APPENDIX E
PRINCIPAL INVESTIGATOR SURVEY RESPONSES

Questions 1-3.

NOTE: Responses to these questions were to confirm who was responding to the survey, from which institution, and which grant awards were included in the PI’s responses. Their responses are not included in these survey results.

Question 4.

Were you able to complete the research funded through your CT Stem Cell Research Grant Funding within the Assistance Agreement timeline? Additional months required?

![Bar Chart]

# of PI's Completing Grant Funding on Time v. # of Extensions Required
n=87

- Completed on Time: 49
- Required an Extension: 38

PI Response
Question 5.

How long have you served as a lead scientist? Indicate in number of years.
And the same responses by ranges of years:

**Lead Scientist - # by Range of Years**

n=89

<table>
<thead>
<tr>
<th>Range of Years</th>
<th># of Lead Scientists</th>
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<tr>
<td>&lt; 1 year</td>
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<td>1 to 5 years</td>
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<td>6 to 10 years</td>
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<tr>
<td>11 to 15 years</td>
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<td>16 to 41 + years</td>
<td>27</td>
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**Lead Scientist - # of Years with Own Lab**

n=71

<table>
<thead>
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<th># of Lead Scientists</th>
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<tr>
<td>Over 41</td>
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</tbody>
</table>
**Question 6.**

*To what extent do you agree or disagree with each of the following statements as a recipient of CT Stem Cell Research Funding?*

<table>
<thead>
<tr>
<th>Statement</th>
<th>PI Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT Stem Cell Research Grant Funding made it possible to increase my lab's technology/equipment (n=87 responses, 6 N/A).</td>
<td>4.28</td>
</tr>
<tr>
<td>CT Stem Cell Research Grant Funding made it possible to increase staff size to support my research (n=88, 4 N/A).</td>
<td>4.46</td>
</tr>
<tr>
<td>CT Stem Cell Research Grant Funding made it possible to obtain funds from other sources (n=87, 18 N/A).</td>
<td>4.18</td>
</tr>
<tr>
<td>My stem cell research would not have been initiated without CT Stem Cell Research Grant Funding (n=90, 0 N/A).</td>
<td>4.63</td>
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**Comments:**

- CT Stem cell Research Grant Funding supported my own salary and lab equipment and supply.
- I was able to receive funding from the Veterans Health Administration and the National Multiple Sclerosis Society to support aspects of the projects initiated by the CT Stem Cell Research Grant Funding.
- The proposed work was high risk and high impact. We have been able to develop new approaches to address a clinically relevant question.
- The CT Stem Cell was instrumental in my career development and getting further funding.
- Our ability to study the disease-modifying effects of neural stem cell grafts would not have been possible without this source of funding.
- Prior to the availability of CT stem cell funds, my lab worked predominantly on adult stem cells. With the CT grant availability, we have expanded our work to also include embryonic stem cells. We also expanded the scope of our adult stem cell work, and the new data obtained with the CT stem cell finding allowed us to obtain a new NIH R01 grant.
• Funding changed my research trajectory and also allowed me to secure additional extramural funding.

• Although technology/equipment was not increased in my specific lab, access to the Stem Cell Center Core facilities extended access to critical and highly expensive equipment that would not have been available otherwise.

• I am a long standing embryonic stem cell researcher, but the initiative has fostered a change to a more applied, translational direction.

• CT Stem Cell Funding allowed my lab to adopt several new technologies including:
  1. Reverse transcription polymerase chain reaction (RT-PCR)
  2. qPCR3.
  3. Western blot
  4. Bulk-loading with calcium sensitive dyes
  5. High pressure liquid chromatography (HPLC) of neurotransmitters such as glutamate, GABA and dopamine

• With seed grant it is not possible to buy equipment, but we introduced some new methodology.

• I have not yet obtained funds from other sources.

• My collaborator, Dr. Charles Giardina, and I successfully competed for a National Institutes of Health award this year based on our stem cell funded work.

• I am semi-independent with a NIDDK Mentored Research Scientist Development Award (K01) studying hematopoietic stem cells. The funding however only covers my salary and basic mouse cost. The CT Stem Cell Research Grant Funding I obtained enabled me to hire a full time research technician to tackle the aspect associated with human pluripotent stem cells, which would not have been initiated were it not for this funding.

• It is awfully hard to purchase equipment on these grants because salaries are so expensive and you have to have money for supplies and services.

• With the current funding crisis at the Federal level, the seed grant was instrumental in allowing me to begin my research career as an independent scientist. I work on induced pluripotent stem cells. However, we still need to compare our findings to human embryonic stem cells, to ensure that our patient specific lines behave appropriately. Differentiation protocols into specific lineages have been worked out in hESCs, so we must compare our patient derived iPSCs to hESCs lines to make sure that the express the appropriate lineage markers.

• National Institutes of Health funding is at a historic low, and the Seed Grant allowed me to retain valuable personnel when it took longer than expected to renew my NIH grant. It also allowed us to explore an entirely new area of research, which has had many positive impacts on my research program.
Question 7.

*Did the availability of CT Stem Cell Research Grants contribute to your reason for doing your research at a CT research institution?*

**Comments:**

- I was involved in the process of setting up stem cell research in CT and provided testimony to the state when the act was being considered.

- I was already at the institution. However, had I not been here, the stem cell grant opportunity would have been an enticement to move to CT. Many states do not have this type of funding.

- This grant caused me to remain in CT for an extra two years longer than I had planned.

- I came here because I knew I could apply for a CT stem cell grant.

- Though I was already in CT, the Stem Cell Research Grant was a major reason for deciding to stay in CT when being recruited by other institutions (I was extended excellent offers from John Hopkins and Brandeis which I declined).
Question 8.

*Indicate the primary type of research you did (are doing) through you CT Stem Cell Research Funding.*

![Diagram showing primary types of research](image)

**Primary Type of Stem Cell Research**

- **Basic**: 55
- **Translational**: 35
- **Clinical**: 0

Question 9.

*Estimate the % of your stem cell research in five years that will be basic, translational and/or clinical.*

![Bar chart showing % of basic research](image)

**% of Basic Research in Five Years**

- **0%**: 4
- **10%**: 4
- **20%**: 6
- **30%**: 8
- **40%**: 11
- **50%**: 21
- **60%**: 2
- **70%**: 6
- **80%**: 5
- **90%**: 5
- **100%**: 13

CONNECTICUT ACADEMY OF SCIENCE AND ENGINEERING
FUNDING

Question 10.

Are you continuing to do similar research to what your CT Stem Cell Research funding enabled you to do? Check all that apply.

Eighty-eight PI’s responded to the question, with several selecting multiple statements for a total of 114 responses.

![Bar chart showing responses to different statements regarding continuation of research](chart_image.png)
Comments:

- Not applicable—still in progress
- The nonhuman primate work is very costly, and I have received additional funds from non-CT sources to enhance our work.
- I have retired.
- I have an NIH grant currently under review that will allow us to extend the research funded by CT Stem Cell to include induced pluripotent stem cells.
- My research has changed based on my research through the CT Stem Cell Research Grant Funding, and I will apply for additional funding.
- Although my 2nd grant from CT is still in process, I have received an R01 [NOTE: The Research Project (R01) grant is an award made to support a discrete, specified, circumscribed project to be performed by the named investigator(s) in an area representing the investigator’s specific interest and competencies, based on the mission of the NIH. Based on data obtained during my first CT grant].
- I studied the same protein for a while afterwards and received a second grant from an independent institution for this work, but am no longer working on this protein.
- The majority of work in my lab prior to, during and after the CT Stem Cell award is focused on Drosophila.

Question 11.

Provide the following information on stem cell research funding applied for in addition to this grant funding. If responding to this survey for multiple CT Stem Cell Grant Funding for similar research, do not include in your response your previous CT Stem Cell grant funding.
Comments:

- Did not apply for additional funding
- I am in the process of revising my NIH proposal. 50% of the preliminary data were obtained thanks to CT Stem Cell Grant.
• I am planning to apply for a NIH grant next year based on the data I received from the currently supported grant.

• I have applied to the National Marfan Foundation. The decision will be made by November 1, 2012.

• Moved back to Japan and grant from the Japanese government is funded.

• NIH funding categories pursued: Small Business Research Funding Opportunity (1)-SBIR, Research Project Award (1)-R01, Exploratory/Development Research Grant Award (2)-R21

• Other source is an internal grant to spur collaboration between UCONN and UCHC

• Other source represents critical collaborative work funded by another state

• Our grant submitted to NIH received favorable reviews despite not getting funded. Resubmission of our grant is in process.

• Outside funding sources are: 1) US Department of Veterans Affairs 2) The National Multiple Sclerosis Society 3) Geron Corporation

• The R21 submitted to NIH will be reviewed soon. Plan on applying again this cycle.

• Will submit another

• Work just commenced and preliminary data will be used to apply for future grants.

STAFFING

Questions 12 and 13

Estimate the # of staff members for each of the following categories who worked with you on your stem cell research PRIOR to receiving your CT Stem Cell Research Grant Funding. Select the “0” option in the drop-down menu if no staff members within a category worked with you on your stem cell research prior to receiving your funding.

Estimate the # of staff members for each of the following categories who were funded through your CT Stem Cell Research Grant Funds by year.

PLEASE NOTE:

- For the years prior to when your CT Stem Cell Research Grant Funding began, select the “Prior Funding” option in the drop-down menu for the # of staff members by category for those years.
- If your CT Stem Cell Research Grant Funds have ended, but you continue to have staff members working with you on your stem cell research, include in your yearly estimate.
- If your CT Stem Cell Research Grant Funds have ended and you have no staff members working with you on your stem cell research or you are no longer doing stem cell research, select the “0” option in the drop-down menu for the # of staff members by category for those years.
The following seven Figures identify total staff by year and staff by grant type, as reported by Principal Investigators who worked on CT Stem Cell Research Program funded projects. (NOTE: PIs are not included in these figures.)
Total Group Grant Funding Staff by Year

n=4

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<tr>
<td>2012</td>
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<td>2013 (Est.)</td>
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Total Group DD and Hybrid Grant Funding Staff

n=2

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<th>Year</th>
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<td>2011</td>
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<td>2012</td>
<td>2</td>
</tr>
<tr>
<td>2013 (Est.)</td>
<td>2</td>
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</tbody>
</table>
Question 14.

Estimate the number of staff members who were new to the stem cell field and the number who relocated to Connecticut as a result of the research that was supported through your CT Stem Cell Research Grant Funding.
RESEARCH RESULTS

Question 15. (see attached)

Provide a lay summary detailing the results and key accomplishments of your CT Stem Cell Research Grant Funding. Your response should include the value your research provides to the citizens and government of the State of Connecticut. Be as clear and concise as possible.

Consider the following in your response:
- Did your research result in notable progress in the field of stem cell research and human health?
- Why is your research important and worthy of state funded investment?
- Did your research result in key scientific breakthroughs? (1,000 character maximum)

• In the first seed grant we identified a mechanism by which to generate neural progenitor cells from hES cells. In the second we compared the utility of hES derived neural progenitors to those derived from iPS cells and adult human brain tissue.

• Our grant was awarded to develop and apply single cell transcript profiling to human embryonic stem cells. The award allowed us to support some of people and buy some of the reagents and equipment required to do this work. As a result of this effort we published the world’s first description of human embryonic stem cells and stem cell differentiation at the single cell level. Single cell resolution provided, and continues to provide, unprecedented insight to the dynamics of stem cell behavior and has enabled a variety of experiments and insights that would not have been possible without this technique. Due, in part, to our successful work funded by the state program, we now
have three major stem cell initiatives in my lab. One of these has been awarded NIH funding, one has served as the foundation for forming a small biotech business in CT, and the last has made a major breakthrough in stem cell reprogramming (submitted for publication). In addition, my lab has trained many PhD and undergraduate students in stem cell and molecular biology. While we only received two years of funding from the state, this funding had a huge impact on the trajectory of the work in my lab and contributed to each of these successes.

