it is likely that human embryonic stem cells can serve as a prime source of HSCs for

hematopoietic reconstitution. The overall objective of this project is to establish a method to generate transplantable HSCs from human embryonic stem cells that could be used for the treatment of a variety of diseases.

26. *Development of efficient methods for reproducible and inducible transgene expression in human embryonic stem cells. Investigator: Yuanhao (James) Li, PhD.* Human embryonic stem cells (hESCs) are an unlimited source of precursor cells that can be directed to differentiate into various types of cells for regenerative medicine and for studies of toxicology and pharmacology. The promises of hESC applications depend on our knowledge and ability to drive hESC differentiation into particular cell types as desired. Genetic manipulations of hESCs are essential for the study of hESC biology and differentiation. The overall goal of this proposal is to develop methods for genetic modification of hESCs. The modified hESC lines would be used to explore new approaches to produce neurons in the inner ear for treatment of hearing loss.

27. *Lineage Mapping of Early Human Embryonic Stem Cell Differentiation, Investigator: Craig Nelson, PhD.* Realizing the full promise of stem cell therapy depends upon our ability to generate medically useful cell types from human embryonic stem cells. The primary objective of our project is to create a "roadmap" of early human embryonic stem cell differentiation that can guide the efficient production of cells for regenerative medicine and cell replacement therapy. In order to draw this map we pioneered single cell analysis methods. These methods allow us to track the identity of individual human embryonic stem cells as they develop and differentiate in culture. By amplifying the genetic material of single differentiating stem cells we can ascertain the precise identity of each cell in a complex population. With funding from our State Seed Grant we have perfected single-cell PCR and single-cell microarray expression assays for use with human stem cells, we have developed statistical methods for analyzing the data derived from these cells, and generated the first single-cell resolution lineage map of human embryonic stem cell development. The findings in this study include a number of previously unknown phenomena, including one that may be critical to preventing cancers derived from stem cell transplants.

28. *Differentiation of hESC Lines to Neural Crest Derived Trabecular Meshwork Like Cells-Implications in Glaucoma, Investigator: Dharamainder Choudhary, PhD.* Glaucoma is the major cause of blindness worldwide. One of the major risk factors for development of glaucoma is an elevated intraocular pressure (IOP). This develops due to resistance to the aqueous humor outflow in the trabecular meshwork (TM) region of eye. The treatment generally constitutes of either to decrease the synthesis rate of aqueous humor or increase the outflow by performing surgery to cannulate the pathway. These treatments are not permanent and patients require repetitive surgeries in many cases. Human embryonic stem (ES) cells offer a unique advantage of generating a differentiated cell line of TM cells, which can be targeted for transplantation in the anterior chamber, to replace the damaged TM cells and populate the structure with the healthy TM cells. Although this is a distant goal it can be accomplished, and the first aim of the current proposal is to develop the optimal conditions for

differentiating human ES cells to a cell type, which displays characteristics similar to TM cells. This will involve the coculture of ES cells with a stromal cell line for induction of differentiation and isolating the mesenchymal precursor cells using CD73-labelling. Our novel approach of targeting the TM cells derived from mesenchymal cells of neural crest origin has a strong potential to act as a viable future glaucoma treatment strategy.

29. *Embryonic Stem Cell as a Universal Cancer Vaccine, Investigator: Bei Liu, M.D.* Long before embryonic stem (ES) cells were used for genetic and developmental studies, it was appreciated that cancer cells have uniformly undergone a process called “de-differentiation”. Tumor and ES cells thus bear many similarities to each other including sharing embryonic antigens that can trigger immune responses. In this proposal, we aim to generate tumor vaccines from plain ES cells. The immunogenecity (the ability to induce immune responses) of these ES cells will be further enhanced by expressing heat shock protein (HSP) gp96 on the cell surface. Our laboratory has significant expertise in using HSP technology, which is currently in late phase clinical trial against kidney cancer, melanoma and colon cancer. The ES cell-based cancer vaccines are expected to stimulate the immune system to recognize a variety of embryonic and cancer antigens that are shared across the broad spectrum of cancers. With the proper inflammatory signal such as HSPs, we will test the concept, for the first time, that ES cells provide a universal cell-based vaccine against cancer. Although our approach is unlikely to induce self-reactive autoimmune diseases, we will rigorously address this possibility along with other safety concerns. Our study, if successfully carried out, could potentially lead to the beginning of direct clinical testing of stem-cell based cancer vaccine to benefit cancer patients in the State of Connecticut.

30. *Quantitative analysis of molecular transport and population kinetics for stem cell cultivation in a microfluidic system, Investigator: Tai-Hsi Fan, PhD.* Precise control of the microenvironment sustaining stem cell proliferation and differentiation is important for advances in regenerative medicine. This project focuses on the design and analysis of biochip-based microculture systems for the culture of undifferentiated embryonic stem cells. Microenvironmental conditions near cell colonies in conventional culture dishes are highly variable. A custom fabricated microsystem, however, can maintain a relatively uniform culture environment to facilitate cell proliferation, control the distribution of cell colonies and the delivery of chemicals for screening analysis, and reduce the expenditure of precious biochemical reagents. Using microculture systems, a several milliliter culture medium is sufficient for a week-long experiment involving parallel trial runs. In this project the hypothesis is that stem cell behaviors in small-scale perfusion culture systems are more predictable and controllable compared with those in conventional culture dishes. Therefore the engineering technology can be used to explore opportunities for advancing stem cell biology and for predicting stem cell behaviors and properties in the growth medium. Through interdisciplinary collaborations, progress has been made toward generating a prototype microfluidic chamber for culturing embryonic fibroblast cells and stem cells. We expect that the understanding of cell behaviors and population kinetics in

microculture systems can be translated to large-scale scaffold and lead to better methodologies for tissue reconstruction.

31. *Cell Cycle and Nuclear Reprogramming by Somatic Cell Fusion, Investigator: Winfried Krueger, PhD.* The increasing ratio of elderly to adults in working age in western societies represents a menacing problem with far reaching socio-economical consequences. Age related degenerative diseases affect personal independence and quality of life and their prevalence in western societies increase unproportionately due to increased numbers of elderly and the extended average lifespan, placing an enormous burden on national social security and health care networks. Somatic reprogramming represents a methodology for conversion of differentiated adult cells into their embryonic stem cell counterparts and these patient-matched stem cells may provide a solution to alleviate the age associated socio-economic national burden. Induced pluripotent stem cells (iPSCs) represent the most prominent advance for somatic reprogramming and cell based therapies in regenerative medicine. It is now critical to understand all molecular requirements for somatic reprogramming in order to facilitate the application of iPSCs for routine clinical intervention. The identity of a basic set of reprogramming factors is known and it is paramount to determine the mechanism for reprogramming and whether these factors are sufficient for reprogramming with equal efficiency of all somatic cells and especially those of the elderly. A single report suggests that cells from aged individuals can be reprogrammed but little is known about efficiency or extent of reprogramming into bona fide pluripotent stem cells. Somatic cells can also be reprogrammed through fusion with existing human embryonic stem cells containing a larger set of natural reprogramming factors. Fusion mediated reprogramming may therefore permit the production of reprogrammed hybrid cells from a larger array of cell sources and ultimately lead to the identification of all possible reprogramming factors capable of modifying the more restrictive nuclear architecture of somatic cells from aged individuals. To enable detection of fusion mediated reprogramming in human cells we have a created a reporter system required for the quantitative assessment of reprogramming efficiency. We are now comparing reprogramming efficiencies in hFFs and human dermal fibroblasts in a cell cycle independent fashion by fusion and factor mediated reprogramming. We find that the two cell sources are not equivalent and are now in the process of identifying the underlying cause.

32. *Generation of insulin producing cells from human embryonic stem cells, Investigator: Mark Carter, PhD*. Diabetes involves the destruction of β-cells in the pancreas, which secrete insulin in response to elevated blood sugar concentrations. Insulin directs the body's tissues to absorb the excess glucose, thus regulating blood sugar levels. When β-cells are absent or not functioning properly, patients must take exogenous insulin to regulate their blood sugar, and face serious health problems, including loss of sensation and circulation in the extremities, poor wound healing, amputation, coma, and death. Current estimates hold the yearly healthcare costs of diabetes in the U.S. at $132 billion, and the Centers for Disease Control and Prevention predicts that 1 in 3 Americans born after 2000 will develop diabetes in their lifetime. While previous attempts to produce β-cells from human embryonic stem cells have produced cells which

secrete insulin, they have not resulted in cells which could effectively function to regulate blood sugar levels, nor have they significantly advanced our understanding of how β-cell lineage commitment is controlled. Our research is focused on studying the roles of the two genes PDX1 and NGN1 in directing differentiation of human embryonic stem cells toward β-cell and other pancreatic lineages. These genes control the expression of groups of other genes, and so by increasing or decreasing the expression of these genes, singly or in combination, then observing the changes in expression of key developmental and pancreatic lineage genes, we hope to learn more about how PDX1 and NGN1 function to control development of the pancreas during embryonic development. Most importantly, we hope to understand how they might be used to direct differentiation of ES cell cultures towards pancreatic lineages. A detailed understanding of such lineage control points will be a requirement for efficient production of safe and effective β-cells from ES cells for clinical use.

Wesleyan University[5]

A. Core Facility

Wesleyan University was a co-recipient with UConn of the grant-in-aid to establish a core facility in Farmington. Dr. Laura Grabel is Co-Principal Investigator along with Dr. Ren-He Xu. As reported under the University of Connecticut update on the core facility, the facility was successful in designing, establishing and providing training sessions for members of research teams from across the State of Connecticut. Dr. Grabel continued to run the outreach seminar program, with seven seminars given at Connecticut colleges and universities this past year. In addition, the first hands-on human embryonic stem cell workshop for undergraduates was held at the Farmington core facility.

