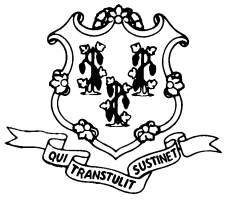
REPORT TO GOVERNOR RELL
AND
THE GENERAL ASSEMBLY

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

JUNE 30, 2010

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



State of Connecticut
Department of Public Health
410 Capitol Avenue
P.O. Box 340308
Hartford, CT 06134-0308

State of Connecticut
Department of Public Health

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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Implementation of Public Act 05-149
2010 Executive Summary

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, 2009 through June 30, 2010.

On June 8, 2010, the State allocated $9.82 million in grants-in-aid to researchers in Farmington, Storrs, and New Haven. Since passage of the enabling legislation in 2005, the State of Connecticut has allocated a total of $49.24 million in support of stem cell researchers. To date, State funding has provided the resources to fully or partially support 128 full or part-time positions at Yale University and 40 full time equivalents at the University of Connecticut. In addition, State funding supports the salaries of three graduate students and a two technicians 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, 2009 to June 30, 2010 was issuance of a fourth Request for Proposal, receipt and review of 89 applications for grants-in-aid, and the allocation of available dollars.

From February 2010 through June 2010, the 15-person Peer Review Committee completed the enormous task of rating and ranking each of the 89 applications for grants-in-aid from Connecticut's research community. During a teleconferenced meeting on May 18, 2010, 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 21, September 15, and November 17, 2009, and on January 19, March 16, May 18, June 7 and June 8, 2010. The SCR Advisory Committee completed their review of applications and allocation of grants-in-aid during the meeting on June 8, 2010. All meetings were 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 $9.82 million to researchers in Farmington, Storrs and New Haven:

Reconstitution of human hematopoietic system by HSCs derived from human embryonic stem cells in humanized mice, Yale University, New Haven, Richard A. Flavell, Principal Investigator, $1,000,000.00

Use of human embryonic stem cells and inducible pluripotent stem cells to study megakaryoblastic leukemia, Yale University, New Haven, Diane Krause, Principal Investigator, $1,000,000.00.

Generation of hematopoietic stem cells and T-cell progenitors from human ESCs, University of Connecticut Health Center, Farmington, Laijun Lai, $1,000,000.00.

Modeling Parkinson's disease using human embryonic stem cells and patient-derived induced pluripotent stem cells, University of Connecticut Health Center, James Yuanhao Li, Principal Investigator, $992,500.00.

Mechanical control of neural stem cell fate, Yale University, New Haven, Angelique Bordey, Principal Investigator, $947,975.00.

*Co-differentiation of hESC-derived retinal and retinal pigment epithelial progenitor*s, Yale University, New Haven, Lawrence J. Rizzolo, Principal Investigator, $832,608.00.

Regulations of Lin28 in Human Embryonic Stem Cell Self-renewal And Differentiation, Yale University, New Haven, Caihong Qiu, Principal Investigator, $750,000.00

Stem Cell Physiology and Chemistry Core, University of Connecticut Health Center, Farmington, Srdjan D. Antic, M.D., Principal Investigator, $500,000.00

Maturation of human embryonic stem (hES) cell-derived cardiomyocytes in vitro using 3D engineered tissue model system, Yale University, New Haven, Lee, Principal Investigator, $200,000.00.

The Role of Dormant Replication Origins in Ensuring Genome Integrity in Human Embryonic Stem Cells, Yale University, New Haven, XinQuan Ge, Ph.D., Principal Investigator, $200,000.00

Regulating Caspase Activity to Enhance Differentiation Efficiency of Human Embryonic Stem Cells, University of Connecticut Health Center, Farmington, Xiaofang Wang, Ph.D., M.D., Principal Investigator, $200,000.00.

Novel roles of long non-coding RNAs in human embryonic stem cells, University of Connecticut Health Center, Farmington, Li Yang, Principal Investigator, $200,000.00.

Molecular mechanisms of germ layer induction in human embryonic stem cells, Yale University, New Haven, Efrat Oron, Principal Investigator, $200,000.00.

Identification and characterization of multipotent cell populations from human adipose tissue for application in regenerative therapies, Yale University, New Haven, Matthew S. Rodeheffer, Principal Investigator, $200,000.00.

Efficient Gene Targeting in Human Embryonic Stem Cell via Recombineering Based Long Arm Targeting Vector, Yale University, New Haven, Chunsheng Dong, Principal Investigator, $200,000.00.

The role of epigenetic factor-HP1 in regulating human embryonic stem cell pluripotency and differentiation, Yale University, New Haven, Ee-Chun Cheng, Principal Investigator, $200,000.00.

In vivo Evaluation of Humans ES, IPS and Adult Brain Derived Neural Progenitor Cell Transplantation and Migration Using MRI, Yale University, New Haven, Erik M. Shapiro, PhD, Principal Investigator, $200,000.00.

Discovering treatments to prevent neurodegeneration in Huntington's Disease using hESCs and patient-derived iPSCs, University of Connecticut Health Center, Farmington, Carolyn Drazinic, M.D., Ph.D., Principal Investigator, $200,000.00.

Generation of a novel source of iPS cells for the treatment of osteoarthritis, University of Connecticut Health Center, Farmington, Rosa M. Guzzo, Ph.D., Principal Investigator, $200,000.00.

To develop efficient methodologies to generate customized anti-tumor effecter T cells from human embryonic stem cells (hES) and induced pluripotent stem cells (iPS) by TCRengineering approach, University of Connecticut Health Center, Farmington, Arvind Chhabra, Principal Investigator, $200,000.00.

Generation of layer V pyramidal neurons from human embryonic stem cells, University of Connecticut, Storrs, Radmila Filipovic, Principal Investigator, $199,945.00.

Nuclear Receptor Control of Human Epidermal Stem Cells, University of Connecticut, Storrs, Brian J. Aneskievich, Ph.D., Principal Investigator, $199,894.00.