- We demonstrated that you can use stem cells as a new form of cancer vaccine.
- The primary aims of this project were, (a) to generate iPSC with or without FOXP3 over expression, and (b) in vitro differentiate to Treg from FOXP3+ iPSC, FOXP3- iPSC, FOXP3+ hESC, and FOXP3- hESC. We have been successful in reprogramming the human fibroblast cell line and Jurkat T cells into iPSC cell with the four reprogramming factors which are either over-expressing or not expressing FOXP3. Thus we can conclude that Foxp3 expression does not affect the generation of iPSC cells. Our next aim was to differentiate these iPSC cells to Tregs in vitro (Aim 1b). For this we cultured iPSC on a monolayer of OP9 stromal cells that express a Notch ligand DL1 for 3 weeks following the addition of survival/proliferation and differentiation. Co-cultures were Tregs. As pre-T cell marker CD3 expression is absent in the cells harvested we used the M210 stromal cells as an alternative strategy, which are more stable in long-term culture than routinely used OP9-DL1 cells. But the yield of differentiated cells was extremely poor. Stimulation in vitro with Notch ligand to differentiate iPSC cells into T cells is a well-established method but recent reports suggest that the iPSC cell derived T cells cannot survive longer, which could be the reason for poor yield. Thus we are optimizing the stimulation strategy. Also, as differentiation of Treg cells from iPSC cells have never been tested before it might be possible that the nature of the stimulating ligands need to be optimized. These different approaches are still ongoing and efficiency of iPSC differentiation to Treg cells will be tested.
- We focus on using human embryonic stem cells and induced pluripotent stem cells (iPSC) to treat bone diseases. To do this we need to develop methods to convert these cell types into cells that can produce bone in patients. We believe that most other researchers’ methods for evaluating success in achieving this goal are not good enough, so we have put a lot of effort into developing methods for evaluating whether we have been able to induce human stem cells to produce bone in mice. A major achievement of our work is that we are the first to produce stem cells that express a fluorescent protein marker when they become bone cells; this provides a rapid and reliable way to assess the success of our methods for producing human bone in mice. We have shown that one published method produces much more bone, and is more reliable, than other methods, and we have developed a new method that also reliably produces bone. To create the cells with bone specific markers, we have used gene targeting with zinc finger nucleases. We will use this technology to correct the genetic defect in iPSC from patients with a genetic bone disease, osteogenesis imperfecta, which we hope will lead to therapy for this debilitating disease.
- This grant was one of the first to support induced pluripotent stem cell research in Connecticut. We were able to establish several patient-matched iPSC cell lines under this grant. In addition, we showed that these cells can be differentiated to hepatocytes (liver cells) and neurons. The grant laid the groundwork for a collaborative disease-directed
grant on drug-induced liver injury (co-PIs Boelsterli, Krueger, Rasmussen).

- These two grants have to do with epigenetic stability of ES cells. Epigenetics is characterized by alterations of chromatin structure that occur on DNA that affect the activity of genes. The seed grant described how ES cells are unstable in terms of epigenetics (and hence gene expression). The established investigator grant consisted of research to find compounds that can stabilize the epigenetic state of ES cells. Our research in these areas has led to improved methods for the culture and maintenance of ES cells, and hence, positively affects the quality of stem cell research in the state. We have been very forthcoming with our results to colleagues in the state.

- 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 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 was 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.

- Despite the remarkable progress made in deciphering embryonic stem cell pluripotency, particularly in murine models, there is a fundamental gap in understanding how human embryonic stem cells (hESCs) regulate the pluripotent state. Our goal is to define the composition and function of the transcriptional network in hESCs network through shRNA-based functional screen. Our work shows that the transcriptional network in hESCs also appears to be different from that in mouse ES cells. Specifically, we demonstrated that although NANOG, OCT4 and SOX2 are required for hESC maintenance, these factors are re-organized into different modules and suppress epiblast-specific cell fates (Wang et al., 2012). We also identified several novel transcriptional factors that are required for the maintenance of the pluripotent state which we currently study in detail. The proposed research is relevant to public health because a thorough understanding of regulation of hESC behavior is a crucial requirement for developing regenerative therapies to effectively treat human disease. Patients with Parkinson’s and Alzheimer’s diseases, spinal cord injuries, autoimmune diseases and birth defects could potentially benefit from this research.

- We attempted to establish a model of Acute Megakaryoblastic Leukemia, an extremely rare disease that generally affects infants. We succeeded in genetically engineering cells to care the leukemia gene of interest and were able to grow them in the lab and turn them in to megakaryocytes, or platelet making cells. Unfortunately, even though the cells contained the leukemia gene, they did not express the leukemia protein as we had expected.

- We developed human radial glia (RG) cells, which are the main progenitor cells in the CNS, from hESC (hESC-RG).
We compared them with RG cells isolated from the human fetal tissue, and concluded that they share numerous molecular, antigen and functional characteristics. Thus in vitro generated RG cells can be successfully used in research without difficulty and ethical problems related to human tissue.

We set up a model system to study how the hESC-RG will behave in the environment of the human brain tissue (important for future cell-replacement studies). This was accomplished by co-culturing hESC-RG cells with fetal brain cells, which increased 9 times their neurogenic capabilities. This effect was due to secreted factors in the media produced by astrocytes. We are now further characterizing the media from co-cultures. We demonstrated that transcription factor Pax6 is crucial for neurogenic differentiation of hESC-RG.

This research demonstrates the close similarities between hESC derived RG and fetal RG cells. It improves our understanding of changing behavior of hESC-RG cells in the environment of the human brain.

- We have established a method to conduct forward genetics screens in hESC and identified a new mechanism for ES cells self renew.

- Adult heart cells have limited restorative potential. Loss of them compromises heart function and ultimately leads to heart failure. The potential capability of human embryonic stem cells turning into every type of cells in the body provides the opportunity to produce more heart cells to repair damaged hearts. The problem is how we can drive the hESCs to become heart cells. The primary of goal of this work is to solve this problem. In the first year of this grant, we tested whether one of the cell-cell communication pathways would help the process. In addition, we also tested whether we can put cells on the synthetic patches, which are placed on damaged hearts to facilitate the engraftment of cells to the damaged hearts.

- Heart disease is one of the leading causes of death and disability in the developed world. Human embryonic stem cells (hESC) may provide an ideal source for production of heart cells for cell-based therapy or for creating functional cardiac tissue replacement. Recently, it has been shown that hESC can be differentiated into functional cardiomyocytes under certain conditions, thereby opening an exciting avenue for generation of patient-specific heart cells for cardiac repair. However, one of the limitations with in vitro differentiation of hESC into cardiomyocytes is their embryonic phenotype based on their size, organization and electric properties even after 2 months of standard 2D tissue culture. In order for stem cell-based therapy to advance, a better understanding of the micro environmental cues that regulate differentiation and maturation of hESC is essential. However, traditional 2D cultures are not physiologic and lack of mechanical stimulation and biological cues from other cell types may be the causes of immature cardiomyocytes as cardiomyocytes exist in an environment with multiple cell types along with extreme dynamic changes of stress and strain.

Thus, in this proposal, we will exploit unique 3D culture systems incorporating mechanical stimulation such as mechanical stretching and shear stress to evaluate hESC-derived cardiomyocyte maturation in vitro. The innovation of this proposal primarily relates to a novel approach for evaluating hESC-derived cardiomyocyte maturation and function in vitro, using two custom-designed 3D engineered cardiac
tissue models. First is the engineered cardiac chamber that allows the direct assessment of cardiomyocyte functionality, force generation, and pressure-volume loops, and is completely novel in its design. Second is the novel perfusion system that allows the culture of various cell types in the presence of a bulk flow field, which is also, to the best of our knowledge, the first demonstration of in vitro perfusion of a gel-based system to study the impact of fluid flow on cell growth, remodeling and micro vascular formation. These engineered tissues represent a more realistic model of a natural ventricle than traditional 2D planar cell cultures, yet provide a more precise level of experimental control than animal or patient studies, and allow long-term cultivation in vitro. The ability to study human cardiac cell differentiation in 3D engineered tissue offering physiologic environment with mechanical cues will not only help to elucidate mechanisms on how cardiomyocytes mature, which is not possible with 2D cultures or in vivo, but also will greatly advance in developing strategies for cardiac repair as this may help to elucidate how new myocardial cells can properly integrate into the adult myocardium followed by the cell-therapy. The overall hypothesis is that the maturation of hESC-derived cardiomyocytes depends on mechanical stimulation within 3D environments.

• Induced pluripotent stem cells (iPSC) are similar to human embryonic stem cells (hESC), but derived from mature somatic cells like fibroblasts or lymphocytes. Since iPSC retain the genetic background of a human individual, they can be used to model human pathology or to screen for individualized therapeutic agents. One of the most important questions in the iPSC field today is whether these cells are indeed similar to hESC derived from human embryos, and whether we will be able to reproducibly and stably differentiate these cells into the desired cell types. Using a novel differentiation protocol, we derived excitatory and inhibitory neuron progenitors from iPSC lines generated in our laboratory and will test whether these iPSC-derived neuronal cells have the structural and functional characteristics of mammalian excitatory and inhibitory neurons. These analyses will be applied to both iPSC and hESC lines growing in culture as well as in vivo, after transplantation of the iPSC-derived progenitors into immature mouse brains. In vivo transplantation will enable analysis of cell cytoarchitecture, location in the CNS and functional integration into the existing circuitry. Determining how excitatory and inhibitory neuron development differs in iPSC as compared to standard hESC lines is essential for determining the validity and reliability of the iPSC model and is an essential prerequisite for their future use in human disease diagnosis and therapeutics.

If our strategy is successful, iPSC from specific individuals can be tested for their ability to reproduce aspects of human disorders in vitro for pharmacological drug screening, as well as correcting disease phenotypes in mouse models of human disorders, including neurodevelopmental and neurodegenerative disorders. Thus our research provides an opportunity of testing their potential to influence future therapeutic strategies and policy.

• The grant from the State of Connecticut has been crucial for developing methodologies and obtaining a number of key findings and that have substantially advanced research in the field of stem cells. Our main intent was to study the neural stem cells that reconstitute lost neurons and promote recovery in a mouse model of perinatal brain injury. We developed methods that allow us to identify and isolate neural stem cells from the mammalian brain and study them in vitro. In young rodents, this type of
neural stem cells (identified by a protein called GFAP) can respond to low oxygen (hypoxia) by generating new neurons that integrate into the brain. We have also been able to reproduce the response of GFAP-positive neural stem cells to hypoxia in a culture dish. The breakthroughs that have emerged from our studies are that GFAP-positive neural stem cells naturally present in the brain of juvenile mice recovering from perinatal hypoxia are able to generate neurons that repair the damaged brain, weeks after the insult. These neural stem cells are no longer able to generate new neurons in the adult brain. To understand this difference, we compared the gene expression profile of these cells at different stages of development. The results indicate dramatic changes in expression of genes controlling the metabolism as well as epigenetic state of glial cells in the young mice. This is very likely to give us leads into how we may be able to use neural stem cells in promoting brain repair under conditions when this is less likely to occur, for example, in adult and aged organisms. This work has important therapeutic implications; for example, it identifies genes that can be modulated in human embryonic stem cells in order to promote their regenerative potential.