B. Research Project

Dr. Grabel reports that they continue to make progress in their second year of funding. Major advances include the development of a rapid, direct protocol for generating NSCs with broad regional identities from human ESCs, the determination that transplant derived granule neurons have electrophysiological properties of endogenous cells, and the demonstration that the use of FACS isolated ESC-derived NSCs decreases the level of subsequent teratocarcinoma formation.

Over this past year, the group published a major article on the role of Hedgehog in ESC neurogenesis, (Cai, C., Thorne, J., and Grabel, L. 2008, Hedgehog serves as a mitogen and survival factor during embryonic stem cell neurogenesis. Stem Cells 26(5):1097-1108); an update on transplantation data (Xu Maisano, Joseph Carpentino[1], Sandy Becker[2], Robert Lanza[2], Gloster Aaron, Laura Grabel, and Janice R. Naegele. Embryonic Stem Cell Derived Neural Precursor Grafts for Treatment of Temporal Lobe Epilepsy. J. Neurotherapeutics, in press); and a review chapter on

[5] [5] The progress report on Connecticut supported stem cell research was provided by Laura Grabel, Ph.D., Department of Biology, Wesleyan University, Middletown, CT

differentiation of ESCs into neuronal subtypes (Ammon, N., Hartman, N., and Grabel, L. Directing the differentiation of embryonic stem cells into distinct neuronal subtypes, In "Stem cells: From tools for studying mechanism of neuronal differentiation towards therapy" H. Ullriich editor, Springer, in press). In addition, Dr. Grabel continued her work with the ethicist Lori Gruen and published a book review in Cell Stem Cell (Grabel, L. and Gruen, L. (2009) Epidosembryos to the rescue. A review of Louis Guenin's book *"The Morality of Embryo Use"* Cell Stem Cell 4:113-114)

C. Internal and External Collaborations

Janice Naegele: The ongoing collaboration with the Naegele laboratory continues to be an integral and essential component of our projects. New contributions include the use of EEG recordings to measure seizure activity and use of the pilocarpine mouse model, which results in recurring seizures.

Gloster Aaron: This interaction has produced the first patch clamp recordings from transplant-derived cells within the granule cell layer of the dentate gyrus and suggest functional integration into the host circuitry. These studies are key to determining the ability of ESC-derived neurons to function in a host brain.

Human Embryonic Stem Cell Core at the University of Connecticut Health Center: This interaction provides us with needed support to grow and differentiate human ESCs and has proven invaluable.

Leonardo Aquilla and the Health Center FACS facility: We are routinely using this FACS facility for key experiments. Flow cytometric analysis has been key to characterizing the role of Hh in NSC survival. FACS isolation of Sox1-GFP+ cells is essential to our ongoing investigation of the how to remove teratocarcinoma-forming potential from the cell population prior to transplant. FACS isolation of human ESC-derived NSCs is currently underway.

Alexander Lichtler and the Health Center Vector Facility. This collaboration facilitated the isolation of the Sox1-GFP/ubiqutin-RFP mouse ESC line described above and will continue to contribute to our transplant work with the human lines. Construction of reporter vectors for out human ESC work is ongoing.

D. Economic Impact

Funding supports the salaries of two graduate students and a technician. In addition, new internal and external support, based upon this project, has led to hiring an additional technician as well as a postdoctoral fellow.

VI. SUMMARY

Passage of the Public Act 05-149 positioned Connecticut as just the third state in the nation, behind only California and New Jersey, in providing public funding in

support of embryonic and human adult stem cell research. Since passage of the enabling legislation in 2005, the State of Connecticut has allocated a total of $39.42 million in support of embryonic stem cell researchers at UCONN, Yale, Wesleyan and the private sector. The allocation of funds has provided ongoing support to the development of two core stem cell research facilities, allowed for the recruitment and retention of world class researchers, and supported new research efforts from established and junior faculty members at the University of Connecticut, Yale University, and Wesleyan University.

Connecticut has successfully positioned itself as a leader in both the national and international stem cell research communities. With robust academic stem cell research programs, with vehicles such as StemCONN and the Interstate Alliance for Stem Cell Research, and with strong collaborative relationships with the International Society for Stem Cell Research, the United Kingdom and Canada, Connecticut will maintain its status as an International Center of Excellence for stem cell research.

APPENDIX A
Public Act 05-149

Substitute Senate Bill No. 934

Public Act No. 05-149

AN ACT PERMITTING STEM CELL RESEARCH AND BANNING THE CLONING OF HUMAN BEINGS.

Be it enacted by the Senate and House of Representatives in General Assembly convened:

Section 1. (NEW) (*Effective from passage*) (a) As used in sections 1 to 4, inclusive, of this act and section 4-28e of the general statutes, as amended by this act:

(1) "Institutional review committee" means the local institutional review committee specified in 21 USC 360j(g)(3)(A)(i), as amended from time to time, and, when applicable, an institutional review board established in accordance with the requirements of 45 CFR 46, Subpart A, as amended from time to time.

(2) "Cloning of a human being" means inducing or permitting a replicate of a living human being's complete set of genetic material to develop after gastrulation commences.

(3) "Gastrulation" means the process immediately following the blastula state when the hollow ball of cells representing the early embryo undergoes a complex and coordinated series of movements that results in the formation of the three primary germ layers, the ectoderm, mesoderm and endoderm.

(4) "Embryonic stem cells" means cells created through the joining of a human egg and sperm or through nuclear transfer that are sufficiently undifferentiated such that they cannot be identified as components of any specialized cell type.

(5) "Nuclear transfer" means the replacement of the nucleus of a human egg with a nucleus from another human cell.

(6) "Eligible institution" means (A) a nonprofit, tax-exempt academic institution of higher education, (B) a hospital that conducts biomedical research, or (C) any entity that conducts biomedical research or embryonic or human adult stem cell research.

(b) No person shall knowingly (1) engage or assist, directly or indirectly, in the cloning of a human being, (2) implant human embryos created by nuclear transfer into a uterus or a device similar to a uterus, or (3) facilitate human reproduction through clinical or other use of human embryos created by nuclear transfer. Any person who violates the provisions of this subsection shall be fined not more than one hundred thousand dollars or imprisoned not more than ten years, or both. Each violation of this subsection shall be a separate and distinct offense.

(c) (1) A physician or other health care provider who is treating a patient for infertility shall provide the patient with timely, relevant and appropriate information sufficient to allow that person to make an informed and voluntary choice regarding the disposition of any embryos or embryonic stem cells remaining following an infertility treatment.

(2) A patient to whom information is provided pursuant to subdivision (1) of this subsection shall be presented with the option of storing, donating to another person, donating for research purposes, or otherwise disposing of any unused embryos or embryonic stem cells.

(3) A person who elects to donate for stem cell research purposes any human embryos or embryonic stem cells remaining after receiving infertility treatment, or unfertilized human eggs or human sperm shall provide written consent for that donation and shall not receive direct or indirect payment for such human embryos, embryonic stem cells, unfertilized human eggs or human sperm.

(4) Any person who violates the provisions of this subsection shall be fined not more than fifty thousand dollars or imprisoned not more than five years, or both. Each violation of this subsection shall be a separate and distinct offense.

(d) A person may conduct research involving embryonic stem cells, provided (1) the research is conducted with full consideration for the ethical and medical implications of such research, (2) the research is conducted before gastrulation occurs, (3) prior to conducting such research, the person provides to the Commissioner of Public Health documentation verifying that any human embryos, embryonic stem cells, unfertilized human eggs or human sperm used in such research have been donated voluntarily in accordance with the provisions of subsection (c) of this section, on a form and in the manner prescribed by the Commissioner of Public Health, (4) the general research program under which such research is conducted is reviewed and approved by an institutional review committee, as required under federal law, and (5) the specific protocol used to derive stem cells from an embryo is reviewed and approved by an institutional review committee.

(e) The Commissioner of Public Health shall enforce the provisions of this section and may adopt regulations, in accordance with the provisions of chapter 54 of the general statutes, relating to the administration and enforcement of this section. The commissioner may request the Attorney General to petition the Superior Court for such order as may be appropriate to enforce the provisions of this section.

Sec. 2. (NEW) (*Effective from passage*) (a) There is established the "Stem Cell Research Fund" which shall be a separate, nonlapsing account within the General Fund. The fund may contain any moneys required or permitted by law to be deposited in the fund and any funds received from any public or private contributions, gifts, grants, donations, bequests or devises to the fund. The Commissioner of Public Health may make grants-in-aid from the fund in accordance with the provisions of subsection (b) of this section.

(b) Not later than June 30, 2006, the Stem Cell Research Advisory Committee established pursuant to section 3 of this act shall develop an application for grants-in-aid under this section for the purpose of conducting embryonic or human adult stem cell research and may receive applications from eligible institutions for such grants-in-aid on and after said date. The Stem Cell Research Advisory Committee shall require any applicant for a grant-in-aid under this section to conduct stem cell research to submit (1) a complete description of the applicant's organization, (2) the applicant's plans for stem cell research and proposed funding for such research from sources other than the state of Connecticut, and (3) proposed arrangements concerning financial benefits to the state of Connecticut as a result of any patent, royalty payment or similar rights developing from any stem cell research made possible by the awarding of such grant-in-aid. Said committee shall direct the Commissioner of Public Health with respect to the awarding of such grants-in-aid after considering recommendations from the Stem Cell Research Peer Review Committee established pursuant to section 4 of this act.