CONNECTICUT'S STEM CELL RESEARCH COMMUNITY

Since passage of the enabling legislation in 2005, the State of Connecticut has allocated a total of $49.24 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.

A. University of Connecticut Stem Cell Research

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, the University has developed state-of-the-art research programs aimed at bringing human stem cell therapies to patients. With support from 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. The University is 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.

Major accomplishments for 2009-2010:

1) The University of Connecticut Stem Cell core facility generated the four human embryonic stem cell lines, CT1, CT2, CT3 and CT4, in the State of Connecticut, through the efforts of Drs Ge Lin and Ren-He Xu. These lines were reviewed and approved by NIH for registration. Once approved all lines are eligible for federally funded research.

2) Scientists from the University of Connecticut Stem Cell Institute made exciting discoveries that were published in international scientific journals. **Some examples are:**

L-L. Chen 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) Mol. Cell 35: 467-478.

Audrey R. Chapman, "The Ethics of Human Embryonic Stem Cell Patents," Kennedy Institute of Ethics Journal, 19 (3) (September 2009): 261-288.

Wang, X., Lin, G., Martins-Taylor, K., Zeng, H., and Xu, R.-H.* Inhibition of caspase-mediated anoikis is critical for bFGF-sustained culture of human pluripotent stem cells. *J. Biol. Chem.* 284: 34054-64, 2009.

Li, Y., Zeng, H., Xu, R.-H., Liu, B., and Li, Z. Vaccination with human pluripotent stem cells generates a broad spectrum of immunological and clinical response against colon cancer. *Stem Cells* 27: 3103-11, 2009.

Zeng, H., Park, J.W., Guo, M., Lin, G., Crandall, L., Compton, T., Wang, X., Li, X.-J., Chen, F.-P., and Xu, R.-H.* Lack of ABCG2 expression and side population properties in human pluripotent stem cells. *Stem Cells* 27(10):2435-45, 2009.

Tanasijevic, B., Dai, B., Ezashi, T., Livingston, T., Roberts, R.M., and Rasmussen, T.P. (2009) Progressive accumulation of epigenetic heterogeneity during human ES cell culture. Epigenetics 4(5): 330-338.

Li,X.J., Zhang,X., Johnson,M.A., Wang,Z.B., Lavaute,T., and Zhang,S.C.(2009). Coordination of sonic hedgehog and Wnt signaling determines ventral and dorsal telencephalic neuron types from human embryonic stem cells. Development 136, 4055-4063.26.

Kanisicak O, Mendez JJ, Yamamoto S, Yamamoto M, Goldhamer DJ. Progenitors of skeletal muscle satellite cells express the muscle determination gene, MyoD. Dev Biol. 2009 Aug 1;332(1):131-41.

Yin D, Wang Z, Gao Q, Sundaresan R, Parrish C, Yang Q, Krebsbach PH, Lichtler AC, Rowe DW, Hock J, Liu P. Determination of the Fate and Contribution of Ex Vivo Expanded Human Bone Marrow Stem and Progenitor Cells for Bone Formation by 2.3ColGFP. Mol Ther. 17: 1967-78, 2009.

Gibson JD, Jakuba CM, Boucher N, HolbrooK KA, Carter KG and CE Nelson. Single-cell transcript analysis of human embryonic stem cells . Integrative Biology 1: 540, 2009.

J Trotter, K Karram, A Nishiyama. NG2 cells: Properties, progeny and origin. Brain Research Reviews, 63: 72-82, 2010.

Villa, M., Pope, S., Conover, J., and Fan, T.-H., Growth of Primary Embryo Cells in a Microculture System, Biomedical Microdevices, 12: 253-61, 2010

Guo Q, Li K, Sunmonu NA, Li JY. Fgfb8b-containing splice forms, but not Fgf8-a, are essential for Fgf-8 function during development of the midbrain and cerebellum. Dev. Biol., 338: 183- 192, 2010.

Gong, G, Ferrari, D., Dealy, C.N., Kosher, R.A. Direct and progressive differentiation of human embryonic stem cells into the chondrogenic lineage. J. Cell Physiology, *In Press,* 2010.

Barisic-Dujmovic T, Boban I, and S H Clark. Fibroblasts/Myofibroblasts that Participate in Cutaneous Wound Healing are not Derived from Circulating Progenitor Cells. J. Cell. Physiol. 222: 703–712, 2010

Economic impact of State Stem Cell funding for UConn:

The stem cell grant program has created and maintained employment for 40 full-time equivalents in 32 labs at the University.

The University is expanding its Technology Incubation Program (TIP), with a new state-of-the-art incubator facility co-located with the UCHC Stem Cell Core Lab and other cell science departments. The facility will provide companies with wet labs ranging from 300 SF to 1000 SF as well as business services. This was a strategic move aimed at assuring that UConn will deliver on the commercial promise of the State's investment in UConn stem cell research by providing resources to build new firms around the University's discoveries and recruit bioscience companies pursuing stem cell therapies to locate at UConn and collaborate with Connecticut researchers advancing commercialization of their discoveries.

Two Federal grants have been obtained through collaboration between the TIP and the UConn Stem Cell Institute to provide access to unique equipment that will further research of for both TIP firms as well as faculty researchers. This specialized equipment, aimed at advancing the speed an efficiency of this research, would be otherwise unattainable for fledgling start ups.

UConn R&D Corp and the TIP have obtained the services of a bioscience business expert to review UConn stem cell grants and match research activities underway with industry interests in the stem cell arena. A key component of this effort is to identify those research activities of keen interest to both large and small companies to seek industry partnerships as well as start up opportunities.

UConn has an advanced infrastructure for technology commercialization that is modeled after national best practices and is designed to take research from the lab to the marketplace. The infrastructure not only offers business and technical expertise and support for faculty and UConn affiliated start ups, it supports industry access to the university and encourages students and faculty to engage in commercialization of technology. The full force of this infrastructure will be utilized to support commercialization of stem cell research at UConn.