• Our studies sought to develop a technique for the screening of approaches to induce stem cell lines to differentiate into kidney epithelial cells. The seed funding provided by the CT stem cell fund allowed us to create the genetically altered mice that were necessary for screening the stem cells, but we were not able to actually proceed to the stem cell screening step. However, we did develop a new mouse strain for stem cell screening that we are making available to the research community.

• Human embryonic stem cells hold great potential in regenerative medicine because they are the only type of cells that have the ability to self-replicate and to generate all types of body cells (i.e., pluripotency). To harness this potential, we first need to understand how the self-replication and pluripotency of these stem cells are controlled by their genes. During first year of funding, I isolated a group of novel small RNAs from human ES cells. I tried to understand whether these small RNAs switch on and off different genes by forming complexes with a class of proteins called Piwi proteins. I also found four Piwi proteins are present in human ES cells. Different piRNA-Piwi complexes directly bind to their corresponding target genes to turn them on or off in all examined organisms, we speculated that these small RNA-Piwi switches will also play a key role in controlling gene activity in human embryonic stem cells, which in turn define these cells’ ability to self-replicate and to produce different types of body cells. To test that, I am developing the antibodies, which can recognize four Piwi proteins in the cells. We will then further study what genes in stem cells are bound and controlled by the piRNA-Piwi complexes (Aim 3), which will allow us to use specific RNAs to switch on and off different genes to control the stem cell behavior for medical applications in the future.

• In order to capitalize on the potential of embryonic stem cells (ESCs) to treat human injury and disease, researchers will have to develop better methods of gene replacement in order to obtain hESCs suitable for gene therapy. One problem hindering hESC technology is the low frequency of gene targeting. Our research focused on monitoring the effect of viral proteins on the frequency of genetic exchange events in various human cell lines, mouse ESCs and on adapting the techniques for use in hESCs. We have shown that expression of a single viral protein can increase genetic exchange events. Furthermore, the cellular pathway that was increased uses a mechanism to exchange segments of DNA reminiscent of that used by bacterial viruses such
as lambda. Interestingly, lambda has evolved a mechanism to exchange genetic information that has also been used to enhance gene targeting in bacteria. Thus, it is possible that viral proteins could be used as a tool to stimulate gene targeting leading to more effective strategies for gene therapy.

- Hematopoiesis is the process of differentiation of all blood cells from hematopoietic progenitors located in the bone marrow. Due to obvious technical and ethical constraints, the study of human hematopoiesis is currently restricted. To circumvent these limitations, small-animal models have been developed in which human hematopoietic progenitors are transplanted into permissive recipient mice. However, the usefulness of these models is limited by two main factors:

  - The mouse environment is different from human. Human hematopoiesis is not optimal in this environment and only partially mimics normal human physiological conditions
  - The low availability of human hematopoietic progenitor cells for transplantation prevents these models from being largely adopted by the scientific community.

In this project, we propose to develop novel models of recipient mice that would provide a better environment for human hematopoiesis. We will then use these recipient mice to optimize protocols of generation of hematopoietic progenitors derived from embryonic stem cells. This would provide an unlimited source of cells and would contribute to make this model of “humanized mice” available to a larger community of researchers.

During our second year of activity, we generated and characterized novel strains of recipient mice which represent highly improved recipients to support human hematopoiesis. We are now using these strains to transplant hematopoietic stem cells derived from embryonic stem cells.

- The grant was designed to look at how human embryonic stem cells become neurons, and how they become dopaminergic neurons – those lost in Parkinson’s disease. We discovered that an mRNA-binding protein called was highly expressed in hESC and its expression increases when these cells divide. We haven’t completed the analysis yet, but we are examining what genes are regulated by this mechanism. This will give us a better understanding of how cell division is regulated in stem cells. This may also give us important new clues as to how we can direct these cells into the neural cell fate. Once we understand this process, we can more easily direct cells into a dopaminergic neuron fate, in the hopes of treating Parkinson’s with these new neurons.

- We developed a method for identifying the expression of genes related to the differentiation of beta cells from human embryonic stem cells. As part of this work we also brought the methods for differentiation of human embryonic stem cells into insulin+ cells into the lab.

- We were able to induce neural crest cells in unprecedented short time, in large numbers and high purity. We are now able to generate human neural crest cells in just 5 days with the use of a simple growth condition in serum free media. These cells are critical for human development as they give rise to many facial features including muscle, bone and cartilage, dentin forming cells, amongst others. Many human malformations cancers and
syndromes are based in failed development of these important cells. Generating them form human embryonic stem cells will allow a better understanding of the afflictions they are involved in and will likely lead to important therapeutic and preventive approaches.

- **Human embryonic stem cells (hESCs)** represent an excellent tool for scientists to learn about how we develop in the womb. These cells are also very useful for applications in tissue engineering and drug screening. Much research is focused on differentiating hESCs into pure populations of different cell type. We propose to develop approaches to efficiently induce hESCs into blood cells in a system that is free of any non-human products. This has never been done before, but it is important to remove animal serum and animal cells from the hESC growth conditions so that the cells can be used for humans in the future. In these two year project, we established a protocol to differentiated hESCs into primitive blood cells in feeder free serum free conditions.

- **mTOR** acts as a molecular switch for lineage expansion in the postnatal sub ventricular zone.

Our work indicates that activation of an intracellular molecule, the mammalian Target of Rapamycin (mTOR) in quiescent neural stem cells of young adults leads to the de-novo generation of immature neurons. mTOR is a highly conserved intracellular signaling hub in all animal species examined and regulates the machinery to synthesize proteins.

Targeted neural stem cells are located in a niche called the sub ventricular zone along the lateral ventricle, which spans the entire forebrain. Although they persist in all mammalian species examined including humans, they are quiescent and do not or rarely generate neurons in adults. As a result, generation of new neurons for brain repair following an insult is limited. Our results suggest that up-regulation of the activity of mTOR is sufficient to activate quiescent neural stem cells leading to the generation of fast dividing daughter cells and immature neurons. Our findings suggest a potential effective way to activate neural stem cells and generate new neurons for endogenous neuron replacement following transient mTOR activation.

- **Human embryonic stem cells (hESCs)** have the properties of unlimited growing, and present an excellent model to understand the molecular mechanisms of early development of human being. Since human beings can never be used for in vivo mutagenesis studies, gene modification in hESCs is a valuable approach to reveal gene function in vitro. Unlike mouse embryonic stem cells (mESCs), hESCs are not easily amenable to manipulation, which makes hESC gene targeting a challenging and laborious process. Here we seek a more practical way to generate long arm (about 10k) targeting vectors based on recombineering in E. Coli. We also aim to improve the delivery of targeting vectors in hESCs as well as the cloning efficiency. Our goal is to set up a platform for highly efficient modified the genes in hESCs.

- This project will lay the foundation for preclinical studies of stem cell therapy for human hearing disorders, including tinnitus, due to damage of the cochlear ganglion, commonly caused by noise, drugs, infections, and aging.

- We published two high quality papers.

- Hematopoietic stem cells (HSCs) are the earliest precursors of all of the cells in the
blood. Hematopoietic stem cell transplantation is widely used in the treatment of cancer, aplastic anemia, and other diseases. Bone marrow and umbilical cord blood are the major source of hematopoietic stem cell transplantation. However, hematopoietic stem cell transplantation is frequently limited by the inability to obtain sufficient numbers of HSCs from these sources. Therefore, the evaluation of alternative sources of cells for hematopoietic stem cell transplantation remains an important goal.

Given that human embryonic stem cells (hESCs) have the dual ability to propagate indefinitely in vitro in an undifferentiated state and to differentiate into all three germ layers, it is likely that hESCs can serve as a prime source of HSCs for hematopoietic reconstitution. By doing a project funded by CT Stem Cell Program, we have produced a new protein that can enhance the generation of HSCs from hESCs. Our studies not only provide a new reagent with which to enhance the generation of blood cells from hESCs, but also provide new insights into how the reagent works in the processes.

- Many studies have suggested that human embryonic stem cells (hESCs) offer enormous potential for treating a variety of degenerative diseases. However, one of the major challenges in ESC-based therapies is that ESC-derived tissues can be rejected by the host immune system. We will induce immune tolerance to ESC-derived tissues by using the same tolerance mechanisms that occur normally in human body. Our studies will not only provide new insight into the mechanisms of tolerance induction, but also have potential clinical applications in overcoming immune rejection of hESC-derived cells and tissues.

- The generation of patient-specific induced pluripotent stem (iPS) cells is a critical step toward understanding the molecular basis of any particular human disorder. This proposal was designed to investigate the epigenetic status of Williams syndrome-derived iPS cells. Williams syndrome (WS) is a complex disorder with distinctive features including craniofacial defects, mental retardation, microcephaly, and short stature. Recent findings of WS patients have pointed to GTF2I as the prime candidate gene responsible for these clinical features. Gtf2i inactivation in mice causes craniofacial, neural tube and skeletal defects. The TFII-I transcription factor, a product of GTF2I, regulates a specific subset of epigenetic modulator genes. Therefore, TFII-I deficiency might disturb embryonic developmental program by changing the histone code of target genes at very early stages of development. We hypothesize that TFII-I is required for maintaining the correct spatial and temporal expression of a specific subgroup of epigenetic marker genes. As a result, TFII-I modulates epigenetic marking-out that is necessary in normal development. We characterized the epigenetic profile in iPS cells derived from the Williams syndrome patients (WS-iPS) and defined the molecular mechanisms of gene regulation in embryonic stem cells after TFII-I ablation. Collectively, combined computational and genomic studies have significantly increased our understanding of the epigenetic marking-out in stem cells and explained some of the WS features. Moreover, this study is going to help to explain the complex human pathologies caused by the large chromosomal deletions and pave the way for discovery of novel clinical therapies.

- Extensive research has been conducted in recent years on signaling, epigenetic, transcriptional, and posttranscriptional mechanisms that regulate stem cells. However, no work has been reported on the regulation of DNA replication of stem cells and on how it may impact on the potency, self-renewal, or differentiation of stem cells.
It is known that maintaining genomic integrity is essential for stem cells. During S phase, the genome must be replicated accurately, completely, and only once. DNA replication initiates from numerous origins licensed before entry into S phase. My research shows that there are many dormant replication origins in mouse embryonic stem cells (ESCs). ESCs use these excess origins to protect genomic integrity by initiating them to rescue DNA replication when forks stall. My results reveal a novel mechanism that ESCs utilize to safeguard their genome. When ESCs differentiate, they license fewer origins and become more sensitive to replication inhibitors. Furthermore, when dormant origins are partially depleted, ESCs are compromised in differentiation but not proliferation. My results reveal a differential sensitivity towards dormant origin depletion between ESC self-renewal and differentiation, likely due to decreased origin licensing and elevated DNA damage response as stem cells differentiate. This makes ESCs better protected against replicative stress.

These findings reveal a novel regulatory mechanism of DNA replication in ESCs to protect the cells against replicative stress and in ensuring ESC genome integrity. Furthermore, this mechanism is differentially regulated during ESC differentiation. My research opens a new territory of stem cell research and lays a foundation for all future studies on DNA replication of ESCs.