(c) Commencing with the fiscal year ending June 30, 2006, and for each of the nine consecutive fiscal years thereafter, until the fiscal year ending June 30, 2015, not less than ten million dollars shall be available from the Stem Cell Research Fund for grants-in-aid to eligible institutions for the purpose of conducting embryonic or human adult stem cell research, as directed by the Stem Cell Research Advisory Committee established pursuant to section 3 of this act. Any balance of such amount not used for such grants-in-aid during a fiscal year shall be carried forward for the fiscal year next succeeding for such grants-in-aid.

Sec. 3. (NEW) (*Effective from passage*) (a) There is established a Stem Cell Research Advisory Committee. The committee shall consist of the Commissioner of Public Health and eight members who shall be appointed as follows: Two by the Governor, one of whom shall be nationally recognized as an active investigator in the field of stem cell research and one of whom shall have background and experience in the field of bioethics; one each by the president pro tempore of the Senate and the speaker of the House of Representative, who shall have background and experience in private sector stem cell research and development; one each by the majority leaders of the Senate and House of Representatives, who shall be academic researchers specializing in stem cell research; one by the minority leader of the Senate, who shall have background and experience in either private or public sector stem cell research and development or related research fields, including, but not limited to, embryology, genetics or cellular biology; and one by the minority leader of the House of Representatives, who shall have background and experience in business or financial investments. Members shall serve for a term of four years

commencing on October first, except that members first appointed by the Governor and the majority leaders of the Senate and House of Representatives shall serve for a term of two years. No member may serve for more than two consecutive four-year terms and no member may serve concurrently on the Stem Cell Research Peer Review Committee established pursuant to section 4 of this act. All initial appointments to the committee shall be made by October 1, 2005. Any vacancy shall be filled by the appointing authority.

(b) The Commissioner of Public Health shall serve as the chairperson of the committee and shall schedule the first meeting of the committee, which shall be held no later than December 1, 2005.

(c) All members appointed to the committee shall work to advance embryonic and human adult stem cell research. Any member who fails to attend three consecutive meetings or who fails to attend fifty per cent of all meetings held during any calendar year shall be deemed to have resigned from the committee.

(d) All members shall be deemed public officials and shall adhere to the code of ethics for public officials set forth in chapter 10 of the general statutes. No member shall participate in the affairs of the committee with respect to the review or consideration of any grant-in-aid application filed by such member or by any eligible institution in which such member has a financial interest, or with whom such member engages in any business, employment, transaction or professional activity.

(e) The Stem Cell Research Advisory Committee shall (1) develop, in consultation with the Commissioner of Public Health, a donated funds program to encourage the development of funds other than state appropriations for embryonic and human adult stem cell research in this state, (2) examine and identify specific ways to improve and promote for-profit and not-for-profit embryonic and human adult stem cell and related research in the state, including, but not limited to, identifying both public and private funding sources for such research, maintaining existing embryonic and human adult stem cell related businesses, recruiting new embryonic and human adult stem cell related businesses to the state and recruiting scientists and researchers in such field to the state, (3) establish and administer, in consultation with the Commissioner of Public Health, a stem cell research grant program which shall provide grants-in-aid to eligible institutions for the advancement of embryonic or human adult stem cell research in this state pursuant to section 2 of this act, and (4) monitor the stem cell research conducted by eligible institutions that receive such grants-in-aid.

(f) Connecticut Innovations, Incorporated shall serve as administrative staff of the committee and shall assist the committee in (1) developing the application for the grants-in-aid authorized under subsection (e) of this section, (2) reviewing such applications, (3) preparing and executing any assistance agreements or other agreements in connection with the awarding of such grants-in-aid, and (4) performing such other administrative duties as the committee deems necessary.

(g) Not later than June 30, 2007, and annually thereafter until June 30, 2015, the Stem Cell Research Advisory Committee shall report, in accordance with section 11-4a of the general statutes, to the Governor and the General Assembly on (1) the amount of grants-in-aid awarded to eligible institutions from the Stem Cell Research Fund pursuant to section 2 of this act, (2) the recipients of such grants-in-aid, and (3) the current status of stem cell research in the state.

Sec. 4. (NEW) (*Effective from passage*) (a) There is established a Stem Cell Research Peer Review Committee. The committee shall consist of five members appointed by the Commissioner of Public Health. All members appointed to the committee shall (1) have demonstrated knowledge and understanding of the ethical and medical implications of embryonic and human adult stem cell research or related research fields, including, but not limited to, embryology, genetics or cellular biology, (2) have practical research experience in human adult or embryonic stem cell research or related research fields, including, but not limited to, embryology, genetics or cellular biology, and (3) work to advance embryonic and human adult stem cell research. Members shall serve for a term of four years commencing on October first, except that three members first appointed by the Commissioner of Public Health shall serve for a term of two years. No member may serve for more than two consecutive four-year terms and no member may serve concurrently on the Stem Cell Research Advisory Committee established pursuant to section 3 of this act. All initial appointments to the committee shall be made by October 1, 2005. Any member who fails to attend three consecutive meetings or who fails to attend fifty per cent of all meetings held during any calendar year shall be deemed to have resigned from the committee.

(b) All members shall be deemed public officials and shall adhere to the code of ethics for public officials set forth in chapter 10 of the general statutes. No member shall participate in the affairs of the committee with respect to the review or consideration of any grant-in-aid application filed by such member or by any eligible institution with whom such member has a financial interest in, or engages in any business, employment, transaction or professional activity.

(c) Prior to the awarding of any grants-in-aid for embryonic or human adult stem cell research pursuant to section 2 of this act, the Stem Cell Research Peer Review Committee shall review all applications submitted by eligible institutions for such grants-in-aid and make recommendations to the Commissioner of Public Health and the Stem Cell Research Advisory Committee established pursuant to section 3 of this act with respect to the ethical and scientific merit of each application.

(d) The Peer Review Committee shall establish guidelines for the rating and scoring of such applications by the Stem Cell Research Peer Review Committee.

(e) All members of the committee shall become and remain fully cognizant of the National Academies Guidelines For Human Embryonic Stem Cell Research, as from time to time amended, and the committee may make recommendations to the Stem Cell Research Advisory Committee and the Commissioner of Public Health

concerning the adoption of said guidelines, in whole or in part, in the form of regulations adopted pursuant to chapter 54 of the general statutes.

Sec. 5. Subsection (c) of section 4-28e of the general statutes is repealed and the following is substituted in lieu thereof (*Effective from passage*):

(c) (1) For the fiscal year ending June 30, 2001, disbursements from the Tobacco Settlement Fund shall be made as follows: (A) To the General Fund in the amount identified as "Transfer from Tobacco Settlement Fund" in the General Fund revenue schedule adopted by the General Assembly; (B) to the Department of Mental Health and Addiction Services for a grant to the regional action councils in the amount of five hundred thousand dollars; and (C) to the Tobacco and Health Trust Fund in an amount equal to nineteen million five hundred thousand dollars.

(2) For the fiscal year ending June 30, 2002, and each fiscal year thereafter, disbursements from the Tobacco Settlement Fund shall be made as follows: (A) To the Tobacco and Health Trust Fund in an amount equal to twelve million dollars; (B) to the Biomedical Research Trust Fund in an amount equal to four million dollars; (C) to the General Fund in the amount identified as "Transfer from Tobacco Settlement Fund" in the General Fund revenue schedule adopted by the General Assembly; and (D) any remainder to the Tobacco and Health Trust Fund.

(3) For each of the fiscal years ending June 30, 2008, to June 30, 2015, inclusive, the sum of ten million dollars shall be disbursed from the Tobacco Settlement Fund to the Stem Cell Research Fund established by section 2 of this act, for grants-in-aid to eligible institutions for the purpose of conducting embryonic or human adult stem cell research.

Sec. 6. (*Effective from passage*) The sum of twenty million dollars is appropriated to the Stem Cell Research Fund established by section 2 of this act, from the General Fund, for the fiscal year ending June 30, 2005.

Approved June 15, 2005

APPENDIX B
Committee Membership Lists

Stem Cell Research Advisory Committee

Member	Affiliation
Robert Galvin, M.D., M.P.H., M.B.A. **Chair**	Commissioner CT Department of Public Health 410 Capitol Avenue P.O. Box 340308 Hartford, CT 06134-0308
Treena Livingston Arinzeh, Ph.D.	Associate Professor Department of Biomedical Engineering New Jersey Institute of Technology University Heights 614 Fenster Hall Newark, NJ 07102-1982
Ernesto Canalis, M.D.	St. Francis Hospital and Medical Center Department of Research 114 Woodland Street Hartford, CT 06105-1299
Gerald Fishbone, M.D.	Hospital of St. Raphael New Haven, CT
Myron Genel, M.D.	Professor Emeritus of Pediatrics Child Health Research Center Yale University School of Medicine Department of Pediatrics 333 Cedar Street P.O. Box 208081 New Haven, CT 06520-8081
David Goldhamer, Ph.D	Associate Professor Interim Director, Center for Regenerative Biology Dept. of Molecular and Cell Biology University of Connecticut 1392 Storrs Road Storrs, CT 06269-4243
Anne Hiskes, Ph.D.	Associate Professor of Philosophy and Director, Program on Science and Human Rights The University of Connecticut 215 Glenbrook Rd. Storrs, CT 06269-4098
Ann Kiessling, Ph.D.	Harvard Institutes of Medicine 4 Blackfan Circle, Room 248 Boston, MA 02115