One patent was filed as a direct result of the state's investment in stem cell research:

"Differentiation of human embryonic and induced pluripotent stem cells". R. Kosher, C. Dealy, G. Gong, D. Ferrari. Provisional patent filed 11/24/09, State of Connecticut (#61/264,170).

There were also two contracts signed between UConn stem cell researchers and Pfizer:

Dr Xue-Jun Li, Title: The Generation of Nociceptor Positive Neurons from Human Pluripotent Stem Cells. Period: 12-28-2009 – 12-27-2010.
Dr James Li for transfer of genetically-engineered human embryonic stem cell line. (January 8, 2010).

State of Connecticut-funded Stem Cell Research projects at the University of Connecticut:

Core grants:

1. Continuing and Enhancing the Human ES Cell Core at University 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 Drs. 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 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 this 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.

B – Group Grant:

Group Leader: David Rowe, MD

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 major 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 (abbreviated hESC) 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.

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

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.

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.

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.

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.

Project 7: 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 cells 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.

Project 8: Cartilage Differentiation from hES derived progenitor cells

Investigator: Caroline Dealy

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.

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.

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 specifically 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.

C – Established Investigator Grants:

1) Mechanisms of Stem Cell Homing to the Injured Heart

Investigator: Linda Shapiro, PhD

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.

2) 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.

3) Therapeutic Differentiation of Regulatory T Cells from iPS for Immune Tolerance

Investigator: Zihai Li MD, PhD, Pramod Srivastava, 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.

4) 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.

5) 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.

6) 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.

7) 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.

8) 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 hESCs 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.

9) Synaptic replenishment through embryonic stem cell-derived neurons in a transgenic mouse model of Alzheimer's disease.

Investigator:Joseph LoTurco, 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.

10) 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.

11) 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.

12) 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.

13) 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 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.

14) 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 human embryonic stem (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 throughput 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.

15) 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.

D – Seed Grants:

1) A 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.

2) Hybrid Peptide/RNA Molecules for Safe and Efficient Gene Silencing in Human Embryonic Stem (ES) Cells

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.

3) 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, differentiation and maturation are well known.

4) 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.

5) 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.

6) 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 operate 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, sometimes 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.

7) 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.

8) 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.

9) 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.

10) 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.

B. Yale University

The $17.3 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 60 laboratories on Yale campus actively pursuing 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 $10 million in funding that Yale received from the State in 2008 and 2009 has further generated a synergistic effect with the current funding to enhance stem cell research in Connecticut. Twelve new projects were awarded in 2009, seven to young investigators with interests in stem cell research and five 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.

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.

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 60 investigators from 28 labs and is supplying hESC cell lines and is growing and differentiating cells (e.g., neurons and erythroid cells) for 16 labs.

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 141 investigators from 37 labs.

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.

Genomics Core. This Core consists of a Cellomics High Throughput Cell Screen system and an Illumina Genome Analyzer that are located on the second floor of the Amistad building. The Cellomics Cell Screen system was provided by the Yale School of Medicine 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, is staffed by a Technical Director with 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 six 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.

New Research Projects

1. Dr. Martin Garcia-Castro, Assistant Professor, Department of Molecular, Cellular, and Developmental Biology, "Molecular profiling and cell fate potential of hESC-derived early neural crest precursors." Neural crest cells (NCCs) are multipotent migratory stem cells critical for the development of vertebrate organisms. NCCs generate, amongst many other different cells, bone, cartilage, muscle, dermis, and teeth in the cranial or head region. In addition, NCCs generate melanocytes, the pigmented cells that protect us from damaging ultra violet light, as well as most of the cells of the peripheral nervous system, special heart cells, secretory and supportive cells of various glands amongst other derivatives. The astonishing capacity of NCCs to generate such a broad range of derivatives is unfortunately matched by their participation in human birth defects. About one third of all congenital birth defects are directly linked to neural crest flaws, and some NCC-related conditions, like cleft lip/palate, occur as often as 1 in 1000 births. NCCs are also associated with various aggressive and frequent tumors, including melanoma and neuroblastoma. The enormous differentiation potential of neural crest stem cells promises to be of great value for therapeutic purposes for degenerative diseases and for severe traumas alike. In order to use these cells for clinical purposes, it is critical to improve our understanding of their basic biology. Very limited work has directly addressed early neural crest events during human development. Recent studies based in the differentiation of hESCs have identified neural crest precursors of relatively late stages (comparable to migratory neural crest cells) that display a limited capacity to differentiate and to maintain this capacity after proliferation. Dr. Garcia-Castro is investigating earlier events related to the human NCCs, using hESCs with the aim to obtain and manipulate NCC precursors from early stages capable of broader differentiation and proliferation capacities. Dr. Garcia-Castro and his lab group are characterizing the expression of a battery of molecular markers expressed by NCCs at early stages of development and they will interrogate their differentiation potential. Furthermore, they will generate new tools to prospectively isolate early NCC precursors, and to test their differentiation potentials in vitro and in vivo. The tools will benefit human research

beyond the neural crest realm, as the candidate molecules to be used are also relevant for muscle development and homeostasis, as well as for cancer research.