- Our research is still an on-going project. We expect that it will result in significant progress in the field of stem cell differentiation, especially, towards the hematopoietic cells via genome-scale delineation of the molecular mechanisms governing the formation of heterogeneous lineages. Although this study does not directly yield a protocol for hematopoietic differentiation, it will instruct the future development of new approaches for directed differentiation of human pluripotent stem cells towards functional cells for disease treatment. Thus, its impact is even much broader and very important to the entire field. Upon the completion of this project, we anticipate two major breakthroughs. First, single cell genomic, epigenomic or proteomic signatures will, for the first time, be generated to delineate the heterogeneous differentiation of human pluripotent stem cells toward hematopoietic cells. Second, an innovative single cell analysis technology will be developed and can generate far-reaching impact to stem cell research.

- Arthritis, the degeneration of joint cartilage, is the leading cause of disability in the US. Despite the high prevalence of osteoarthritis (OA), current treatments are ineffective. In addition to a decreased quality of life, OA imposes a significant economic burden in the state of Connecticut. Research in our lab aims to develop novel strategies to treat or reverse cartilage degeneration. We generated compelling evidence that a patient’s own cartilage cells may be used to develop therapeutic strategies to regenerate cartilage tissue. Using human cells isolated from normal and OA cartilage, we generated several lines of “induced pluripotent cells” (iPS cells) that can form any cell type. Our extensive analyses indicated that the novel iPS cells from cartilage bear a superior capacity to generate cartilage cells as compared to iPS cells from skin. Our published studies further described new efficient methods to generate cartilage cells from iPS cells. From a translational perspective, these studies represent a critical first step towards identifying the most suitable stem cell source for the regeneration of joint cartilage tissue.

- We are currently preparing three papers for submission to high impact journals, most in large part done with funds from the CT Stem Cell Research Grant. Without
this funding, my research would likely have taken an entirely different direction. Specifically, we will publish on the fundamental mechanisms of stem cell maintenance and how they can be co-opted under pathological conditions.

This has been critical to our more recent investigations of pediatric brain tumors, one of the leading factors in pediatric and adolescent mortality. It is believed that our basic research will have significant implications for both the mechanisms of tumorigenesis and also for the generation of pure populations of cells for transplantation in the case of regenerative medicine.

We are in the midst of preparing drafts for several manuscripts which we believe will be scientific breakthroughs. Specifically, we have identified a molecule critical for stem cell maintenance--Dynamin--as well as a novel cellular substrate of Notch signaling--the filopodium. Furthermore, we will provide evidence to support previously unproven hypothesis in mammalian systems.

- The research in our laboratory on human embryonic stem cells focuses on the question of how a new class of molecules, namely tiny bits of RNAs, controls the cell fates of stem cells. We are researching on new ways that can utilize such tiny RNAs to make useful cells from stem cells. We have made several important discoveries, both on how stem cells work and on new methods that can help the community to further stem cell research. These discoveries have generated 4 manuscripts (published, in submission or in preparation). For example, we have found a new way to use such tiny bits of RNA to make more blood cells from stem cells, and powerful new methods to analyze the function of such small RNAs in stem cells. These progresses have helped to gain insights on how to direct stem cells to become cells useful for human health, which potentially can be used as new therapeutics. These are all breakthroughs that are only possible with the state funding.

- Our research focuses on potential applications of stem cells in the field of neurology and we are developing stem cell-based therapies for treating intractable seizures in temporal lobe epilepsy. We use mouse models of temporal lobe epilepsy and generate specific types of neurons from mouse and human embryonic stem cells (ESCs). We transplant the neural progenitors derived from ESCs into the affected brain regions in mice with epilepsy, then monitor the incidence of seizures using electroencephalography (EEGs) for periods of up to 100 days to show the disease-modifying effects of the stem cell grafts. These studies are complemented by electrophysiological and histological analysis examining the connections formed by the transplanted neurons, the number of cells that survive long-term, and whether they replace cell types injured by the epileptic seizures. The research funded by this grant led to five published journal articles and 3 book chapters. The funding also allowed us to train over 10 undergraduate students, a postdoctoral scientist and 3 research technicians, and strengthened a multi-laboratory collaboration in this area.

- A major advancement in the field of stem cell research over the past 5 years has been the ability to generate human neural cells in the laboratory by generating induced pluripotent stem (iPS) cells from skin fibroblasts. We are examining whether this technology can be used as a model system to study the biological effects of alcohol on human neurons to better understand the processes leading to alcoholism. We have generated iPS cells from 16 subjects, 8 from controls and 8 from subjects with alcohol
dependence. Initial results comparing neurons from these subject specific samples, indicates that there may be greater molecular adaptations in excitatory neurotransmitter systems in neurons from alcoholics in response to the repeated exposure to alcohol in vitro. We are additionally investigating the use of this model system to understand the biological effects in human neurons of genetic variations that have been reported to be risk factors for alcoholism. Use of iPSC cell technology may allow scientists to better understand why tolerance to alcohol develops in some but not all persons at risk for alcohol use problems and to better inform the treatment of alcohol use disorders.

- In 2006 I was awarded funding for a seed grant to investigate a novel embryonic stem cell based procedure to generate pluripotent stem cells. Only 4 months after commencement of the award both the Yamanaka and Thomson groups published landmark papers on the production of pluripotent stem cells for which Dr. Yamanaka received the Nobel prize the year. I therefore changed my research direction and established Dr. Yamanaka’s procedures for reprogramming of donor cells. Importantly, I was able to utilize this technology and establish state of the art procedures for the generation of donor specific liver cell like cells, a resource to my knowledge unavailable up to now in Connecticut and critical for the development of transplantable liver tissue and the study of drug and toxin induced idiosyncratic liver damage. This research is currently funded as a sub project in a disease directed group grant under the leadership of Dr. Boelsterli. I expect this research to have significant spin-offs, both through attracting federal funds and commercialization. This advance would have been impossible in the absence of the initial seed grant.

- A micro culture system can reduce the expenditure of precious biochemical reagents used in cell culture experiments and provide opportunities to investigate the highly transient and localized cellular responses to environmental change. The greatest advantage of a microculture system is the ability to regulate local culture conditions. We developed a microculture system to provide rigorous control of flow shear stress and mass transfer rate for the delivery of nutrients to growing mouse embryonic stem cells. The initial goal for the study, self-renewal of embryonic stem cells in a microculture system, was achieved. We have successfully sustained the growth of primary mouse embryonic fibroblasts and mouse embryonic stem cells in the system and demonstrated that a perfusion-based microculture environment is capable of growing and maintaining embryonic stem cells as pluripotent cells. The optimal perfusion rate was found to balance the nutrient renewal and waste removal while ensuring the presence of cell secreted factors. The methodology and parameters established in this study are essential for further investigation of regulatory controls required for the differentiation of human embryonic stem cells or iPSC cells in a microenvironment.

- Skin cells must be renewed throughout our lifetime to replace those lost to wear and tear as well as in wounds and burns. Skin stems cells are the replicating population that accomplishes this replacement and we studied the effect of natural and clinically used compounds to improve this regeneration. We were able to identify the target inside of cells for those compounds and prove which compounds were most successful. This basic research may ultimately help in the selection of compounds for clinical use in helping wounds heal or dietary improvements to maintain skin health.

- The CT Stem Cell Research Grant is a seed grant that supports pilot research using human embryonic stem cells (hESCs) to understand the effects of Huntington’s Disease
(HD) on brain cells, and to find potential treatments to slow progression of this severe disorder that kills brain cells. The stem cell research funding allowed one year of salary support for a full-time post-doctoral fellow beginning September 2012. Another part-time post-doctoral fellow, who is enrolled in a degree-seeking master’s translational research program, also recently joined the laboratory because of the exciting potential of this research project. Already since September 2012, progress has been made in characterizing the HD mutant stem cell lines, and the resulting unpublished data will definitely advance the field beyond the current knowledge of HD. We are in the process of obtaining more data so that a complete paper can be prepared for publication, which will contain key scientific ideas that have yet to be considered by other scientists in the field. This research would not have been possible without the support of the CT Stem Cell Research Grant, especially given the uncertain climate for supporting research, particularly with non-federally approved stem cell lines. It is important research, worthy of state funded investment, because any breakthroughs in understanding HD leads to breakthroughs in understanding other disorders of protein aggregate toxicity, such as Alzheimer’s Disease, Parkinson’s Disease, and Amyotrophic Lateral Sclerosis (ALS). All of these disorders cause brain cells to die over time, because cells lose the ability to get rid of toxic proteins efficiently as people get older.

- Our basic stem cell research has potential to have many positive effects to the State of Connecticut. After less than one of year of funding, we have applied a grant from the National Institute of Health, which totals approximately 3.7 million. In addition, a patent generated from the work is being considered which may benefit the UConn Health Center and the State of Connecticut. Although still early in the grant funding from the State of Connecticut in its first year, a number of potential payouts from this proposal are being considered.

- The importance of state funding cannot be overestimated. In my case, we are testing a completely novel theory - that there are fundamental constrains on stem cells in mammalian tissues that prevent them from repairing damages that go beyond the normal wear and tear - and, without state funding, we would not have been able to move our research forward. With state funding, we are now at the point where we have obtained sufficient evidence to seek funding from other sources. We believe understanding this mechanism is essential for using stem cells therapeutically to repair tissues damaged by disease, injury or aging.

- 1. In the first funded project, Project 7 accomplished its goal to develop a method to direct the differentiation of hESC and iPSC into the chondrogenic lineage. This was a key finding for the field. The method is superior to other methods. The studies resulted in 1 publication and 1 submitted manuscript and the method is the subject of a patent application by the University of Connecticut, and the creation of the UConn-affiliated CT-biotech startup company (Chondrogenics, Inc.), which is the first company to come out of the CT Stem Cell Program.

- 2. The second funded project is in progress. This project is awarded to Chondrogenics (Dealy, PI) with a UCHC subcontract. The project goal is to develop the commercial potential of a stem cell based therapy for osteoarthritis and cartilage injury. In its first year the project is evaluating the efficacy of the hESC/iPSC derived chondrogenic cells for in vivo repair of OA damage in a small animal model. In its first year the project generated 1 publication, 1 book chapter and 1 editorial. The project is an important
example of how the CT Stem Cell Program has led to new discovery with direct application to a human health problem, and to creation of economic growth in the state through founding of a new CT biotech company.

- Our main goal in this work is to determine the cause of a form of childhood leukemia called Acute Megakaryoblastic Leukemia that as a very poor prognosis. The cells that become leukemia in this disease are called megakaryocytes, and their normal job in the body is to produce platelets, which are necessary for preventing bleeding. In order to better understand what goes wrong to cause this leukemia to occur, are determining the differences between normal non-leukemia cells and the leukemia, and we are assessing the changes that occur in cells when the mutations that cause leukemia are over expressed. In our recently published papers, we elucidated, for the first time, two novel mechanisms underlying how these cells normally develop, and how the cells become more proliferative (like leukemia) when key genes are mutated. Our findings now allow us to make a link between the causes of leukemia and normal cell development. By identifying the mechanisms that are altered in leukemia, we can identify novel targets for treatment of this leukemia.

- Through our basic research on stem cells we have learned a number of things which have helped us to understand how these cells work and how they develop into other cells. Our research funded through the State of Connecticut has led to a number of major publications, some of which are highly cited in the field. Our most recent publication supported by these funds involves the discovery of a new type of molecules in stem cells that might not only be important for stem cell function, but which also appear to be implicated in an important human disease, Prader-Willi Syndrome. Thus, this research has not only led to advances in our understanding of basic stem cell biology, but may eventually lead to translational benefits in the treatment of a human disease.