Julius Landwirth, M.D., J.D. **(Resigned 5/20/09)**	Associate Director, Yale Interdiciplinary Center for Bioethics and Donaghue Initiative in Biomedical and Behavioral Research Ethics Yale Interdiciplinary Center for Bioethics 87 Trumbull Street New Haven, CT 06520-8209
Stephen Latham, Ph.D., J.D.	Deputy Director Yale's Interdisciplinary Center for Bioethics P.O. Box 208209 New Haven, CT 06520-8209
Robert Mandelkern	Parkinson Disease Representatiave to CT Stem Cell Coalition
Saraswathi Nair, M.D.	Norwalk Pathology Associates 35 Maple Street Nowalk, CT 06850
Paul Pescatello, Ph.D., J.D.	President & CEO CT United for Research Excellence, Inc. 300 George Street, Suite 561 New Haven, CT 06511
Jeffrey Seemann, Ph.D.	Dean, College of the Environment and Life Sciences University of Rhode Island Kingston, RI 02881
Milton B. Wallack, DDS	295 Washington Avenue Hamden, CT 06518

Stem Cell Research Peer Review Committee

Member	Affiliation
Linzhao Cheng, Ph.D.	Associate Investigator and Co-Director Stem Cell Program, Institute for Cell Engineering Johns Hopkins School of Medicine Broadway Research Building, Room 747 733 North Broadway Baltimore, MD 21205
Steven Goldman, M.D., Ph.D.	Rykenboer Professor and Chairman Department of Neurology University of Rochester Neurologist-in-chief Strong Memorial Hospital 601 Elmwood Avenue, MRB/Box 645 Rochester, NY 14642
Dieter C. Gruenert, Ph.D.	Senior Scientist California Pacific Medical Center Research Institute Adjunct Professor, Department of Laboratory Medicine University of California, San Francisco Adjunct Professor, Department of Medicine University of Vermont 475 Brannan Street, Suite 220 San Francisco, California 94107
D. Leanne Jones, Ph.D.	Assistant Professor Department of Biology Salk Institute for Biological Studies P.O. Box 85800 San Diego, California 92186-5800
Michael Kyba, Ph.D.	Assistant Professor Center for Developmental Biology The University of Texas Southwestern Medical Center at Dallas 6000 Harry Hines Boulevard, NB5.208 Dallas, Texas 75390-9133
Majlinda Lako, Ph.D	Senior Lecturer Institute of Human Genetics University of Newcastle upon Tyne International Centre for Life Central Parkway Newcastle upon Tyne, NE1 3BZ United Kingdom

M. William Lensch, Ph.D.	Instructor in Pediatrics, Harvard Medical School Senior Scientist, George Q. Daley Laboratory Division of Hematology/Oncology Children's Hospital Boston 300 Longwood Avenue Boston, Massachusetts 02115
Linheng Li, Ph.D.	Associate Investigator Stowers Institute for Medical Research 1000 East 50th Street Kansas City, Missouri 64110
Hanna Mikkola, M.D., Ph.D.	Assistant Professor Department of Molecular, Cell and Developmental Biology Institute for Stem Cell Biology and Medicine University of California, Los Angeles 621 Charles E. Young Drive South, 2204 Los Angeles, California 90092
Martin Pera, Ph.D.	Institute for Stem Cell and Regenerative Medicine University of Southern California 1501 San Pablo Street, ZNI-535 Los Angeles, California 90033-2821
Gary S. Stein, Ph.D.	The Gerald L. Haidak, M.D. and Zelda S. Haidak Distinguished Professor and Chair of Cell Biology Professor of Medicine Deputy Director, University of Massachusetts Memorial Cancer Center Department of Cell Biology University of Massachusetts Medical School 55 Lake Avenue, North Worcester, MA 01655
Miodrag Stojkovic, Ph.D.	Deputy Director Principe Felipe Centro de Investigacion C/ E.P. Avda. Autopista del Saler 16-3 (Junto Oceanografico) 46013 Valencia, Spain
Catherine M. Verfaillie, M.D.	Director, Stamcel Instituute Leuven Katholieke Universiteit Leuven Onderwijs & Navorsing 1 Herestraat 49, bus 804 3000 Leuven Belgium

Leslie Weiner, M.D.	Chair, Department of Neurology Keck School of Medicine of USC 2025 Zonal Ave., RMR 506 Los Angeles, California 90033
Ian Wilmut, Ph.D.	Professor Reproductive Science Centre for Reproductive Biology Reproductive and Developmental Sciences The Queen's Medical Research Institute The University of Edinburgh 47 Little France Crescent Edinburgh EH16 4TJ, Scotland

APPENDIX C
Stem Cell Research Application and Guidelines

Connecticut Stem Cell Research Grants Program

Letter of Intent Submission Deadline- October 31, 2008

Proposal Submission Deadline – December 8, 2008

Proposal Instructions

It is the intent of the Connecticut Stem Cell Research Advisory Committee to fund the best basic and translational stem cell research proposals that Connecticut scientists can offer. The Advisory Committee intends to maintain a program of outstanding science that will continue Connecticut's pioneering role as an international center of excellence and leadership in stem cell research.

Purpose

The Connecticut Stem Cell Research Grants Program, authorized in the Connecticut General Statutes (C.G.S.) §§ 19a-32d through 19a-32g, supports the advancement of embryonic and/or human adult stem cell research in Connecticut.

Proposals must describe the applicant's organization, the applicant's plans for stem cell research, proposed funding for such research from sources other than the State of Connecticut, and proposed arrangements concerning financial benefits to the State of Connecticut as a result of any patent, royalty payment or similar rights developing from any stem cell research made possible by the awarding of such grants.

The Connecticut Stem Cell Research Advisory Committee, in consultation with the Commissioner of Public Health, administers and monitors the grant program. Connecticut Innovations, Incorporated serves as administrative staff of the Advisory Committee, reviewing applications, and preparing and executing assistance agreements for the grants.

Ten million dollars is available in the Connecticut Stem Cell Research Grants Fund through June 30, 2009. For each of the fiscal years ending June 30, 2010 to June 30, 2015, inclusive, ten million dollars will also be available.

Definitions

Embryonic Stem Cells: cells created through the joining of a human egg and sperm or through nuclear transfer that are sufficiently undifferentiated such that they cannot be identified as components of any specialized cell type.

Nuclear Transfer: the replacement of the nucleus of a human egg with a nucleus from another human cell.

Eligible Applicant: a nonprofit, tax-exempt academic institution of higher education, a hospital that conducts biomedical research, or any entity that conducts biomedical research or embryonic or human adult stem cell research.

ESCRO Committee: an Embryonic Stem Cell Research Oversight (ESCRO) committee means a committee established in accordance with the National Academies' Guidelines for Human Embryonic Stem Cell Research, as amended from time to time.

Overview

It is the intent of the Connecticut Stem Cell Research Advisory Committee to consider funding any form of stem cell research, but priority will be given to human embryonic stem cell research that is not currently eligible for federal funding. Other types of stem cell research will also be eligible, with priority given to studies with clear potential relevance to human health. Animal models are not excluded from consideration, but applicants will need to demonstrate a direct relevance to human stem cell biology and its therapeutic implications.

Who May Submit

Connecticut researchers engaged in the advancement of embryonic or human adult stem cell research are encouraged to submit proposals. Research must be conducted at an eligible academic institution, hospital or company. Except under extraordinary circumstances, all research must be conducted in Connecticut. Researchers at such entities may apply for any category of grants. The researcher's institution, hospital, or company must undertake responsibility for financial administration of the grant and for overall compliance with rules governing research at that entity. Except as specified, applicants at academic research institutions must be faculty members. Non-tenure track faculty members may apply if their institutional policies permit them to hold independent grants. Postdoctoral fellows may apply for seed grants with the support of a faculty sponsor. Applicants from hospitals or companies must be permitted by their organization to hold research grants.

When to Submit

Submit a one page letter of intent, in PDF format due to Connecticut Innovations by **4:30 p.m. on** by **October 31, 2008.**

Completed, signed electronic copies of proposals, in PDF format, **December 8, 2008.**

No additional proposals or supplemental materials will be accepted after the deadline.

Where to Submit

1. Letters of intent in **PDF** format should be sent electronically to stemcellinfo@ctinnovations.com

2. A signed electronic copy of the proposal in **PDF** format should be sent to stemcellinfo@ctinnovations.com

Refer questions to Chelsey Sarnecky: 860-257-2355 or stemcellinfo@ctinnovations.com

Special Considerations for Human Embryonic Stem Cell (hESC) Research

A priority for the Connecticut Stem Cell Research Grants Program is to support research on hESC that is not currently eligible for federal funding. The State is committed to the highest standard of ethical oversight and transparency, and expects all grant recipients to be in full compliance with all applicable laws, regulations and guidelines, including a review and approval by the Institutional Review Board (IRB) and Embryonic Stem Cell Research Oversight (ESCRO) Committee, when applicable, regarding this type of research.

The grantee's institution, hospital or company must establish an ESCRO committee, or establish an affiliation with an existing ESCRO committee, established in accordance with the National Academies' Guidelines for Human Embryonic Stem Cell Research, as amended from time to time, http://www.nap.edu/books/0309096537/html to oversee all hESC research at the institution, hospital or company. Each grantee's institution, hospital or company must submit a list of members of the ESCRO committee along with a copy of the policies and procedures of the ESCRO committee and the ESCRO committee approval for the research project prior to the release of funds. The Advisory Committee reserves the right to delay or rescind funding if it is not satisfied that the ESCRO committee is appropriately established and constituted. If an applicant institution, hospital or company does not have an established ESCRO committee, the application must summarize the entity's plans and timetable for establishing or affiliating with an ESCRO committee.