2. Dr. Kevan Herold, Professor, Department of Immunobiology, "Induction and differentiation of beta cells from human embryonic stem cells." Type I diabetes (T1D) is an autoimmune disease with strong genetic susceptibility, characterized by a gradual infiltration of the pancreatic islets with auto-reactive T-cells, progressive decrease in β-cell mass, impaired insulin secretion and finally hyperglycemia and insulin-dependence. A true "cure" of the disease will require prevention of the autoimmune process as well as regeneration or replacement of the lost β-cell mass. New immunologics, such as anti-CD3 monoclonal antibody (mAb), and others, may arrest β-cell destruction, but there has been little evidence for spontaneous recovery of normal β-cell function even when the immune process ceases. Human embryonic stem cells (hESC) could provide a limitless source of cells needed for replacement of lost β cells. Promising results, using hESCs to produce β-cells have been described using a series of cell culture manipulations. There were two problems with the differentiated cells, however. First these cells did not respond in a normal manner to glucose, the primary secretagogue in vivo. Second, transplantation of the cells into mice led to acquisition of glucose responsiveness, but tumors were also induced. The factors responsible for differentiation of the β-cells in vivo were not identified. Moreover, the need for transplantation of the cells into mice and the development of tumors would preclude use of the cells in patients. Dr. Herold has recently found that co-culture of mature islets with β cell precursors can deliver signals to induce glucose responsiveness. The goals of the proposed studies are therefore to establish methods for differentiation of hESC into insulin+ cells at the Yale Stem Center facility. He and his lab group will use hESC lines that are available in the Yale Stem Cell Core. They will test whether transcription factors tagged with fluorescent labels can be used to visualize cell differentiation in vitro, and whether this visualization system will allow them to identify cells that acquire glucose responsiveness and separate them from cells that develop into tumors. Finally, they will test whether mature islets can induce glucose responsiveness of differentiated hESC.

3. Dr. Valerie Horsley, Assistant Professor, Department of Molecular, Cellular, and Developmental Biology, "Transcriptional control of keratinocyte differentiation in human ES cells." As the body's largest organ and its first defense against external pathogens, the skin is crucial for life. The skin creates an external barrier to our external environment but can be compromised during injury, burns, cancers and genetic skin diseases. As a treatment for these skin disorders, hESC replacement therapies have great potential. Skin grafts can be generated ex vivo from patient skin keratinocytes but generating enough cells for tissue engraftment is time consuming and the grafts have limited potential since they do not form hair follicles or sweat glands. Thus, cell-based therapies are not currently used extensively in the clinic to treat burns or other genetic diseases that compromise skin. hESC based therapies might be able to overcome these current limitations because large numbers of cells are able to be generated and they may have a broader differentiation spectrum. However, current methods to generate keratinocytes from hESCs are inefficient. Uncovering the specific mechanisms that drive keratinocyte

differentiation in hESCs will allow Dr. Horsley to exploit these mechanisms to generate large numbers of keratinocytes from hESCs for therapeutic purposes and to generate tissue replacements for skin disorders like burns, extensive wounds and genetic abnormalities.

The transcriptional control of lineage specification is well established for many cell types. Dr. Horsley hypothesizes that specific transcription factors are required for the direction of keratinocyte fate in hESCs. Interestingly, studies of early stage epidermis in the mouse identified that transcription factors are highly enriched during epidermis formation. The objectives of Dr. Horsley's research are to: 1) define the transcriptional network that defines keratinocyte specification of hESCs and 2) analyze the function of specific transcription factors during keratinocyte formation in hESCs. They will generate reporter lines for the two stages of keratinocyte differentiation from hESCs and use these lines for analysis of candidate and whole genome expression of transcription factors during keratinocyte differentiation of hESCs. Their second aim is to functionally analyze the role of four candidate transcription factors that are enriched in developing mouse epidermis in keratinocyte formation in hESCs using gain- and loss-of function studies. These experiments will dissect the mechanisms of human skin epithelial cell differentiation and potentially allow Dr. Horsley to more efficiently generate differentiated keratinocyte cell populations for use in human therapies.

4. Dr. Yingqun Huang, Assistant Professor, Department of Reproductive Sciences, "Molecular function of Lin28 in human embryonic stem cells." Highly expressed in human and mouse embryonic stem cells, Lin28 is among four factors (including Oct4, Sox2, and Nanog) that can together reprogram human skin cells to induced pluripotent stem cells, or iPS cells. Despite its critical role in hESCs and in the production of iPS cells, the molecular function and mode of action of Lin28 are largely unknown. Dr. Huang has recently discovered that in the mouse Lin28 plays an important role in the regulation of expression of genes important for the maintenance of the unique proliferation properties of embryonic stem cells. Specifically, Dr. Huang and her research group have found that Lin28 selectively binds to a subset of messenger RNAs (mRNAs) and enhances their ability to make proteins. These mRNAs encode proteins important for embryonic stem cell proliferation. Dr. Huang's research is focused on using the knowledge she has gained with mouse embryonic stem cells to extend the analysis of Lin28 to hESCs. Thus, she has developed four specific aims: 1) to characterize the expression of Lin28 in hESCs throughout the cell cycle; 2) to characterize the interactions between Lin28 and its associated mRNAs, in order to gain insight into how this protein selects its targets and regulates mRNA function in the cellular milieu; 3) to identify Lin28-interacting proteins that may work in concert to mediate Lin28 function; and 4) to perform a genome-wide search for new Lin28 targets to establish a more comprehensive and global understanding of Lin28 function. She anticipates that results derived from these studies will shed new light on the molecular function of Lin28, thus not only contributing greatly to the general hESC field of research, but also to the technology of generating patient-specific iPS cells.

5. Dr. Jeffery Kocsis, Professor, Department of Neurology, "Cellular transplantation of neural progenitors derived from human embryonic stem cells to remyelinate the nonhuman primate spinal cord." Myelin is the insulating material surrounding single nerve fibers which increases the velocity of the nerve impulse and is essential for the proper functioning of the nervous system. Myelin in the brain and spinal cord is formed by cells called oligodendroctyes. In diseases such as multiple sclerosis myelin is lost leading to neurological disease. Dr. Kocsis and his research group have established a focal demyelinating lesion in the nonhuman primate (NHP) spinal cord and have transplanted oligodendrocyte precursor cells (OPCs) that were derived from human embryonic stem cells. Their initial histological data obtained at 6 weeks post-transplantation indicate that the transplanted hESCs survive in the immunosuppressed NHP demyelinated spinal cord, do not form tumors and associate with demyelinated axons. Neurological examination of the NHPs indicates no adverse effect of the transplanted cells. These early results are encouraging in that they indicate feasibility of this transplantation approach for demyelinating disorders. Human OPCs require a longer period of time to form myelin than do rodent OPCs. Ongoing studies will examine the myelinating potential of the transplanted human OPCs at longer time points to determine if they form functional myelin.