- Parkinson’s disease has been a widely attempted application for stem cell therapy. It is promising because the immediate cause is the loss of dopamine neurons in a small area of the brain which can be compensated by treatment with a dopamine precursor. But this drug, L-Dopa, often produces debilitating side effects and the benefits wear off. Replacing the dopamine neurons using fetal brain tissue has been promising in rodents, and had some variable benefits in human trials. Instead of using aborted fetuses, creating the same cells by differentiation of embryonic stem cells would provide a more reliable source and move us closer to a clinical treatment. Our project, which could not have been funded under existing US rules, has therefore successfully derived neurons from human embryonic stem cells and shown their survival and integration into the brain of immuno-suppressed monkeys. We have acquired critical information about how to convert them into dopamine neurons. Next we will attempt to derive and show functional benefits of reprogrammed skin cells, which should be superior by not involving embryos, and should also be immune compatible with the patient.


BDNF-hyper secreting human mesenchymal stem cells promote functional recovery, axonal sprouting, and protection of corticospinal neurons after spinal cord injury.

• Using our CT state stem cell seed grant funds we have refined our knowledge of the
human adipocyte progenitor populations in vivo. These cells will provide superior
starting materials for regenerative medicine applications. Additionally, identification
of these cells will allow us to focus future studies on how these cells are regulated
in obesity and metabolic disease, potentially leading to the development of novel
therapeutic strategies.

• We used a new analytic method developed in the laboratory to study a type of
modification of cells that is used to regulate many important cellular processes. This
modification, termed “tyrosine phosphorylation”, is often used to signal cells to grow
or to change into more specialized cell types. We found that in human embryonic stem
cells, this type of modification is not nearly as prevalent as in other more specialized
cell types. Through biochemical experiments, we found evidence that this low level of
tyrosine phosphorylation is due to low levels of activity of the proteins that perform the
modification (these are called tyrosine kinases). This led us to explore how different cell
types, including stem cells and some cancer cells, are very different in how quickly they
add and remove phosphate groups from proteins. This is likely to be an important and
previously unappreciated form of regulation, and we have preliminary evidence that
this suppression is critical for maintaining stem cells in a state that can go on to form
many different cell types. While it is early to tell if this is a truly major breakthrough, it
has important implications for how we might treat stem cells and manipulate them for
regenerative medicine.

• Based on the CT Stem Cell Funding, we can start our new lab in CT, and focus on basic
epigenetics research on mouse and human ES cell from beginning. We have several key
scientific breakthroughs in the ES cell field.

• We have established a new and efficient method to modify human embryonic stem
cells. A cell line is licensed to Pfizer Inc. Furthermore, our interactions have led to the
ongoing collaboration between my lab and the Primary Pharmacology Group in Pfizer,
including funding from the company to develop new methods. These techniques
are important for the ongoing project, which aims to create a cell-based model for
Parkinson’s disease.

• We generated Angelman syndrome (AS) and Prader-Willi syndrome (PWS) iPSCs
from patients’ skin cells. We proved that the iPSCs were stem cells and that they had
normal chromosomes, except for the mutation that leads to AS or PWS. We determined
that DNA methylation in the critical region was correctly maintained through the
reprogramming process in both AS and PWS iPSCs. We then converted the iPSCs into
neurons (brain cells) and determined that the gene expression patterns that are neuron-
specific are observed in our iPSC-derived neurons from AS patients. We showed that
PWS iPSCs are lacking gene expression from all of the appropriate genes and that
neurons made from AS iPSCs lack the appropriate protein. We also proved that the
neurons are functional and can send and receive signals. Together, these findings were
the first iPSC model of a human imprinting disorder. We are currently using these
models to test two different potential therapies that may cure AS.

• The overall goal of our research is to better understand (and ultimately predict) the
individual susceptibility factors in drug-induced liver injury (DILI). DILI is a major
clinical problem that also hampers the development of new drugs. The underlying
mechanisms are largely unknown, and the susceptibility factors are highly dependent
on the genetic background of the susceptible patients. With the use of patient-specific
induced pluripotent stem (iPS) cells that can be redifferentiated into liver cells
(hepatocytes) we hope to be able to assess the functional changes and mechanisms of
the toxic response of these individual liver cells to DILI drugs and to identify key genes
that are altered. We will first focus on a number of antibiotics that have caused clinical
DILI. This is a new project - no data yet.

• The key objective of our research is to generate effector T cells from human pluripotent
stem cells [hPS: human embryonic stem cells (hES) and induced pluripotent stem cells
(iPS)] for generating customized anti-tumor T cells through TCR engineering approach.
Our research is presently in progress. We are pleased to share that we have made
significant progress towards generating hematopoietic stem cell (HSC) precursors from
hPS cells. Interestingly, we have discovered that the hPS derived embryoid bodies (EB)
can facilitate an in-situ differentiation of HSC precursors. These are novel findings.
We are currently confirming our preliminary observations and are working towards
defining the mechanism of this process. We strongly believe that our findings would
provide a better understanding of T cell generation from hPS cells and would also lead
to novel discoveries of immense translational implications for cancer immunotherapy
field. Our work would not have been possible without the state funding, and our
progress demonstrates that we are effectively utilizing the funds for establishing a
productive research program of immense translational potential.

• 1) established hESC lines with lentivirus-based, drug-inducible knockdown of
Caspase-3 via shRNA
2) found that Caspase-3 knockdown indeed inhibits BMP4-induced hESC
differentiation. In addition, we have optimized the hemangioblast-HSC differentiation
system using feeder-free, serum-free, and defined system with stable and much
enhanced differentiation efficiency.
3) Found caspase-3 knockdown can affect hematopoietic stem cell differentiation
4) Found new method to differentiate hESc to mesenchymal stem cell
5) using hESC-MSC to treat multiple sclerosis with better effect than bone marrow
derived MSC.

Based on the research, we are going to commercialize the hESC-MSC to cell therapy
product, which will benefit millions of patient in the future.

• The proposed project will establish a paradigm to generate currently unavailable
human cortical motoneurons from limitless pluripotent stem cells derived from normal
and SPG3A patients, which will offer an otherwise inaccessible tool for studying the
birth, maturation, and degeneration of human cortical motor neurons. Moreover, the
specific mechanisms underlying the axonal maintenance and degeneration observed
in these cells may provide insights into other common neurological diseases involving
axonopathy, such as peripheral neuropathies, multiple sclerosis and motor neuron
disease. These cells will also provide a target for screening pharmaceuticals that stop
the process of motor neuron degeneration and a source for potential future cell therapy
for cortical motor neuron-related diseases.

• Our long-term goal is to use embryonic stem cell-derived neural progenitors to
suppress seizures and cognitive and behavioral deficits in mouse models of temporal
lobe epilepsy. These studies could set the stage for relevant clinical applications.
Epilepsy is associated with the loss of inhibitory interneurons and we hypothesize transplanted stem cells can replace them. We asked: can embryonic stem cell-derived neural progenitors survive, differentiate into neurons, migrate, function, and hook-up to host circuitry when transplanted to the dentate gyrus of the hippocampus, the site of damage in epilepsy?

Scientific advances

1. Mouse embryonic stem cell-derived neural progenitors can replace the upper blade of the dentate gyrus of the hippocampus in a fluid injection lesion model, becoming granule neurons. This observation demonstrated the ability of the embryonic stem cell-derived material to survive and respond to cues in the environment that direct their maturation.

2. The precise location of the transplanted cells determines their fate: they become neurons in the dentate gyrus where they are surrounded by neurons, and oligodendrocytes in the fimbria where they are surrounded by oligodendrocytes.

3. Transplanted neural progenitors can migrate within the hippocampus using a specific chemical signal, CXCL12. We can use this information to target the cells to specific sites.

4. A major concern with embryonic stem cell-derived material is the formation of teratocarcinomas. We showed that isolating neural progenitors away from the undifferentiated stem cell population decreases the rate of tumor formation.

5. Transplants that replace the dentate gyrus upper blade are vascularized, suggesting a role for blood vessels in transplant survival and differentiation.

6. The transplanted cells migrate on the surface of blood vessels within and outside of the graft.

7. Using a pilocarpine-based mouse model of temporal lobe epilepsy, mouse embryonic stem cell-derived neural progenitors transplanted to the dentate gyrus become inhibitory interneurons that fire appropriately and hook-up to the host circuitry. These exciting observations demonstrated the ability of the stem cell-derived material to function as the endogenous neurons do, bringing us a step closer to effective reversal of seizures and their aftermath.

8. We have developed a direct and rapid protocol for generating neural progenitors from human embryonic stem cells. This represents a significant advance over previously published methods.

9. Using a specific reporter cell line (NKX2.1:GFP) and treatment with the signaling molecule Sonic hedgehog, we are able to produce and isolate large numbers of interneuron progenitors from human embryonic stem cells. This represents a major advance in the field, as interneuron progenitors will be useful in the treatment of a number of neurological conditions.
10. Preliminary transplants of the human embryonic stem cell-derived interneuron progenitors demonstrate the cells survive and can differentiate into interneurons in the mouse dentate gyrus. This represents a major advance, as many groups have reported difficulty both in obtaining this cell population and in seeing the cells survive following transplant.

Why the research is worthy of state funding

1. Human embryonic stem cell-based research has great promise for understanding and treating a wide variety of diseases and should not be subject to the changing winds in Washington for funding.

2. There is great value in creating a hub for stem cell research in the state for economic development and jobs creation.

3. As shown in the accompanying data, we have generated jobs, trained skilled workers, and attracted skilled workers and leaders in the field to the state.

4. In our collaborative efforts at Wesleyan, we have trained a number of graduate students who have gone on to postdoctoral and faculty positions at Yale and the University of Connecticut Health Center. These individuals remained in Connecticut after their training in large part due to the Stem Cell Initiative.

Publications related to the funding:


stem cells in experimental models of neurodegenerative diseases. In: Stem Cells and
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Maisano, X; Litvina, E; Aaron; Grabel,L; and Naegele, J. (2012) Differentiation and
Functional Incorporation of Embryonic Stem Cell-Derived GABAergic Interneurons
in the Dentate Gyrus of Mice with Temporal Lobe Epilepsy. Journal of Neuroscience

formation in embryonic stem cell-derived neural progenitor (ESNP) hippocampal

Stem Cells Reviews and Reports, in press.

Grabel, L. (2011) Prospects for Pluripotent Stem Cell Therapies: Into the Clinic and Back
to the Bench, Journal of Cellular Biochemistry, 113(2):381-387

- Nanog, Oct4, and Sox2 are the core regulators of mouse (m)ESC pluripotency. Although
their basic importance in human (h)ESCs has been demonstrated, the mechanistic
functions are not well defined. We identified general and cell-line-specific requirements
for NANOG, OCT4, and SOX2 in hESCs. We show that OCT4 regulates, and interacts
with, the BMP4 pathway to specify four developmental fates. NANOG represses
embryonic ectoderm differentiation but has little effect on other lineages, whereas SOX2
and SOX3 are redundant and repress mesendoderm differentiation. Thus, instead of
being panrepressors of differentiation, each factor controls specific cell fates. Our study
revises the view of how self-renewal is orchestrated in hESCs.

- Our research focuses on cartilage repair. This is important because a traumatic injury
of cartilage of the joint need to be repaired in order to avoid the occurrence of what
is called post-traumatic osteoarthritis. The state of CT has a significant population of
young and middle aged patients targeted by these injuries. One simple example is that
of our athletes who complete nationally at the college level. This is also useful for our
service men and women who get injured during routine exercise or on the battlefield.
Therefore, it is necessary to devise a strategy aimed at replenishing damaged cartilage.
The reason why we need to use cells to treat these injuries is because cartilage cannot fix
itself like bone does during fracture repair. We have shown that we can turn stem cells
into cartilage forming cells. Then we began establishing conditions for these cartilage
cells to resemble the cells that are embedded in human joints. To deliver cells into a
site of injury, one needs a vehicle. We are working with experts in tissue engineering to establish the best vehicle for these cells to fix cartilage defects. The candidate is a water-based gel that is naturally present in shellfish. One of our scientific breakthroughs is that we can control the progression of ES-derived cartilage cells using specific culture conditions. This is very important because the current treatment strategies lack the ability to make implanted adult stem cells make only cartilage of the joint.