If research on non-federal hESC lines is to be conducted in a research environment that also receives federal funding support, the institution, hospital or company must have established a detailed policy for the segregation of funding in compliance with federal funding restrictions. The policy must be in place before the release of funds.

Types of awards

Applications will be considered for (1) Seed Grants, (2) Established Investigators, (3) Group Projects, and (4) Core Facilities. For this funding cycle, total annual funding for Seed Grant Awards will be at least 10% of the total annual budget for the Connecticut Stem Cell Research Grants Program.

1. Seed Grant Awards: These awards are intended to support the early stages of projects that are not yet ready for larger scale funding whether from federal or nonfederal sources. Junior researchers in hospitals and companies are particularly encouraged to apply. In academic institutions, priority will be given to junior faculty members at the start of their independent careers. Established investigators new to stem cell research may apply for seed grants. Postdoctoral fellows, or equivalent, may apply with the support of a faculty sponsor or equivalent. A letter from the sponsor indicating support of the proposal must be included with the application and must describe the applicant's level of independence, as well as other resources/funding available for the project.

Requested funding for a Seed Grant Award may be up to $200,000 (including indirect costs) and may be expended over 2 years. The yearly budget must not exceed $100,000. Project Descriptions for Seed Grant applications are limited to 5 pages (inclusive of the main text, methodology, figures and legends). Other proposal requirements are described under "Guidelines for Preparation of Proposals."

2. Established Investigator Awards: These awards are intended for investigators with a track record of independent research including prior grant support and regular peer reviewed publications.

Requested funding for an Established Investigator Award may be up to $500,000 (including indirect costs) and may be expended over 4 years. Funding is encouraged to be evenly budgeted over the duration of the award. Project Descriptions for Established Investigator applications are limited to 10 pages (inclusive of the main text, methodology, figures, and legends). Other proposal requirements are described under "Guidelines for Preparation of Proposals."

3. Group Project Awards: These awards are intended to support coordinated approaches to ambitious strategic goals that are beyond the scope of a typical single laboratory. Priority will be given to projects involving collaboration across disciplines and/or institutions, and proposals should include explanations of the need for collaboration, along with plans for managing the collaborative process, including division of responsibilities among collaborators and timelines for achieving expected project

milestones. If more than one institution, hospital or company is involved, the proposed budget must specify how funding is to be distributed between collaborating entities. As with other grants, eligibility for funding is restricted to researchers at Connecticut institutions, hospitals or companies. Group Projects may have multiple co-principal investigators, but one individual must be identified as the lead investigator and primary contact with the Connecticut Stem Cell Research Program.

Requested funding for a Group Project Award may be up to $2 million (including indirect costs) and may be budgeted for up to 4 years. Descriptions for Group Project applications are limited to 50 pages (inclusive of the main text, methodology, figures, and legends). Other proposal requirements are described under "Guidelines for Preparation of Proposals."

4. Core Facilities Awards: These awards are intended to provide shared core facilities for stem cell researchers at eligible Connecticut institutions, hospitals or companies.

Applications will be considered for additional support of already established cores and for new cores that are beyond the means of most individual labs, that will be made widely accessible to the Connecticut stem cell research community, and that are likely to advance stem cell research throughout the State. Proposals must include an explanation of the need for a new core or expansion of an existing core, along with estimates of likely capacity and usage. Previously funded cores should provide specific details in their budget justification about the necessity of additional funding; including explanation of how new and existing funding will be integrated without overlap.

Applicants should demonstrate a proven expertise in the relevant technology and ability to provide a high quality service. Funds may be used to cover equipment, salaries or other costs associated with establishing and operating cores. Cores will also be allowed to establish a reasonable fee-for-service schedule in order to recover additional costs associated with their operation. Proposed fees must be specified and approved by the institution, hospital or company.

Requested funding for a Core Facilities Award may be up to $2.5 million (including indirect costs) and may be budgeted for up to 4 years. Project Descriptions for Core Facilities applications are limited to 50 pages (inclusive of the main text, methodology, figures, and legends). Other proposal requirements are described under "Guidelines for Preparation of Proposals."

Note: Group Project Awards may include shared equipment as part of their budget. Core Facility Awards are distinct, however, in that they are intended specifically to provide services to the wider Connecticut research community, rather than being restricted to participants in a specific collaborative project or to members of the host institution, hospital or company.

Note: Group Project Awards and Core Facilities Awards may under special cases include startup funds for investigators yet to be hired. Such proposals require detailed justification, including the identification of the person to be hired *and* a detailed description of his/her contribution to the specific project. Release of funds will be contingent on the investigator accepting and taking up the position. Justification must include the need for additional recruitment and an explanation of how the funding will be used to support the overall goals of the project. Funds may not be used for general research infrastructure not directly related to the goals of the Connecticut Stem Cell Research Grants Program.

Selection Criteria

The criteria to be employed in the evaluation shall include, but not be limited to, the following:

A. Scientific merit of the proposed research
B. Conformance to high ethical standards
C. Ability to perform the proposed research
D. Commitment of host institution, hospital or company and (where applicable) collaborators to the proposed project, including cost sharing
E. Potential for collaboration across disciplines and institutions, hospitals or companies
F. Benefits (including financial benefits) to the State of Connecticut
G. Alignment with funding priorities as determined by the Connecticut Stem Cell Research Advisory Committee

I. Proposal Review

The Connecticut Stem Cell Research Peer Review Committee will review all proposals and make recommendations to the Connecticut Stem Cell Research Advisory Committee with respect to the ethical and scientific merit of each proposal. The Peer Review Committee will utilize the National Academies' Guidelines for Human Embryonic Stem Cell Research, as amended from time to time, http://www.nap.edu/books/0309096537/html and C.G.S. §§ 19a-32d through 19a-32g.

The Advisory Committee, in consultation with the Commissioner of Public Health, will make the funding decisions. The Advisory Committee reserves the right and discretion to fund one or more components or defined parts of an application's proposed research project. In the event of such a determination, the applicant will be required to submit a revised budget reflecting the Advisory Committee's funding decision and such other information as the Advisory Committee may require.

At time of application, an applicant may send to Connecticut Innovations the name(s) of any reviewers with whom there is a conflict of interest and who should not be considered as reviewers.

Decisions regarding funding are anticipated on or after April 2009.

II. Funding

Notification of funding approval will be made by Connecticut Innovations.

The institution, hospital or company will then sign an assistance agreement indicating that the institution, hospital or company is in compliance with the requirements of applicable Connecticut General Statutes, Executive Orders and other administrative requirements. The institution, hospital or company must establish an ESCRO committee or become affiliated with an ESCRO committee that will review and approve proposals involving the use or creation of human embryonic stem cells and must submit a list of members of the ESCRO committee along with a copy of the policies and procedures of the committee and the ESCRO approval for the research project prior to the release of funds. The Advisory Committee reserves the right to delay or rescind funding if it is not satisfied that the ESCRO committee is appropriately established and constituted.

The funding period begins on the effective date specified in the assistance agreement. Expenditures incurred before the effective date of the assistance agreement may not be charged against the project. Funding not used in a competed grant year may be used in a subsequent grant year to discharge expenses incurred but not yet paid in the competed grant year. Any other carry over funding shall be expended

only in accordance with the terms specified in the assistance agreement.

Transmittal of Funds

Funds will be transmitted to the institution, hospital or company over the duration of the grant according to each year's budget request. Multi-year projects will receive the first installment immediately following the signing of the assistance agreement for the project, and subsequent installments will be transmitted after technical and fiscal progress reports are received and approved.

Audit of Funds

Expenditures by institutions, hospitals or companies may be subject to audit. Entities submitting proposals for funding must agree to cooperate by providing information for audit and a full review of the project.

III. Guidelines for Preparation of Letter of Intent and Proposals

Letter of Intent

Applicants are asked to submit a letter of intent that includes the following information:

- Title of proposed project
- Type of award
- Estimate of requested funding amount
- Contact information for Principal Investigator
- Brief description of proposed project

Although a letter of intent is not required, is not binding, and does not enter into the review of a subsequent application, the information that it contains allows staff to estimate the potential review workload and plan the review.

Proposal

Signed electronic proposals must have pages numbered at the bottom, with one-inch margins, and with 12 point font. They may be single-spaced and shall be printed only on one side. Any reprints, appendices, or other materials to be considered with the proposal must be attached to the original proposal as well as electronic copies. The electronic copy of the proposal and all attachments should be sent in one (1) PDF file.

The total length of the proposal is dependent upon the type of award being sought and is outlined above under the heading "Types of Awards." Proposals that do not follow the prescribed format or are incomplete when they are submitted or otherwise do not conform to the requirements of these Proposal Instructions may be rejected as ineligible for consideration.

Proposals shall include the following:

1. Cover Page (Attachment I)

Use the format provided in Attachment I. A proposal is incomplete if any of this information or required signature is omitted. The Cover Page must be signed by the Vice President for Research or other authorized officer to confirm institutional approval for the application including financial as well as other types of regulatory compliance (see #8 Special Considerations).

A separate page (Attachment I), should be completed by an investigator at each participating institution, hospital or company. For projects with multiple investigators, the lead investigator should be indicated.

Please make sure to note whether or not your proposal contains privileged or proprietary information and mark these portions in bold text.