6. Dr. Qi Li, Associate Research Scientist, Department of Pathology, "Neural stem cell responses to hypoxia." Dr. Li is utilizing two mouse strains and brain microvascular endothelial cells and neural stem cells isolated from them in addition to commercially available human neuronal stem cells. She and her lab group have confirmed that the C57BL/6 mouse strain exhibits significantly more cognitive defects than the CD1 stain, mimicking the range of cognitive handicaps observed in the human very low birth weight premature infant population. They have correlated this with decreased factors that have been shown to enhance the development and stabilization of blood vessels and the proliferation of neural stem cells. They have shown that two of these factors are "master regulators" of endothelial and neural stem cell behaviors. One of these factors, an enzyme called glutathione synthase kinase-3 beta (GSK-3□), appears to regulate the other factor, hypoxia inducing factor-1 alpha (HIF-1□), a protein that in turn regulates the production of several proteins that are themselves regulators of several cellular functions. Knowing this hierarchy of signaling pathways has enabled Dr. Li to manipulate the responses of neural stem cells and endothelial cells to hypoxic injury. Specifically, by inhibiting GSK-3□ selectively they were able to convert C57BL/6 neural stem cell and endothelial cell migration and proliferation to levels found in CD1 cells, effectively "rescuing" the cells and identifying a potential therapeutic target.

Using microarray analyses, they have also been accruing and analyzing data on the differential expression of genes altered by hypoxic insult in these two mouse strains which may lead to a deeper understanding of individual responses to hypoxia and a wide range of potential drugable targets. Lastly, they have developed the expertise to assess the effects of individual blood vessel cells (endothelial cells) on human neural stem cell migration using both tissue co-culture methods and implantation of human neural stem cells into embryonic mouse brains. The use of human neural stem cells co-cultured with murine endothelial cells from C57BL/6 and CD1 mice

(mimicking the SVZ neurovascular niche) and implanting human neural stems into the neurogenic zones of the developing mouse brain will lead to insights into the dynamic neural stem cell-endothelial cell interactions that modulate neural stem cell behavior and may eventually lead to the development of novel therapies that will optimize the repair and recovery phases following nervous system injury.

7. Dr. Jun Lu, Assistant Professor, Department of Genetics, "MicroRNA regulation of hESC fates." The power of human embryonic stem cells (hESCs) to become any given cell type in a human body is the underlying principle to develop hESC-based therapies to replace damaged cells in human diseases. In order to make any desired cells, hESCs need to undergo sophisticated changes to switch from a stem cell state to a committed path toward another cell type, a fate switch process that is not fully understood. Prior research efforts have been focusing on genes that make proteins. However, such protein-making genes only make up less than 2% of the human genome, leaving the majority of the human genome poorly explored. Recently, Dr. Lu and others have produced data to support the idea that a class of new genes, termed microRNAs, deserves strong attention in hESC research. Unlike protein-making genes, microRNAs do not make proteins themselves, but control the levels of protein production in cells.

Dr. Lu's research hypothesizes that microRNAs contribute to the hESC fate switch process. He and his lab group aim to systematically produce a roadmap of microRNA-based regulation in hESCs and to identify microRNAs that modulate the hESC fate switch. They propose to accomplish these objectives through four achievable steps. They will first chart all the early cell types produced by hESCs when they undergo cell identity transformation, by documenting all the microRNA changes during these processes. They will then look for microRNAs that may reinforce or compromise the stem cell state, by testing their ability, one by one, to regulate important known protein factors. They have already identified candidate microRNAs that can do this. So in the third step, they will test whether these candidate microRNAs can mold hESCs into other cell types. Finally, additional candidate microRNAs that have potentially different molding ability will be explored and studied in detail. Dr. Lu anticipates that the proposed work will lead to critical knowledge in a previously underappreciated dimension in hESC research, providing important clues to novel hESC-based therapies.

8. Dr. Stephanie Massaro, Instructor, Department of Pediatrics, "The influence of aberrant notch signaling on Rb mediated cell cycle regulation in megakaryopoiesis & acute megakaryoblastic leukemia." Acute Megakaryoblastic Leukemia (AMKL) is a rare form of leukemia that affects megakaryocytes, which are platelet-making blood cells. The disease most commonly strikes very young children. Approximately 30% of pediatric patients diagnosed with AMKL are infants who have a specific genetic abnormality that involves two genes, RBM15 and MKL1. These genes may play important roles in normal blood cell development. However, when they are incorrectly linked together, they may contribute to leukemia development by altering Notch signaling, a normal cell signaling pathway responsible for cellular growth and maturation. This aberrant Notch signal may cause a failure of the tumor

suppressor protein Rb and result in uncontrolled growth of immature megakaryocytes. This abnormality is associated with an extremely poor outcome, with an average survival time of only eight months from diagnosis despite aggressive medical therapy.

Given that AMKL primarily afflicts infants, Dr. Massaro believes this leukemia arises during fetal blood development. Thus, it is essential to study the interactions of RMB15 and MKL1 with regulators of cell growth, such as Notch and Rb, using hESCs in order to understand their impact on fetal blood maturation and leukemia development.

Dr. Massaro's research is focusing on the following aims: 1) to establish the pattern of embryonic megakaryocyte development and test the hypothesis that RBM15 and MKL1 are differentially expressed during hESC-derived megakaryopoiesis, 2) to define the role of Notch in hESC-derived megakaryopoiesis and to determine whether RMB15 and/or MKL1 impact Notch signaling, and 3) to demonstrate the downstream effects of Notch signaling on Rb mediated cell cycle regulation during hESC-derived megakaryopoiesis.