- SEED GRANT: Dopamine releasing neurons can potentially be used for the treatment of Parkinson’s disease. Using the CT Stem Cell Funding, we adopted the technology for deriving dopamine neurons from human stem cells. We then characterized the physiological properties of such neurons. Our results are published in Stem Cell & Development (Belinsky et al., 2011). Next, we characterized dopaminergic signaling in stem cell colonies, and stem cell-derived neuroprogenitors and neurons. In addition, we tested if dopamine can improve the number and quality of derived neurons (paper under review).

CORE GRANT: Across the state of Connecticut there are more than 20 laboratories currently working on experimental procedures to derive neurons from stem cells. A critical step in this type of research is the evaluation of neuronal identity and neuronal functionality by direct electrical recordings. Two main factors have prevented the CT Stem Cell laboratories to pursue physiological measurements: (1) Lack of equipment; and (2) Lack of expertise. To overcome these obstacles, we built a core facility that provides both the necessary equipment and experimental skills to perform electrical and optical measurements from young neurons.

- Chronic sublethal hypoxia caused by premature delivery is a significant cause of neurodevelopmental handicaps among low birth weight premature infants. Patient IQs improve variably over time and recovery from neurodevelopmental handicaps is thought to depend upon the responses of distinct neurogenic regions and neurovascular niches (the subventricular zone and the subgranular zone), and are differentially responsive to insult and stimuli. Interestingly, significant improvement in academic functioning over time in this population has been reported, but the repair/recovery mechanisms involved are not yet understood. The variability of the improvement requires a better understanding of the mechanisms involved in modulating neurogenesis occurring in the subventricular zone (SVZ) in order to providing a greater and more complete recovery. With this in mind, we used a murine animal model to mimic and therefore study the chronic sublethal hypoxia of the premature newborn. Our previous data showed that CD1 postnatal day 3 (P3) pups survive a 30-day exposure to hypoxia (9.5% O2), while C57BL/6 pups expire at day P13 under similar conditions. Our in vitro study showed that CD1 neural stem cells (NSCs) exhibit higher proliferation in SVZ compared to C57BL/6 pups in both baseline and hypoxic insult, while C57BL/6 NSCs exhibited significantly increased apoptosis after hypoxic insult, indicating that CD1 NSC survival & self-renewal ability is greater than that observed in C57BL/6 NSCs. Following a P3 to P11 hypoxic insult, murine behavior activity measurements showed that C57BL/6 pups exhibit hyperactivity while the CD1 pups do not, suggesting a correlation of behavioral recovery with SVZ NSC self-renewal and survival capabilities of these two mouse strains. We also demonstrated the role of glycogen synthase kinase 3 beta (GSK-3b; a multifunctional kinase expressed in all eukaryotes whose activity is inhibited by serine and stimulated by tyrosine) as a signaling node in the two mouse strains.
• We have made significant progress in several important aspects of the proposed study during the project period. First, we have begun a more systematic approach to elucidate the signaling pathway that is critical for glial differentiation by performing a high throughput screen for compounds that promote glial differentiation and increase transcription of the key transcription factor Olig2. In addition to the proposed transplantation experiments, we have successfully generated a transgenic mouse line that allows one to ablate NG2 glial cells and study the consequences on axonal growth during development and after injury. The findings from these studies will be used in future experiments to provide a better method for promoting glial differentiation from stem cells and for utilizing manipulated NG2 glial cells to promote axonal regeneration.

• The funding helped us to define the conditions under which we could obtain smooth muscle cells from embryonic stem cells, and thereafter from iPS cells. In addition, it showed the feasibility and drawbacks of using ES-derived or iPS-derived cells for vascular regeneration in vitro.

• A goal of this project is to determine if over expression of particular transcription factors, such as Sox5 and Satb2 will result in specification of particular deep and upper cortical layer specific neurons. We have tested the effect of over-expression of Sox5 or SATB2 transcription factors on generation of layer specific neurons from pluripotent human cells (hESC and iPSC), compared the effect of transcription factors at two distinct stages of neuronal progenitor cells: early and late neuronal progenitor cells.

We have discovered that there is a particular time window which allows cell fate specification, based on expression of layer-specific transcription factors in human embryonic stem cell- derived neuronal progenitor cells. It is not clear however whether increased expression of transcription factors will influence their projections. To address that issue, transplantation experiments proposed for second year of the projects are under way.

Project funded to Joseph Loturco, in which laboratory I am performing my research, which was focused on the effect of doublecortin on migration potential of hNP was published in Stem Cells journal this Year, and I am first author. Manuscript from my own project is presented in SFN meeting and is in preparation. These findings will improve understanding of timing of development of cortical neurons and ways to facilitate generation of parivicular subtypes of cortical neurons.

• This 4-year project with 7-month no-cost extension (March 2, 2007–Dec. 31, 2011) aimed to reveal how human embryonic stem cells (hESCs) are regulated by molecular signals to determine their early fates. Specifically it focused on two groups of antagonizing signaling molecules TGFβ and BMPs.

We completed all the three aims proposed in the grant. (1) We screened for target genes of the TGFβ and BMP signaling in hESCs via collaboration with Dr. James Thomson of University of Wisconsin-Madison; (2) analyzed how several important target genes regulate the fate of both hESCs and human induced pluripotent stem cells (hiPSCs), another pluripotent stem cell type derived from adult cells; and (3) revealed how another signaling molecule bFGF prevents hESCs and hiPSCs from cell death, differentiated hiPSCs into functional neurons in the brain and spinal cord, and identified genetic variations in hiPSC lines during their derivation and culture.
These discoveries are important for understanding how to quality control and sustain human pluripotent stem cells, and how to tease them to become therapeutically desired cell types in response to cues. This knowledge is essential to promote both basic research and translational application of the stem cells.

This grant funded, fully or partially, jobs of 7 staff members including the PI, 4 postdoctoral fellows, 1 graduate student, and 1 visiting student, who together published 17 research papers.

- We have successfully established an hESC-based model for spinal muscular atrophy (SMA), which exhibits disease gene isoform specificity, cell type specificity, and phenotype reversibility. Our model provides a unique paradigm for studying how motor neurons specifically degenerate and highlights the potential importance of antioxidants for the treatment of SMA.

- We published a significant paper in the journal Differentiation that described methodology for using ES cells to begin to repair damaged intestinal mucosa (Cao et al., Differentiation, 2011).

- Bone marrow transplantation is a potentially life saving procedure for many cancer patients. One of the critical limiting factors limiting the application of this procedure is the identification of match donors for hematopoietic stem cells. Our research aims at deriving transplantable hematopoietic stem cells from human pluripotent stem cells including from patient specific induced pluripotent stem cells. If successful, every patient in need will be able to have access to at least one match donor stem cells. The benefit for patient care will be profound and will change the management for many cancer types.

One year into the funding, we have established protocols that produce hematopoietic progenitor cells with much higher efficiency. The derivation process and regulation is unique to human cells, as we have observed different controls in mouse systems. This knowledge would not have been obtained using any other systems.

- Our work is still in progress but we have made fundamental observations regarding the role of adhesion in stem cell differentiation and function that can be directly applied to mitigate current obstacles to stem cell therapy. A universal problem in tissue engineering/replacement with stem cells is obtaining enough cells of desired tissue type and once implanted, the body often rejects the cells as foreign. We have found that loss of a certain adhesion molecule promotes rapid, specific and abundant differentiation of mouse ES cells into skeletal muscle both in vitro and in vivo and have developed a reagent to block this adhesion that induces differentiation as well. Furthermore, we have also found that lack of this protein also allows cells engrafted into tissues to evade the immune system and thrive to repair function to the damaged tissue. Elucidation of these critical stem cell functions is fundamentally important to their future clinical utility.

- This research, only in its seventh month of funding, has not produced any major translational milestones. It is, however, on schedule, as milestones are being reached in accordance with the proposed timeline.
• Age-related macular degeneration is the leading cause of vision-loss among older individuals. There are few treatment options. One treatment in clinical trials is to transplant retinal pigment epithelium (RPE) derived from human embryonic stem cells. Although transplants in rodents retard the progression of disease, they fail to restore vision if the disease is advanced. To solve this shortcoming, my lab has made progress in engineering a culture model of the retina using human embryonic stem cells (hESC).

The key progress has been to develop a method for growing in one culture RPE and retinal progenitor cells, all derived from human embryonic stems cells. We confirmed earlier studies that hESC-RPE and hESC retinal cells differentiate independent of one another, but we are the first to demonstrate that co-culture furthers the maturation of both tissues. Further, we developed a panel of genes to screen protocols for their ability to promote maturation. This ability will allow us to study how RPE-retinal interactions are established, test drugs that might augment or eliminate transplant surgery, and serve as a transplantable tissue for late-stage disease. SC-RPE and hESC retinal cells differentiate independent of one another, but we are the first to demonstrate that co-culture furthers the differentiation of both tissues. Further, we developed a panel of genes to screen protocols for their ability to promote maturation. This ability will allow us to study how RPE-retinal interactions are established, test drugs that might augment or eliminate transplant surgery, and serve as a transplantable tissue for late-stage disease.

• The ability to culture and use undifferentiated human embryonic stem cells (hESCs) as a model for embryonic developmental decisions has great potential in basic research to define the mechanisms that generate skin cells and also for therapeutic purposes for treating skin diseases with patient specific tissue replacements. The skin is a particularly useful model for therapeutic use of ES cell therapies since skin grafting is quite efficient and tissue grafts can be generated ex vivo. However, a major challenge for ES based therapies is the ability to generate homogeneous cell populations that are specified to a specific lineage.

In our work funded by a CT Stem Cell Research Grant, we have successfully identified mechanisms that drive keratinocyte specification using hESCs. We have shown that manipulating these mechanisms can lead to the generation of keratinocyte progenitor cells. We not only are able to generate more keratinocytes from hESCs, but we also have defined the timing and mechanisms that control skin keratinocyte formation. The data generated by has illuminated how the development of human skin cells occurs and in the future will allow us to more efficiently generate differentiated cell populations for use in human therapies.

• The project has the goal of making new stem cell models for Alzheimer’s disease based on human neurons. We have obtained iPS cells (induced pluripotent stem cells) from Alzheimer’s disease patients and are making those cells into neurons through direct reprogramming. We are developing methods to measure changes in synaptic function. This system of cells should allow for the preclinical screening of novel drugs targeted to ameliorate the decay of synapses that occurs in Alzheimer’s disease.

• We developed and tested a method to enhance the ability of neurons made from human embryonic stem cells to migrate or move across biological surfaces and through brain tissue. Improving the ability of neurons to migrate or move through tissue may be essential for stem cell replacement therapies that will require cells to move away from
the place they are initially transplanted into. This work was published in the journal Stem Cells.