2. Project Summary (Attachment II)

Use the format provided in Attachment II. The summary shall include a statement of objectives and the scientific methods to be employed written in lay language. Limit summaries to the space provided on Attachment II. Note: Because the Project Summary will be available to the public, do not include proprietary information in the Summary.

3. Table of Contents

4. Project Description

Page limits for each type of Award are defined above under the heading "Types of Awards." The description of the project shall include the following subsections:

a. Project Objectives and Significance of Proposed Work

Describe the goals and objectives of the project. Discuss the rationale for choosing these objectives. Explain how these objectives compare to the state of the art and what distinguishes this proposed work from other efforts.

b. Project Plan

Describe the technical plan over the life of the project, how the proposed work will be organized into tasks and how the tasks are interrelated. Define clear, quantitative milestones and provide an expected schedule for reaching these milestones, including regulatory approvals where applicable. For projects involving several co-investigators and/or institutions, hospitals or companies, describe the expected contributions of each participant. Summarize the technical tasks that must be accomplished, with special emphasis on new or innovative technologies required for success of the project. Describe the technical challenges and the approach to overcoming any barriers. Assess the probability of success of this project.

c. Intellectual Property

Describe the plans and timeline to protect the intellectual property. Describe the plans and timeline for licensing the technology. As required by C.G.S. §§ 19a-32d through 19a-32g, applicants must submit *"proposed arrangements concerning financial benefits to the state of Connecticut as a result of any patent, royalty payment or similar rights developing from any stem cell research made possible by the awarding of such grants-in-aid."*

In evaluating proposed arrangements, it is expected that the State of Connecticut shall be entitled to royalties from the awardees and certain of its affiliates, at a minimum rate of 5 percent, on revenues generated from the exploitation of any invention or intellectual property that is conceived, created or developed during the stem cell research and development activities, and during the term of the funding or at any time during the 12-month period immediately following the term of funding, and which was made possible (in whole or in part) by, or otherwise resulted (in whole or in part) from the funding.

d. Bibliography

List the existing research and technology base that supports the proposed work. Please note that the bibliography shall not be included within the page limitations.

5. Evidence of Commitment

a. Commitment of Institution, Hospital or Company and other Collaborators

Describe the commitment of the institution, hospital or company and that of other collaborators to this project.

b. Commitment of the Key People

- Describe their qualifications
- Describe the focus of each person's efforts
- Estimate the percentage of time each person will devote to this project
- Describe the project management plan

c. Commitment to Sharing Resources

The Connecticut Stem Cell Research Grants Program expects grant recipients and their institutions, hospitals or companies to share reagents, data and protocols developed in connection with these grants. In particular such resources shall be made freely available to other Connecticut-based researchers. Describe plans for sharing such anticipated resources. If this is expected to involve significant costs to the recipient institution, hospital or company, the budget may include a component to cover these costs.

d. Financial Commitment from other Sources

Describe financial commitments to the project from other sources. As required by C.G.S. § 19a-32e, applicants must submit "proposed funding for such research from sources other than the state of Connecticut."

e. Available Facilities and Major Items of Equipment

Describe the facilities and major equipment available for this project.

6. Biographical Sketches

Submit a brief biographical sketch, including patents, selected publications, and recently funded projects for each principal investigator (four page maximum per person). For Seed Grant Awards, provide a biographical sketch for the applicant and, if appropriate, for the faculty sponsor.

7. Budget

a. Budget Detail (Attachment III)

Each proposal must contain a budget for each year of support requested and a cumulative budget for the full term of requested support. Identify each year's request ("First year," "Second year," or "Cumulative Budget") at the top right of each page. Use the prescribed budget format provided in Attachment III. Companies should prepare the budget on a quarterly basis.

Salaries and Wages: List the names of the principal investigator(s) and other senior associates and the estimated amount of time dedicated to this project (number of academic-year, summer, or calendar-year person-months if proposal is from academic institution) for which funding is requested. Salaries requested must be consistent with the regular practices of the institution, hospital or company.

Hospitals and companies may not use Connecticut Stem Cell Research Grant Funds to augment the existing salaries of investigators. For proposals from academic institutions, Connecticut Stem Cell Research Grant Funds may not be used to augment the total salary or rate of salary of faculty members during the period covered by the term of faculty appointment. Nor may funds be used to reimburse faculty members for consulting or other time in addition to a regular full-time institutional salary covering the same general period of employment. For postdocs, graduate students and

technical staff, etc., list only the total number of persons and total amount of salaries per year in each category.

Fringe Benefits: If the usual accounting practices of the institution, hospital or company provide that its contributions to employee benefits (social security, retirement, etc.) be treated as direct costs, funds may be requested to defray such expenses as a direct cost.

Equipment: The Connecticut Stem Cell Research Grants Program wishes to avoid expensive duplication of research infrastructure wherever possible. Therefore, any budget requests for major equipment must be carefully justified.

Identify items exceeding $1,000 or more and a useful life of more than one year as Permanent Equipment. Special purpose research equipment having a unit acquisition cost of $10,000 or more purchased or leased with project funding is subject to reasonable research equipment inventory controls, maintenance procedures, and organizational policies that enhance its multiple or shared use on other projects, if the other use does not interfere with the work on the project for which the equipment is acquired.

Travel: Funds may be requested for fieldwork necessary to carrying out the project and up to $5,000 per year to travel to conferences to present findings or to further the research. (Documentation of expenses will be required in subsequent fiscal reports).

Other Direct Costs: The budget should itemize other anticipated direct costs, including materials and supplies, publication costs, and computer services. Other examples include payments to service charges, and construction of equipment or systems not available off-the-shelf.

Publication Costs/Page Charges: The budget may request funds for the costs of publishing the results of the project, including costs of reports, reprints, page charges, other journal costs and necessary illustrations.

Cost of sharing reagents: If the project is expected to generate reagents or data that will be of general value to the research community, the budget may include a component to cover the reasonable costs of generating and distributing such resources.

Indirect Costs: Budgets may include indirect costs, which may not exceed 25 percent of the Modified Total Direct Costs (MTDC). MTDC are described in Attachment A of OMB Circular A122 and consist of all salaries and wages, fringe benefits, materials and supplies, services, travel, and sub-grants and subcontracts up to the first $25,000 of each sub-grant or subcontract (regardless of the period covered by the sub-grant or subcontract). Equipment, capital expenditures, charges for patient care, rental costs and the portion in excess of $25,000 shall be excluded from MTDC. Participant support costs shall generally be excluded from MTDC.

b. Budget Explanation/Justification

In a separate section titled “Budget Explanation/ Justification,” clearly delineate the specific use and justification of funds. Breakdowns should be as accurate and specific as possible. For equipment funding requests, describe and justify each piece of requested equipment. Identify location of use. If comparable equipment is available at the institution, hospital or company, explain why it cannot be used.

Include in this section a detailed description of the contributions from the institution, hospital or company and collaborators.

8. Special Considerations

Several situations require written assurance that appropriate institutional, hospital or company clearance procedures are in place:

1. Projects that involve the use of recombinant DNA and/or hazardous reagents.
2. Projects that involve use of human eggs, embryos and/or human embryonic stem cells.
3. Projects that involve the use of human subjects.
4. Projects that involve the use of animal subjects.

All proposals must be in compliance with federal, state and local laws and all applicable permitting requirements. Prior to conducting research involving human embryonic stem cells, documentation verifying that any human embryos, embryonic stem cells, unfertilized human eggs or human sperm used in such research have been donated voluntarily as required by C.G.S. §§ 19a-32d through 19a-32g must be provided to the Commissioner of Public Health on a form available from the Connecticut Department of Public Health http://www.ct.gov/dph/cwp/view.asp?a=3142&q=389700&dphNav_GID=1825&dphNav=|

9. Appendix

Letters of commitment from the institution, hospital or company and collaborators should be included. For applicants at the postdoctoral fellow stage, a letter of support from the faculty sponsor should also be included.

IV. Project Administration

Responsibility for general supervision of all project activities rests with the institution, hospital or company.

Adherence to Original Budget Estimates

A reallocation of 10 percent or more in the aggregate of the total approved annual budget requires the prior written approval of Connecticut Innovations. Reallocation of more than 20% in the aggregate of any approved annual budget requires the prior written approval of the Advisory Committee. The written request to re-budget, signed by the principal investigator and the authorized institution, hospital or company representative, must fully explain the need for re-budgeting and must describe the impact if any on the conduct of the research.

Changes in Personnel

Timely notification to Connecticut Innovations (who will notify the Advisory Committee) is required for any change in any principal investigators before or after signing the assistance agreement. The notification must describe the impact, if any on the conduct of the research. A change in principal investigator that occurs after the peer review process is completed and prior to the signing of the contract may result in the denial or rescission of funding by the Advisory Committee. All changes involving senior personnel must be approved by the Advisory Committee. If the principal investigator terminates employment with the institution, hospital or company, the entity may terminate the project, or when appropriate, propose to the Advisory Committee a substitute principal investigator to continue the project.

Funding cannot be transferred from the institution, hospital or company except when the grantee moves to another eligible entity within Connecticut and the transfer receives the prior approval of the Advisory Committee.

Equipment

Title to equipment purchased or fabricated with funds or matching funds vests in the institution, hospital or company.

Project Reports

Principal investigators are required to submit **Annual Technical Progress Reports**. Reports shall:

- summarize activity during the 12-month period then ended;
- describe progress with reference to scheduled milestones;
- identify any significant scientific developments and all invention and intellectual property disclosures;
- describe collaborative work;
- describe any problems encountered; and
- include a detailed summary in lay language suitable for the public and press on a form provided by Connecticut Innovations

Institutions, hospitals and companies are required to submit **Semi-Annual Fiscal Reports** for each project.