Methods have been established to derive megakaryocytes in vitro from hESCs. Dr. Massaro is evaluating the cellular and genetic characteristics of these megakaryocytes as they develop in culture. She will manipulate this system in order to stimulate Notch signaling and to examine specific downstream targets, including regulations of the cell cycle in the resultant megakaryocyte population.

9. Dr. Yibing Qyang, Instructor, Department of Internal Medicine, Section of Cardiology, "Derivation and functional characterization of heart cells from human embryonic stem cells." Heart disease is the leading cause of mortality in the developed world. Human embryonic stem cells (hESCs) may provide an ideal source for production of heart cells for cell-based therapy. In order to characterize hESC-derived heart cells and then use them for cell-based therapy for heart diseases, it is very important to establish an efficient protocol to generate heart cells from hESCs. Dr. Qyang compared cardiac differentiation between NIH-approved hESC lines and non-NIH approved line, and discovered that two hESC lines have the best cardiac differentiation potential. By modulating signaling pathways critical in the regulation of cardiac differentiation, his research lab has established a highly effective protocol to generate heart cells from hESCs. They next examined whether heart cells produced from hESC cultures adopt a fully differentiated cardiac phenotype. They discovered that these hESC-derived heart cells exhibit normal Ca^{2+} transient rhythm and responded to both β-adrenergic and electric stimulation. In addition, heart cells derived from hESCs show predominantly ventricular action potential. This cardiac differentiation strategy will help them readily isolate heart cells for tissue engineering and cardiac repair and regeneration.

A mixture of cells enriched for heart cells can be isolated from hESC culture, but due to their heterogeneity they fail to engraft and may cause abnormal electrical activity after implantation. Thus, it is critically important to isolate pure cell populations enriched for cardiac cells that will engraft and promote cardiac repair.

Dr. Qyang and his lab have successfully engineered DNA constructs so they can study two highly purified hESC-derived cell populations: ventricular cardiomyocytes (VCM) and cardiovascular progenitor cells (CPC).

Another challenge for cell-based therapy is to improve upon routes of delivery because cell suspension injection often results in poor engraftment. Tissue engineering has the capacity to greatly alleviate this problem. Using a recently developed cell sheet technology, they propose to establish and characterize engineered heart tissues in cell culture using these hESC-derived CPC and VCM. As a proof of principle study to generate engineered heart tissues for comparing VCM and ICPC derived from hESCs in a 3D and more physiologically relevant context and for future cardiac repair *in vivo*, they first established a cell sheet with highly enriched heart cells derived from murine embryonic stem cell culture using cell sheet engineering technology. They will use the same technology to generate human VCM and ICPC-based cell sheets. This research will lead to the production of pure, well-characterized CPC and VCM, and novel engineered heart tissues from hESC cultures, which ultimately could be used for cell-based therapy for heart failure. In addition, pure CPC and VCM derived from hESC cultures will provide an unprecedented cellular model to dissect human heart development at the level of CPC function and VCM maturation. Furthermore, CPC and VCM will provide ideal cells for cardiotoxicity screening which is important for many therapeutic applications in cardiac medicine. There are many stem cell scientists throughout Connecticut who would like to derive heart cells from hESCs for cell-based therapy for heart failure as well as for basic research into human heart development; and Dr. Qyang will be happy to share with them our discoveries and expertise in order to facilitate their research.

10. Dr. Richard Sutton, Associate Professor, Department of Internal Medicine, Section of Infectious Diseases, "Genome-wide screen to identify hESC-specific DNA transcription elements." Human embryonic stem cells (hESC) have enormous potential in treating illnesses in man. Much is known about the genes that are turned on in these cells, but little is known about what controls the genes being turned on and off. Dr. Sutton and his research group have developed a new method in which small pieces of human DNA are placed in a 'vector' based upon human immunodeficiency virus or HIV. To cover all human DNA this requires 10 million vectors. These vectors can be made as defective viruses in cells, and then transferred to hESC such that each hESC only gets one. If the piece of DNA is read as active, then a special fluorescent protein is made, which allows us to identify and collect those cells. The pieces of 'active' DNA may then be amplified and deciphered by using new sequencing methods, and when compared to other cells it may be determined which pieces are active only in hESC. A subset of the DNA pieces may be recovered and tested again in hESC and other cell types to confirm the results. A few of the DNA pieces may be subdivided to determine what parts make them active in hESC. At the end of four years, Dr. Sutton hopes to have a better understanding of which DNA pieces are best able to turn on genes in hESC. It should be pointed out that the method is general enough to apply to any human cell type that can be cultured.

11. Dr. Sandra Wolin, Professor and Vice Chair, Department of Cell Biology, "Investigating the role of nuclear RNA quality surveillance in embryonic stem cells." The objective of this exploratory project is to determine the contributions that nuclear RNA surveillance pathways make to ES cell pluripotency and self-renewal. The genomes of mouse and human embryonic stem (ES) cells are hyperactive, with many sequences expressed at levels up to 10-fold higher than differentiated cells. The expressed RNAs include many RNAs that may be deleterious, such as transcripts of intergenic regions, variant noncoding RNAs and pseudogenes. This low-level promiscuous transcription, by keeping many tissue-specific genes in a transcriptionally competent state, is thought to be important for maintaining ES cell pluripotency.