• While this work and grant are still in progress, we have achieved notable progress in our field. Derivation of new induced pluripotent stem cell lines from our disease of interest has allowed us to better understand the region. We have uncovered novel differences in regards to how the region is regulated between different classes of patients. We are hoping to uncover key genes whose expression is dysregulated in all classes to better understand the etiology of our disease. To date, very little is known about the deletion of a specific region on one of the chromosomes leads to our particular disease of interest. Animal models cannot completely recapitulate the disease phenotypes, especially since how the region is regulated differs between mouse and human. The development of human tissue culture models have allowed us to begin to understand how the region is regulated specifically in humans, and to uncover novel differences between different classes of patients. From these experiments, we plan to produce a list of genes that are specifically altered in our disease and gain a better understanding of the underlying causes. Ultimately, we plan to use our tissue culture model to screen for compounds that restore normal gene expression levels and set the stage for the development of new therapeutic strategies. State funding has been instrumental in our studies because iPSC technology is still in its infancy and we need to compare our tissue culture models to hESCs to ensure that they differentiation in the cell types of interest appropriately. There is variability in the individual clones from patients, and to interpret our results we need to compare them to hESC-derived cells to ensure that the differences that we observe are from the disease and not the reprogramming method, etc. With the ethical debates and current federal funding rates, this work would have a really hard time getting funded at the federal level.

• Human cells function based in part upon which of the 25,000 genes are turned on and which are turned off. In order to fully understand how stem cells work and to develop the power to turn human stem cells into different types of cells such as brain cells or muscle cells, it is essential to determine which genes are turned on or off in both stem cells and brain or muscle cells. Many studies have been conducted to determine this information for human embryonic stem (hES) cells as this will inform us about how hES cells have the amazing potential to generate all possible cell types. However, all of these studies have overlooked an important aspect of gene functions - a process called alternative splicing (AS). Alternative splicing is a process by which the quality and function of a gene that is turned on can be altered - essentially, each gene can come in a variety flavors or colors. So, it is not only important which of the 25,000 genes are turned on or off, but also which flavor or color of those genes that are turned on. These different flavors or colors, for instance, make a given gene function in different ways. AS is extremely important as ~95% of human genes come in a variety of flavors/colors. The main goal of this project is to determine the flavors and colors of the genes that are turned on in undifferentiated hES cells and in hES cells that are changing into different cell types, such as brain or muscle. This project will allow us to obtain a more thorough understanding of how hES cells function, which is essential knowledge for the long term goal of directing the differentiation of hES cells into specific cell types.

During this project we have developed technologies that allow us to which flavors or colors are being used for all 25,000 human genes at once. During the past year of the project, we have worked on analyzing the tremendous amount of data we
have generated using this technology. We have used this technology to analyze undifferentiated H9 and CT2 hES cells grown in three different conditions. In addition, we have used this technology to study H9 cells as they change from naive stem cells into functional brain cells. While we still need to fully analyze the data we have obtained, our results suggest that there are many differences in the flavors or colors of each gene when the cells are treated differently. This will have a large impact on the field as it is critical to understand how these cells are behaving and it is clear that how the cells are grown can make a considerable difference on their properties. Thus, a more thorough analysis of this process will significantly increase our understanding of how genes function in hES cells.

• We ran into technical difficulties with the human ES cells and were unable to do the intended experiments. We switched to a mouse and worm system instead and eventually published two papers on the topic. Our subject matter was very basic in nature and didn’t directly contribute to human health, but does increase our understanding of how stem cells in the germ line undergo cell division and meiosis.

• Lin28 is a protein that is abundantly expressed in human embryonic stem cells. We and other researchers have previously demonstrated that Lin28 plays an essential role in maintaining stem cell viability and helps to keep them from differentiating. In addition, Lin28 is able to facilitate reprogramming human somatic cells to induced pluripotent stem cells, or iPSCs, which has an important application in generating patient-specific iPSCs for tissue repair and even organ replacement in the future. We are interested in how Lin28 works. During the past several years of research supported by CT Innovations Inc., we have begun to figure this out. We now know that Lin28 controls the efficiency by which stem cells express some important genes by helping them produce higher levels of proteins. Without help from Lin28, the levels of the proteins encoded by these genes would be too low to maintain the normal growth and survival of stem cells. Proteins are made from messenger RNA molecules (mRNAs) transcribed from their respective genes. We have found that Lin28 recognizes and attaches to specific features in the mRNAs of the genes to help and improve their protein-producing efficiency. These findings not only shed new light on the fundamental biology of stem cells, but also will prove important for harnessing the power of Lin28 for regenerative medicine.

While we were working with hESCs, we unexpectedly discovered that Lin28 also has important roles in cancer stem cell function. Cancer stem cells, or CSCs, have the ability to give rise to both more CSCs (by self-renewal) and daughter cells of other types (by differentiation); as a result these cells have the capacity to regenerate tumor cells. CSCs have been suggested to contribute to tumor recurrence, metastasis and the development of drug-resistance. We found that high Lin28 expression levels in breast cancer and high Lin28 levels in combination with high Oct4 levels (another stem cell factor) in ovarian cancer are powerful predictors of poor prognosis. Importantly, we found that these are at least in part due to Lin28’s ability to specifically enhance the protein-coding ability of several oncogenes (cancer-promoting genes), including the human epidermal growth factor receptor 2 (HER2), which is a major therapeutic target in breast cancer. Together, these findings hold potential for the development of new strategies for the diagnosis and treatment of breast and ovarian cancer. In summary, our studies not only represent a major step forward in our understanding of Lin28 function in stem cells, but also uncover novel mechanistic insights linking Lin28 expression to human cancers.
The above research and discoveries would not be possible without the funding from the CT Innovations.

- In embryonic stem cells, many more genes are active than in differentiated cells. This increased gene activity is believed to be important for keeping embryonic stem cells in a state that allows them to differentiate into many types of cells. Because more genes are active, we hypothesized that embryonic stem cells may need special pathways to identify and degrade unneeded or harmful gene products. To test this idea, we characterized two pathways that could function to degrade harmful RNA molecules in these cells. Our work revealed one of these pathways is important for the growth of embryonic stem cells, and that both pathways contribute to degrading unneeded RNAs. Thus, our work identified new processes that contribute to the unique properties of embryonic stem cells. This knowledge will be helpful for designing more translational experiments, such as those that use the special properties of embryonic stem cells in order to repair or replace diseased tissues.

- Human embryonic stem cells (hESC) have enormous potential in treating illness in man. Much is known about which genes are turned on in these cells, but little is known about what controls the genes being turned on and off. Knowing the intimate details of the regulatory elements or ‘enhancers’ that actually activate all of the genes in hESC will be critical as these cells (and their genes) are to be used in man in clinical trials in the not too distant future. Regulatory elements are typically ‘cis-acting’, meaning that they act on the same piece of DNA, and are non-coding, meaning that they do not code for protein. Current methods of investigating enhancers examine them one at a time, which is very labor-intensive—we wished to develop a general method in which we could identify all of the hESC enhancers at once, across the entire human genome of close to three billion base pairs or units of DNA. If developed properly, this method could then be used to investigate the enhancers of hundreds of other cell types that are culturable, not just hESC.

To do so, we have developed a new method in which small pieces of human DNA (~2000 base pairs for each piece) are placed into a special ‘vector’ based upon human immunodeficiency virus (HIV). To cover all human DNA in a representative and complete manner this required 5 million individual vectors. The reason to use HIV is that it is very efficient in terms of delivery of genes or pieces of DNA to cells and delivery is essentially permanent or irreversible, for the lifetime of the cell that received the vector. We have spent three years of this grant perfecting the method of doing this, and to a large extent succeeded in our efforts. Additionally, we were able to use a special method in order to deliver this type of vector to hESC in an efficient manner and examine the entire population of cells using a technique called FACS analysis.

**Question 16.**

Which of the following outcomes can you attribute to your research through the CT Stem Cell Research Grant Funding?
Comments:

- Grant still in progress
- Publication has not happened yet, but that is the goal
- Several research outcomes are plausible, but they will take much more funding, research and time.
- Research through the CT Stem Cell Research Funding has enabled us to identify new funding opportunities and has also fostered new collaborations. As a postdoctoral trainee, the program has enabled me to formulate new ideas that I anticipate will be the mainstay of my research program when I become an independent investigator.
• A number of these issues are being considered. However, because it is only year one of the funding, these issues need to be further developed and matured.
• Too early - project just started.
• I established a new company to commercialize a patented stem cell product as a potential therapeutic.
• Presentations at the New York Stem Cell Foundation Meeting, 2011, 2012
• We published one research paper and are in the process of preparing two additional papers to be published.

Question 17.

If yes to any of the above, please briefly comment and indicate the number, if appropriate (100 character maximum).

Licenses Issued

• A cell line created was licensed to Pfizer, Inc.

New Laboratory Established

• A cell culture facility for hESCs was established within the department.
• A human cell culture laboratory in the P.I. research center
• Because of the CT funding, I established my research lab besides the core service lab.
• Dr I-Ping Chen has established a laboratory, with NIH funding based on our work, to study disease iPSC
• Established a new laboratory in 2011
• hES cell restricted cell culture, low oxygen cell culture
• Horsley laboratory started with this funding
• I was able to acquire an independent research position at Cedars-Sinai
• I was promoted to Assistant Professor in Residence, in part due to being able to secure independent funding.
• My laboratory at UConn Health Center
• My laboratory relocated and is a new lab based on a new research direction from this grant
• My stem cell laboratory was established to carry out the work.
• New company founded, got lab space from UConn TIP program
• State funding allowed us to establish stem cell infrastructure and research facilities in our laboratory.
• The new laboratory is the lab of Chondrogenics, Inc., which is the first company to form from the CT Stem Cell Program. Chondrogenics is a UConn TIP company, and the Chondrogenics lab is in TIP space.
• The postdoc performing the project has started her own laboratory
• The seed grant enabled me to be promoted and begin my own lab
• We have established a few part of our laboratory dedicated to stem cell research

New Practice (Clinical, Tool Instruments, Procedure/Technique)

• Better cell culture procedures (3)
• Blood vessel graft engineered using stem cells, hopefully in the future they will have properties that will enable them to be implantable
• Development of new strain of mice, and protocols of HSC differentiation
• I established the technique to generate iPSC cells at UCHC using this grant
• Microfluidic devices and instruments for single stem cell analysis
• Microscale perfusion stem cell culture system was developed.
• New procedures for iPSC, and hepatic differentiation
• Stem cell technology is a new technique to my laboratory; we will be developing a new screening technique as well.
• Technology and procedures for long-term EEG recordings in mice
• The new procedure and research method is Chondrogenics’ novel method for directing the differentiation of hESC/iPSC into the chondrogenic lineage without EB formation
• Use of inhibitors of Notch signaling pathways to promote keratinocyte progenitor formation
• We developed a new method to generate neural progenitor cells
• We have adopted >10 stem cell techniques (new for us) and have pioneered 3 new techniques
• We have been successful in reprogramming the human fibroblast cell line and Jurkat T cells into iPSC cell with the four reprogramming factors

New Research Method

• A method for non-viral genomic integration of transgenes was developed and tested.
• A method to co-culture multiple cell types on a biodegradable scaffold and recreate a tissue in culture
• A new method created in my seed grant is the cornerstone for the ongoing stem cell research
• Better cell culture procedures (3)
• Characterization of mouse model to identify neural stem cells in mouse brain
• Co-culture with human fetal brain tissue
• Derivation of mesenchymal stem cells from human embryonic stem cells.
• Derivation of specific types of neurons from embryonic stem cells
• Developed novel approaches for expanding megakaryocytes in vitro and then inducing differentiation
• Employed novel approaches and techniques
• Established iPSC and liver cell differentiation
• Established method to transplant human stem cells into the nonhuman primate spinal cord
• Faster and more efficient derivation of neural crest cells
• Funding resulted in the generation of new research directions and new animal models of cartilage degeneration.
• I now use iPSC models of the diseases that I used to study using mouse models.
• Method for differentiating embryonic stem cells into dopaminergic neurons
• Methods for identifying cell differentiation in vivo; distinguishing human and mouse bone tissue
• Methods of differentiating smooth muscle cells from ES cells
• New method for cardiomyocyte differentiation from hES cells
• New protocols for making hematopoietic cells that could be potentially transplanted.
• Perfusion culture method
• Production of stem cells from standard cell lines
• Protocols for single cell genomic and proteomic measurements
• Protocols of HSC differentiation
• RNAi in human ES cells
• Several new techniques were pioneered due to the research project, including stem cell co-culture techniques for Notch
• Single cell transcript analysis
The new method should impact on hESC research to decipher the function of individual genes and related pathways.