Failure to submit required reports or the submission of incomplete or inadequate reports could result in deferral of subsequent installment payments or termination of support and forfeiture of funds.

The Advisory Committee and/or their designees reserve the right to conduct site visits for funded projects.

Principal investigators are required to submit a **Final Report** within 90 days after the expiration of a assistance agreement**.** This report must include information needed for purposes of program management, evaluation, fiscal accountability, and informing the public about the results of research supported under the Connecticut Stem Cell Research Grants Program.

Acknowledgment of Support and Disclaimer

Any publication, oral presentation, or meeting abstract based on research activity supported by the funding must contain the following acknowledgment: "This material is based upon work supported by the State of Connecticut under the Connecticut Stem Cell Research Grants Program. Its contents are solely the responsibility of the authors and do not necessarily represent the official views of the State of Connecticut, the Department of Public Health of the State of Connecticut or Connecticut Innovations, Inc."

Documents as Public Records

All documents submitted to the Connecticut Stem Cell Research Grants program will become a matter of public record and will be available to the public, except as described below. Information or material that Connecticut Innovations and the institution, hospital or company mutually agree to be of a privileged nature will be held in confidence to the extent permitted by law. Without assuming any liability for inadvertent disclosure, Connecticut Innovations will seek to limit dissemination of such information only to its employees, selected employees at the Connecticut Department of Public Health, the Connecticut Stem Cell Peer Review Committee, and to the Connecticut Stem Cell Research Advisory Committee. Accordingly, a proposal which indicates the inclusion of "Proprietary and Privileged Information" on the cover page, will be released to the Connecticut Stem Cell Peer Review Committee, and to the Connecticut Stem Cell Research Advisory Committee only after those reviewers have signed a non-disclosure document reflecting applicable state law. Applicants are required to identify the words or paragraphs on specific pages of the application that contain trade secrets or other proprietary information. Notwithstanding the foregoing, all applicable laws governing access to public records will be observed.

Inventions, Software, and Copyrights

As required by C.G.S. §§ 19a-32d through 19a-32g, applicants must submit *"proposed arrangements concerning financial benefits to the state of Connecticut as a result of any patent, royalty payment or similar rights developing from any stem cell research made possible by the awarding of such grants-in-aid."* The State of Connecticut encourages the publication and distribution of the results of the project performed under its funding. The Commissioner of Public Health retains the right to use published materials resulting from the performance of work under Connecticut Stem Cell Research Grants Program funding for state purposes.

Award Documentation

Applications selected to receive funding will be required to execute an Assistance Agreement and Royalty Agreement in forms approved by the Advisory Committee.

Attachment I CT Stem Cell Research Proposal

Cover Page

Attachment I should be completed by the principal investigator of each participating institution. For projects with multiple investigators, the lead investigator should be indicated.

Indicate type of project:

_____ Seed Grant

_____ Established Investigator Grant

_____ Group Project Grant

_____ Core Facility Grant

Title of Project

Institution/Hospital/Company

PI Name(and sponsor name where applicable) **Signature (s)**

I certify that the statements herein are true, complete and accurate to the best of my knowledge. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. I agree to accept responsibilities for the scientific conduct of the project and to provide the required progress reports if a grant is awarded as a result of this application.

PI Department/Business Address

PI Phone **PI Email**

Amount Requested $

Authorized Representative **Title**

I certify that the statements herein are true, complete and accurate to the best of my knowledge, and accept the obligation to comply with all terms and conditions of the Connecticut Stem Cell Research Grants Program ***and all applicable laws and ethical standards*** if a grant is awarded as a result of this application. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties.

Signature **Date**

Items included in Project (check where appropriate)

☐ Proprietary and Privileged Information **(identify such words or paragraphs on specific pages in bold type)**

- ☐ Recombinant DNA and/or Hazardous Reagents
- ☐ Human Eggs, Embryos and/or Human Embryonic Stem Cells
- ☐ Animal Subjects
- ☐ Human Subjects

Attachment II CT Stem Cell Research Proposal

Project Summary (in Non-Scientific Language)

Attachment II should be completed by the principal investigator of each participating institution, hospital or company. For projects with multiple investigators, the lead investigator should be indicated.

Title of Project

Amount requested $

Principal Investigator

Institution/Hospital/Company

Collaborator (s)

One sentence description. This Project's purpose is to

Project Summary (Limit to this side of form)

Attachment III CT Stem Cell Research Proposal Budget

To be completed by each Institution/Hospital/Company

Budget for [Year] [Quarter] ___
Cumulative Budget ___

A. Senior Personnel PI, CO-PI's, Faculty and Other Senior Associates (List each separately with Title and Organization on Budget Explanation page. Show number in brackets.)	**Grant Funded Person-Mos.**	**Funding Requests**
1.		
2.		
3.		
4. ( ) Others (List individually on Budget Explanation Page)		
5. ( ) Total Senior Personnel (1-4)		
B. Other Personnel (Show numbers in brackets)		
1. ( ) Post-Doctoral Associates		
2. ( ) Other Professionals (Technician, Programmer, Etc.)		
3. ( ) Graduate Students		
4. ( ) Other -Specify		
Total Salaries And Wages (A&B)		
C. Fringe Benefits (If charged as Direct Costs)		
Total Salaries, Wages, and Fringe Benefits (A+B+C)		
D. Permanent Equipment (Describe on Budget Explanation Page)		
E. Other Direct Costs (Describe details on Budget Explanation Page)		
1. Materials And Supplies		
2. Publication Costs/Page Charges		
3. Computer Services		
4. Other		
Total Other Direct Costs		
F. Indirect Costs (Describe on Budget Explanation Page)		
G. Total Costs (A Through F)		
H. Projected Revenues		
I. Total Contributions from Other Sources		

APPENDIX D
Listing of Published Articles, Manuscripts and Posters

2006 Stem Cell Grants-in-Aid
Publication List

Advincula, M.C.; Liu, Y.; Gronowicz, G.; Habibovic, P.; Goldberg, A.J. and Kuhn, L.T. 2009. A rapid, reproducible method for coating tissue culture polystyrene disk inserts with carbonated hydroxyapatite (Manuscript to be submitted).

Ammon, N., Hartman, N., and Grabel, L. Directing the differentiation of embryonic stem cells into distinct neuronal subtypes, In "Stem cells: From tools for studying mechanism of neuronal differentiation towards therapy" H. Ullriich editor, Springer, In press (3/09).

Baikang, P. and Rowe, D., and Shin, D-G., "Improving Gene Regulation Network Reconstruction by Integrating Indirect Prior Knowledge", *Proc. of IEEE BIBM*, 2008, Philadelphia, PA.

Cai, C., and Grabel, L. (2007) Directing the differentiation of embryonic stem cells to neural stem cells. *Developmental Dynamics*, 236(12):3255-66.

Cai, C., Thorne, J., and Grabel, L. (2008) Hedgehog serves as a mitogen and survival factor during embryonic stem cell neurogenesis. *Stem Cells* 26(5):1097-1108.

Carpentino, J., Hartman, N., Grabel, L., and Naegele, J. (2008) Region-specific differentiation of ES-derived neural progenitor transplants into the adult mouse hippocampus following seizures. *Journal of Neuroscience Research* 86: 512- 524.

Cheng, E.C., Luo, Q., Bruscia, E.M., Renda, M.J., Troy, J.A., Massaro, S.A., Tuck, D., Schultz, V., Mane, S.M., Berliner, N., Sun, Y., Morris, S.W., Qiu, C., Krause, D.S. Role for MKL1 in megakaryocytic maturation. *Blood* 2009 Mar 19;113 (12):2826-34.

Chen, L-L. and G.G. Carmichael: Altered nuclear retention of mRNAs containing inverted repeats in human embryonic stem cells: functional role of a nuclear noncoding RNA. (2009) submitted to *Molecular Cell*.

Chen, L-L. and G.G. Carmichael: Gene regulation by SINES and inosines. (2008) *Cell Cycle* 7: 3294-3301.

Chen, L-L., J. DeCerbo and G.G. Carmichael: *Alu* element-mediated gene silencing. (2008) *EMBO Journal*. 27:1694-1705.

Cheng, Ee-chun, Qing Luo, Emanuela M. Bruscia, Matthew J. Renda, James A. Troy, Stephanie A. Massaro, David T. Tuck, Vincent Schulz, Shrikant M. Mane, Nancy Berliner, Yi Sun, Stephan W. Morris, Caihong Qui and Diane S. Krause. Role for MKL1 in megakaryocytic maturation. *Blood.* Prepublished online January 9, 2009, DOI10.1182/blood-2008-09-180596.

Fang, P., Petersen, P.H., Zhou, Y., Zou, K., and Zong, W. (2007) Numb and Numbl are essential for specifying neural progenitor fates but dispensable for maintaining cell adhesion and polarity. (Manuscript in preparation).

Fu, Y., Rodgers B, Fu Y, Wang Y-H, Balic A, Aguila L, Roberts C and Mina M. *IADR 09*.

Gangaraju, V., and Lin, H. (2009) Formation of Neural Crest Cells from Mouse Embryonic Stem Cells. miRNAs: Key Regulators of Stem Cells. *Nature Rev. Mol. Cell Biol.* 10, 116-125.

Gong, G., Ferrari, D., Dealy, C.N., and Kosher, R.A. (2009). Directing differentiation of human embryonic and induced pluripotent stem cells into the chondrogenic lineage. Abstracts of Presentations, University of Connecticut Health Center, New England Musculoskeletal Institute, 3rd Annual Research Day, March 20, 2009.