As ES cells must balance the need to keep many genes transcriptionally competent with the need to protect themselves from deleterious transcripts, nuclear RNA surveillance may play a key role by targeting intergenic transcripts and variant defective noncoding RNAs for decay. Although the role of these pathways in ES cells is unknown, a nuclear poly(A) polymerase, TRAMP, functions with an exoribonuclease complex to degrade intergenic transcripts and unstable mutant noncoding RNAs in yeast. In vertebrate nuclei, the ring-shaped Ro protein binds the ends of misfolded noncoding RNAs in its central cavity and targets them for decay. Consistent with a role for Ro in sequestering defective RNAs in ES cells, Ro binds variant misfolded U2 snRNAs in mouse ES cells, but is not detected bound to these RNAs in other cells. Our first aim is to determine the spectrum of RNAs targeted by the TRAMP pathway in mouse and human ES cells and by the Ro pathway in human ES cells**.** Using RNA interference to deplete components, followed by examination of specific classes of noncoding RNAs, we will determine which RNAs are targets of these pathways. Our second aim is to determine if RNAi-mediated knockdown of Ro and/or TRAMP impairs ES cell function**.** Following depletion of either Ro or TRAMP components, we will determine if these pathways are required for ES cell self-renewal or pluripotency. The proposed studies should elucidate an important aspect of ES cell biology and advance our understanding of the role of nuclear RNA surveillance pathways in mammalian cells.

12. Dr. Tian Xu, Professor and Vice Chair, Department of Genetics, "piggyBac Transposon for Genetic Manipulation and Insertional Mutagenesis in Human Embryonic Stem Cells." The ability to perform genetic manipulations in hESCs is of central importance to study hESC biology. However, researchers lack critical methodologies to manipulate the hESC genome. Development of efficient technologies to genetically manipulate hESCs will enable researchers to address many interesting and fundamental aspects of hESC biology that are currently difficult to approach.

To efficiently manipulate the hESC genome, Dr. Xu proposes to use the newly developed, bipartite piggyBac (PB) transposon system for mammals as a tool for insertional mutagenesis. There are multiple reasons why PB should be developed as a tool for functional annotation of the hESC genome. It transposes efficiently in a genome-wide manner in mouse embryonic stem cells and all human cell types tested. PB also can carry antibiotic-resistant or visible markers and can be easily

mapped upon insertion into the genome. In addition, Dr. Xu has shown that PB can induce loss-of-function as well as gain-of-function mutations, and that it can interrogate not only protein-coding genes but also small RNA genes and regulatory regions. Finally, PB-based genetic manipulations permit the utilization of forward genetic screens to address complex biological processes controlled or driven by several genetic components or pathways in an unbiased fashion. These advantages suggest that PB, as a tool, will have wide applicability to the study of many aspects of hESC biology.

As an example of a complex process that can be addressed using PB, Dr. Xu's lab will use PB insertional mutagenesis to screen for hESC transformation and malignancy. They will use the soft agar assay for transformation as well as injection into immune-compromised mice for malignancy. While cancer research has identified a host of oncogenes and tumor suppressors that are important for transformation and malignancy in terminally-differentiated cells, hESCs may require fewer genetic alterations or a different set of genetic mutations altogether. The identification of genes that drive transformation and malignancy in hESCs will elucidate interesting mechanistic differences between the biology of pluripotent cells and terminally-differentiated cells. In addition, it will provide crucial information to researchers about the kinds of genetic alterations that should be avoided in hESC cells for therapeutics.

Recruitment of Leading Stem Cell Researchers

The State stem cell funding has aided Yale's ability to recruit eleven faculty members to the Yale Stem Cell Center. These include:

- Haifan Lin, Ph.D., Duke University (2006)
- Caihong Qiu, PhD, Albert Einstein College of Medicine (2007)
- Natalia Ivanova, PhD, Princeton University (2008)
- Jun Lu, PhD, Broad Institute of MIT and Harvard (2009)
- Shangqin Guo, PhD, Harvard University (2009)
- Valentina Greco, Ph.D., Rockefeller University (2009)
- Yibing Qyang, PhD, MGH, Harvard Stem Cell Institute (2009)
- Valerie Horsley, PhD, Rockefeller University (2009)
- Matthew Rodeheffer, PhD, Rockefeller University (2009)
- In-Hyun Park, Ph.D., Children's Hospital, Harvard Medical School (2009)
- Andrew Xiao, Ph.D., Rockefeller University (2010)

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 New York and New Jersey. In addition, 33 high level non-faculty researchers have been supported with CT funds in the past three years and together these individual represent some of the best stem cell researchers in the world.

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:

September 9, 2009: University of Connecticut Stem Cell Institute Seminar Series. Keynote Speaker: Dr. Yibing Qyang, Instructor, Yale Stem Cell Center. The title of his talk was, "Towards Human Heart Repair Using IsI1 + Cardiovascular Progenitors." This talk was attended by more than 100 stem cell researchers from the University of Connecticut.

October 23, 2009: Yale Stem Cell Center Second Annual Retreat. Keynote Speaker: Dr. Ronald D. G. McKay, Senior Investigator, Laboratory of Molecular Biology, National Institute of Neurological Disorders and Stroke. This Retreat was attended by over 250 Yale faculty, staff, and students and members of the CT Stem Cell Research Advisory Committee. In addition to oral presentations by Yale faculty members, it also included a poster session with 19 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.

October 30, 2009: New England Stem Cell Consortium (NESCC) First Annual Junior Investigator Symposium. As members of the NESCC, University of Connecticut, Wesleyan University, and Yale organized and participated in this symposium targeted at graduate students and postdoctoral associates interested in stem cell research. It was attended by over 95 participants from New England.

January 29, 2010: Wesleyan University Conference, "Stem Cells in to the Clinic: Biological, Ethical, and Regulatory Concerns." Valerie Horsley, Assistant Professor of Molecular, Cellular and Developmental Biology at Yale University spoke on "Intrinsic and Extrinsic Control of Skin Stem Cells. It was attended by staff from Connecticut Innovation, the Connecticut Department of Public Health, and University of Connecticut, University of Connecticut Health Center, Wesleyan, and Yale faculty, students, and staff.

May 17, 2010: Yale Stem Cell Center and Wesleyan University hosted the Connecticut Stem Cell Research Retreat at Yale University. The Program included eight talks from University of Connecticut, Wesleyan, and Yale faculty. In addition, participants attended workshops on the new NIH hESC Guidelines and High-Throughput Sequencing. It was attended by over 250 stem cell researchers from academic institutions and pharmaceutical companies throughout the State and members of the CT Stem Cell Research Advisory Committee.

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 7 universities in the Northeastern United States (Harvard, UConn, Univ. of Maine, Univ. of Massachusetts, Univ. of Vermont, Wesleyan and Yale), affirming the leading position of Connecticut in stem cell research.

Economic Impact on the State of Connecticut

This funding has effectively leveraged other resources to boost the state economy. At Yale, over 70% of the $17.3M in state funds, after initial investment in equipment, has been used for salary support, and has created 128 full-time and part-time positions in Connecticut. Moreover, this funding allowed us to attract more than $40M of research support from Yale and $7.65M research funds from outside the state, and this number is rapidly growing. These resources continue to generate more jobs and to develop our State's competitive edge in economic development.

C. Wesleyan University

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 eight seminars given at Connecticut colleges and universities, some institutions not visited previously, this past year. Funds also supported Connecticut Stem Cell Research Retreats, at which researchers share their initiative-funded data and core facilities report updates. In addition, the second hands-on human embryonic stem cell workshop for undergraduates was held at the Farmington core facility.

Research Projects

Directing production and functional integration of embryonic stem cell-derived neural stem cells Investigator, Investigator: Laura Grabel, PhD. In the third year of funding we continue to make progress. The goal of this project is to understand the conditions that promote neurogenesis of embryonic stem cells, in vitro and after transplant to the brain. Under Objective 1, using the human ESC lines H9 and H1

and a rapid, direct protocol for generating neural stem cells we reported last year, we are now characterizing these cells further and examining the effect of growth factors and signaling molecules on their pattern of differentiation. We have also generated, in collaboration with Alex Lichtler at UConn Health Center, a derivative of H9 that expresses a lentiviral vector encoding a constitutive reporter as well as a neural stem cell reporter. Under Objective 2, we continue to study the fate of ESC-derived neural stem cells transplanted to the hippocampus. Recent analysis reveals a gradual decline in stem cell markers and increase in neuron-specific markers. We have also completed our analysis of the role of SDF-1a in the migration of transplanted ESC-derived NSCs in the dentate gyrus and have quantitative data that suggest this chemokine helps direct transplant dispersal. We continue to work with FACS isolated forebrain progenitors derived from human ESCs based on expression of Forse1 and are preparing to transplant these cells into the mouse kainate model of epilepsy. A number of chapters and reviews have now appeared or are in press. We are currently preparing a manuscript on the role of SDF-1 (CXCL12) in transplant migration and are writing two invited neurogenesis reviews.

Brain grafts of GABAergic neuron precursors derived from human and mouse ES cells for treating epilepsy, Investigator: Janice Naegele, PhD. The goal of this project is to generate inhibitory interneurons from ES cells and transplant them into sites of damage in a mouse model of temporal lobe epilepsy. The hope is that these neurons will dampen the hyperactivity that leads to recurring seizures. In the first year of funding we have established a long-term video/EEG recording system that accurately measures clusters of recurring seizures in pilocarpine-treated mice. Cell protocols are now in use that enrich for GABAergic neurons, and these cells are being transplanted to the dentate gyrus of treated mice. Preliminary results, including patch clamp analysis, suggest these cells are able to generate interneurons in the hippocampus and ongoing studies will determine their effect on the severity and frequency of seizures. Publications in the past year include several reviews and chapters on cell-based therapies for epilepsy and manuscripts in preparation on the GABA cell-enrichment protocol and the fate of neural progenitors transplanted to the dentate gyrus in pilocarpine-treated mice.

Internal and External Collaborations

Laura Grabel/Janice Naegele/Gloster Aaron: The ongoing collaboration between these three Wesleyan University colleagues continues to be an integral and essential component of our projects. Grabel provides the ES cell expertise, Naegele the experience with epilepsy models, and Aaron the background in neurophysiology essential to test the function of transplanted cells. New contributions include the use of EEG recordings to measure seizure activity and use of the pilocarpine mouse model, which results in recurring seizures.

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.

Economic Impact

Funding supports the salaries of three graduate students and two technicians, as well as a postdoctoral fellow. In addition, new internal and external support, based upon these projects, has led to hiring an additional technician. These projects have created seven new positions.

V. 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 $49.24 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.

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
Richard H. Dees, Ph.D.	University of Rochester P.O. Box 270078 Rochester, NY 04627-8110
Gerald Fishbone, M.D.	Hospital of St. Raphael 1450 Chapel Street 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
Ronald Hart, Ph.D.	Rutgers University Cell Biology and Neuroscience W.M. Keck Center for Collaborative Neuroscience 604 Allison Road Piscataway, NJ 08854
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
Stephen Latham, Ph.D., J.D. Resigned: 06/08/2010	Deputy Director Yale's Interdisciplinary Center for Bioethics P.O. Box 208209 New Haven, CT 06520-8209
Robert Mandelkern	CT Co-Coordinator Parkinson's Action Network
Paul Pescatello, Ph.D., J.D.	President & CEO CT United for Research Excellence, Inc. 300 George Street, Suite 561 New Haven, CT 06511
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
Guoping Fan, Ph.D.	Associate Professor Dept. of Human Genetics UCLA 695 Charles Young Drive South Los Angeles, CA 90095
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
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
William Lowry, Ph.D.	Assistant Professor Maria Rowena Ross Chair in Cell Biology and Biochemistry University of California, Los Angeles 621 Charles Young Drive South Los Angeles, CA 90095
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
Leslie Weiner, M.D.	Chair, Department of Neurology Keck School of Medicine of USC 2025 Zonal Ave., RMR 506 Los Angeles, California 90033