Grabel, L and Gruen, L. (2009) Epidosembryos to the rescue. A review of Louis Guenin's book *"The Morality of Embryo Use" Cell Stem Cell* 4:113-114.

Gruen, L and Grabel, L. (2007) Ethics and Stem Cell Research, Introductory Paper. Metaphilosophy 38:137-152. Also published by Blackwell as a book, *Stem Cell Research, the Ethical Issues*.

Gruen, L., and Grabel, L. (2006) Scientific and ethical roadblocks to human embryonic stem cell therapy. *Stem Cells* 24: 2162-2176.

Gruen, L., Grabel, L., and Singer, P. (2007) Guest editors of special issue of Metaphilosophy 38 "Stem Cell Research: the Ethical Issues". Blackwell . Also published by Blackwell as a book, *Stem Cell Research, the Ethical Issues*.

Guo, Q.X. and Li, J.Y.H. (2207). Distinct functions of the major Fgf8 spliceform, Fgf8b, before and during mouse gastrulation. *Development* 134:2251-60.

Kazmi, S., Bikang, P., Wong, A., Nori, R., Wang, H-W, Kim, Y-A, Rowe, D., and Shin, D-G, "Meta Analysis of Microarray Data Using Gene Regulation Pathways", *Proc. of IEEE BIBM*, 2008, Philadelphia, PA.

Komitova, M., Zhu, X., Serwanski, D.R., and Nishiyama, A. NG2 cells are distinct from neurogenic cells in the subventricular zone. *J Comp Neurol* 512:702-715, 2009.

Kuhn, L.T.; Advincula, M.C.; Liu, Y.; Gronowicz, G.; Habibovic, P. and Goldberg, A.J. 2009 Biomimetic method for coating tissue culture polystyrene with carbonated hydroxyapaptite. *J. Dent. Res*. 88.

Kuhn, L.T., Goldberg, A.J.; Liu, Y. and Rowe, D. 2009 Fluorescent quantification of osteogenesis using GFP-transgenic mouse reporter cell technology. Regenerative Medicine-Advancing Next Generation Therapies, March 5-8, 2009.

Kuo, L., Zhao, Y., Xie, W., Yu, F. (2008) A preliminary study on combining classifiers for selecting differentially expressed genes. 2008 *JSM Proceedings, Business and Economics Statistics Section [CD-ROM],* Alexandria, VA: American Statistical Association: pp 2984-2990.

LaMonica, K., Bass, M., and Grabel, L. (2009) The Planar Cell Polarity Pathway Directs Parietal Endoderm Migration. *Developmental Biology*, in Press.

Lin, H. and Yin, H. (2009) A novel epigenetic mechanism in *Drosophila* somatic cells mediated by PIWI and piRNAs. *Cold Spring Harbor Symposia on Quantitative Biology: Control and Regulation of Stem Cells*, Volume 73 (in press).

Liu, Y.; Advincula, M.; Goldberg, A.J. and Kuhn, L.T. 2009 Differential effects of fibrillar and non-fibrillar collagens on osteogenesis of GFP-reporter preosteoblasts. New England Musculoskeletal Institute Research Day, March 21, 2009.

Liu, Y.; Advincula, M.; Wang, Y.-H.; Goldberg, A.J. and Kuhn, L. 2009 Fluorescent quantification of osteogenesis of GFP-transgenic mouse calvarial osteoblasts on biomimetic coatings. *Society for Biomaterials*, April 22-25, 2009.

LoTurco, J.J., Kriegstein, A.R. Manipulating midbrain stem cell self-renewal. *Cell Stem Cell.* 2008 May 8,2(5):405-6. (Review Paper)

Manent, J.B., Wang, Y., Chang, Y., Paramasivam, M., LoTurco, J.J. Dcx reexpression reduces subcortical band heterotopia and seizure threshold in an animal model of neuronal migration disorder. *Nat Med.* 2009 Jan:15(1):84-90. Epub 2008 Dec 21.

Maisano, Xu, Joseph Carpentino, Sandy Becker, Robert Lanza, Gloster Aaron, Laura Grabel, and Janice R. Naegele. Embryonic Stem Cell Derived Neural Precursor Grafts for Treatment of Temporal Lobe Epilepsy. *J. Neurotherapeutics*, In press (3/09).

Nagalakshmi, U., Wang, Z., Waern, K., Shou, C., Raha, D., Gerstein, M., Snyder, M. The transcriptional landscape of the yeast genome defined by RNA sequencing. *Science*. 2008 June 6;320(5881):1344-9. Epub 2008 May 1. PMID:18451266.

Nishiyama, A., Komitova, M., Suzuki, R., Zhu, X. NG2 cells (polydendrocytes): multifunctional cells with lineage plasticity. *Nat Rev Neurosci* 10:9-22, 2009.

Paic, F., Igwe, J.C., Ravi, N., Kronenberg, MS, Harrington, P., Kuo, L., Sin, D-G., Harris, S.E., Rowe, D.W., Kalajzic, I. (2009) Identification of differentially expressed genes between osteoblasts and osteocytes, Preprint (to be published).

Peng, F., L. Wang, X. Yu, J. Huang, X. Jiang, D. Rowe, M. Wei. Biomimetic Hydroxyapatite/Collagen Scaffold for Bone Repair, *Society for Biomaterials 2009 Annual Meeting and Exposition*, April 22-25, 2009, San Antonio, Texas.

Peng, F., L. Wang, X. Yu, X. Jiang, J. Huang, Z. Xia, D. Gynther, D. Rowe, M. Wei. Hydroxyapatite/Collagen Biomimetic Co-precipitated Scaffold for Bone Repair, *StemCONN09*, March 23-24, 2009, New Haven, Connecticut.

Rodgers, B., Fu, Y., Wang, Y-H., Baltic, A., Aguila, L., Roberts, C and Mina, M. Derivation of Neural Crest from Mouse Embryonic Stem Cells. Poster #40 *StemConn 09*.

Shin' D-G., Hong, S-H., Joshi, P., Nori, R., Pei, B., Wang, H-W., Harrington, P., Kuo, L., Kalajzic, I., Rowe, D., "PBC: A Software Framework Facilitating Pattern-Based Clustering for Micoarray Data Analysis", *Proc. of ISIMB Int'l Join Conf. on Bioinformatics, Systems Biology and Intelligent Computing*, August 3-6, 2009, Shanghai, China. (To be published)

Theodorou, E., Dalembert, G., Heffelfinger, C., White, E., Weissman, S., Corcoran, L., Snyder, M. A high throughput embryonic stem cell screen identifies Oct-2 as a bifunctional regulator of neuronal differentiation. *Genes Dev*. 2009 Mar 1;23(5):575-88. PMID:19270158.

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Wang, J., Saxe, J.P., Tanaka, T., Chuma, S., and Lin, H. (2009) Mili interacts with Tudor Domain Containing Protein 1 (Tdrd1) in regulating spermatogenesis. *Curr. Biol*. (Cover story, in press).

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Wang, Z. and Snyder, M. 2009. An RNA Seq pipeline for mapping transcriptomes. In preparation.

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Wu, J.,* Habbegger, L.,* Rozowski, J., Wang, Z., Gerstein, M. and Snyder, M. 2009. Complex transcriptome diversity revealed during human embryonic stem cell differentiation by sincle, long and paired end RNA sequencing. In preparation. To be submitted to *Nature*.

Xie, C.-Q., Jeong, Y., Fu, M. Bookout, A.L., Garcia-Barrio, M.T., Sun, T., Kim, B.-h., Xie, Y., Root, S., Zhang, J., Xu, R.-H., Chen, Y.E., and Mangelsdorf, D.J. Expression profiling of nuclear receptors in human and mouse embryonic stem cells. *Mol. Endocrinol***.** Feb. 5, 2009.

Xu, R.-H., Sampsell-Barron, T.L., Gu, F., Root, S., Peck, R.M., Pan, G., Yu, J., Antosiewicz-Bourget, J., Tian, S., Stewart, and Thomson, J.A. *NANOG* is a direct target of TGFb/Activin mediated SMAD signaling in human ES cells. *Cell Stem Cell* 3(2):196-206, 2008. Specifically previewed by Jiang, J. and Ng, H.-H. in *Cell Stem Cell* 3(2):127-128.

Yongxing Liu, Maria Advincula, Yu-Hsiung Wang, Peter Maye, A. Jon Goldberg and Liisa T. Kuhn. 2009 Promoter-Driven Fluorescence Reveals Cell Patterns of Osteogenesis on a Biomimetic Calcium Phosphate Surface. (Manuscript to be submitted)

Zhao, Y. (2008). Contributions to Microarray Data Analysis. Ph.D., Dissertation. Statistics Department, University of Connecticut.

Zhong, W., Waern, K., Nagalakshmi, U. and M. Snyder 2008. Methods for Scoring transcribed regions and boundaries using RNA Sequencing (in preparation).

Zhou, J., Xu, R.-H., and Wang Y. Nanoporous membrane-encapsulated feeder cells for culture of human embryonic stem cells. *Int'l J. Functional Informatics & Personalized Med.* 2(1):77-88, 2009.

Zhou, J., Q. Wang, L-L. Chen and G.G. Carmichael: On the mechanism of induction of heterochromatin by the RNA-binding protein vigilin. (2008) *RNA* 14: 1773-1781.

Zhu, X., Hill, R.A., and Nishiyama, A. NG2 cells generate oligodendrocytes and gray matter astorcytes in the spinal cord. *Neuron Glia Biol* 4(1), 19-26, 2008.