The new procedure and research method is Chondrogenics’ novel method for directing the differentiation of hESC/iPSC into the chondrogenic lineage without EB formation.

To establish a paradigm for generating cortical motor neurons.

Transposon for forward genetics screen in ES cells.

Using fluorescent protein marked iPSC and hESC to study bone formation in a mouse model.

We are also collaborating with multiple core laboratories to perform the research.

We are experimenting with in vivo injection of iPSC-derived neural progenitors in mice.

We developed a mouse strain for rapid screening of stem cell differentiation.

We developed a new way to analyze genomic data and published this.

We established a hematopoietic differentiation protocol in a serum free and feeder free conditions.

We established a transient gene knockdown technique, which works very well with hESCs and iPSCs.

We established ES cell culture systems.

We have been successful in reprogramming the human fibroblast cell line and Jurkat T cells into iPS cell with the four reprogramming factors.

We have developed a way to rapidly produce neurons from stem cells through transcription factor reprogramming.

We have developed protocols for isolating and studying human adipocyte progenitors.

We have established a new method that allows high efficiency siRNA knockdown in hESCs and iPSCs.

We have found that hPS derived EB can facilitate in-situ T cell differentiation of HSC precursors.

We pioneered on 3 methods to assay for small RNA-mediated regulation in stem cells.

Whole genome lentiviral library.

New Theory

Behavior of stem cell precursors in vitro.

Better immunosuppressive efficacy with hES-MSC.

Contribution of nuclear organization to inflammation.

Developed novel concepts.

hPS derived EB can facilitate in-situ T cell differentiation of HSC precursors.
I generated a new hypothesis as to how neuron specific imprinting of UBE3A occurs

Neural stem cells able to generate new neurons are diffusely present in brain tissue of juvenile mice

New epigenetic stem cell theory (1)

New theories about reprogramming and differentiation

Nuclear RhoA for ES differentiation

On epigenetics in ES cell research

Post-transcriptional regulation of gene expression is important in stem cell differentiation

Reprogramming is a loosely ordered probabilistic process

Some research led to a published new theory of one type of gene regulation

Stem cell as a cancer vaccine was a new concept.

The factors in EGM media promote formation of bone and cartilage progenitor cells

The new theory is that avoiding EB formation leads to more homogenous hESC/iPSC derivatives

This lead us to postulate that growth arrest in grafted cells might be a major consideration in cell therapeutics

We are currently finishing a draft on new cellular substrates related to Notch signaling

We discovered a new pathway for degrading harmful gene products in embryonic stem cells

We have developed new theories on how small RNA regulate stem cell fate

We have uncovered novel mechanisms of regulation of our region of interest.

We uncovered a novel mechanism the controls stem cell number is mammalian tissues

**Novel Human Stem Cell Line**

- Four new induced pluripotent stem cell lines established from human fibroblast donors
- Created 23 novel iPSC lines
- Derivation of 10 novel lines of human induced pluripotent stem cells from normal and osteoarthritic cartilage cells.
- Derived from in vitro explants
- Development of new cell lines
- Engineered stem cell lines (5)
- Genetically engineered human pluripotent stem cell lines that allow precise control of certain gene expression.
• hES cells derivitized with knock in GFP reporter constructs using Zn finger technology.
• hESC and iPSC lines with osteoblast specific fluorescent protein marker genes
• Human stem cell line containing leukemia gene t(1;22)
• I generated stem cell lines from individuals with Angelman syndrome and Prader-willi syndrome
• New hES cell lines were brought from Australia to perform the research at UConn, that otherwise would not have been possible.
• Several human iPSC cells and engineered derivatives created
• The new human cell lines are lines of iPSC from human patients with cartilage disease
• T-MSC
• Two induced pluripotent stem cell lines were derived from patients with congenital disease
• We have derived new iPSC lines from different classes of patients.

Patent Application

• A patent for the mouse strain was applied for through Yale.
• Patents for the new method are being pursued in the US, Canada, Europe and Japan by UConn
• Pipeline for the extraction of maximally informative Biomarker sets
• Provisional application filed for new strains of mice
• Single cell analysis tools
• UConn Health Center is considering to patent associated technology from the project.
• US provisional patent: 61684509
• We have filed for one provisional patent and another intellectual property disclosure

Patents Issued

• Patent has not been issued yet
• Patents are still in progress
• Single cell analysis tools

Peer-Reviewed Research Papers and Journals

• 1 (2012) Alcoholism: Clinical and Experimental Research
• 1 published in Proceedings of the National Academy of Sciences
• 5 articles, 3 peer-reviewed book chapters
• 6 papers have been published with research supported by CT stem cell grants
• 8 manuscripts since 2008 (4 stem cell related), 2 manuscripts currently under review with 4 first authorships
• A manuscript describing this mouse strain has been submitted for publication
• A paper was published in Stem Cells
• Cell Stem Cell - 6 April 2012 (Vol. 10, Issue 4, pp. 440-454)
• Fourteen peer-reviewed papers published, and data accumulated for others
• Genomic and epigenomic signatures of stem cell differentiation
• Gibson et al., Integrative Biology, 2009
• I have published 11 papers since the beginning of the award period
• In preparation - possibly 2, maybe 3
• One paper on JoVE, in press. The other paper is under revision.
• published several articles in the international journal and presented at the international meetings
• Radmila Filipovic, Saranya Santhosh Kumar, Chris Fiondella, Joseph Loturco (2012); Increasing Doublecortin Expression Promotes Migration of Human. Stem Cells 30:1852–1862
• Resulted in two publications in peer-reviewed journals.
• Several papers and abstracts on cell therapies
• Submitting manuscript to Development based on this work
• There have been 2 peer-reviewed papers, 1 review and 1 book chapter from this work
• Two articles on osteoblast differentiation in final stages for submission
• Wang et al., Cell Stem Cells, 2012
• We are currently preparing a manuscript for publication, and there should be at least one additional manuscript
• We are in the process of submitting our findings for publication
• We are preparing a publication from the latest work.
• We are putting the finishing touches on two papers describing our work.
• We have manuscripts in preparation, based on the novel findings from the new iPSC lines.
• We have published a number of papers on our research, some in high impact journals.
CONNECTICUT STEM CELL RESEARCH PROGRAM ACCOMPLISHMENTS
APPENDICES

- We have published a total of 12 peer-reviewed research papers from the CT funded stem cell research
- We have published on review article. A manuscript based on our findings is under preparation.
- We published one initial manuscript describing how we can evaluate the chondrogenic potential of mesenchymal-like stem cells from pluripotent cells
- We published three papers in which the data were generated by the CT funding

Replication of Research Findings

- Confirmed safety and efficacy of the human cells we are transplanting in the nonhuman primate
- Confirmed that human embryonic stem cells can differentiate into retinal pigment epithelium and retinal progenitors
- During the course of our Notch investigations, we have to some extent replicated other findings in the process of novel questions
- Extended prior work using fetal cell transplants
- Human iPSC derived cell derived neural cells replicated some findings from rodent neural cell culture models of alcohol effects
- Methods of differentiating smooth muscle cells from ES cells
- Other literature
- Replicated other’s findings that liver cells can be produced from pluripotent cells
- Replicating recent reports of differentiation methods
- Reproducing published methods for cardiomyocyte differentiation
- Reprogramming using specific transcription factors, components important for generation of liver cell like cells
- We have been able to recapitulate how the region is regulated in humans in our tissue culture model.
- We have replicated findings that were known in mice in our human model.
- We have replicated our own results showing hES cell grafts continue to proliferate in the nervous system
- We have reproduced some results from other labs

Research Findings/Knowledge Creation

- Built a knowledge base for the future studies
• Contribution of human cytokines to hematopoiesis
• Contribution of nuclear organization to liver cell metabolism
• Data generated for use as preliminary data to get funds from other sources.
• Datasets of gene expression in neural stem cells in brain after injury
• Determined and compared the functional properties and transcriptome of fetal and stem-cell derived RPE
• Determined that the tissue source of iPS cells influences their cartilage-forming capacity.
• Distinct Lineage Specification Roles for NANOG, OCT4, and SOX2 in Human Embryonic Stem Cells
• Established the remyelination potential of the human cells
• Foxp3 expression does not affect the generation of iPS cells.
• Genomic and epigenomic signatures of stem cell differentiation
• hESC and iPSC treated with EGM media produce bone in vivo
• I find a novel mechanism of how stem cells guard their genomic integrity
• Identified novel transcription factors required for the maintenance of hESCs
• Impact of various differentiation methods on phenotypic stability after injecting into primates
• Improved methods for iPS and liver cell differentiation
• Induction of neural crest cells based in wnt signaling alone
• Mechanistic investigations on isoniazid toxicity are under way.
• New datasets of gene expression in stem cells
• New findings are the novel method and evaluation of repair of cartilage damage by the cells in vivo in animals
• New knowledge on the regulation of cardiomyocyte differentiation by Wnt
• New understanding of how megakaryocyte maturation is regulated
• New understanding of stem cell differentiation toward cardiac tissue
• Novel method for functional identification of enhancers
• Nucleic acid oligonucleotides can be applied to manipulate cell behavior
• Our findings may explain why stem cells in normal tissues cannot repair damages that goes beyond the normal wear and tear
• Our work has uncovered a new way in which embryonic stem cells handle unneeded gene products
• PiggyBac transposon could efficiently mobilize in hES cells
• Post-transcriptional regulation of gene expression is important in stem cell differentiation
• Research findings are reported in two journal papers, 1 conference paper, and two poster presentations.
• Several novel lines of investigation have resulted directly from the CT Seed grant
• Showed ability of transplanted cells to modify disease
• The knowledge obtained so far is setting up the stage for obtaining additional funding from NIH.
• The novel mouse strain will allow researchers to perform high throughput screening of stem cells.
• The published paper is the first to show a genetic manipulation of hESCs can enhance their migration across tissue.
• Unequivocal in vivo bone cell differentiation from hES and iPS cell lines.
• Various relating to heterogeneity and population complexity in stem cell culture and manipulation. Dissection of the reprogramming process.
• We extended the translational regulation of genes by LIN28
• We found that there is a need for just the right level of heterogeneity to support proper chondrogenesis in vitro and that cells will positively respond to growth factors in a time-specific manner
• We have already performed seminal work with the hES cell lines that will advance knowledge about the pathology of the disease.
• We have been able to make several interesting cell cycle observations from this work
• We have defined the molecular functions of the important stem cell factor Lin28 in both hESCs and cancer stem cells
• We have developed protocols for isolating and studying human adipocyte progenitors.
• We have discovered a number of new things
• We have discovered that the hPS derived EB can facilitate in-situ T cell differentiation of HSC precursors.
• We have established a hESC-based cell model for SMA.
• We have established a novel method to genetically modified human embryonic stem cells
• We have gained new insights into how stem cells function
• We have gained new knowledge about how our region of interest is regulated and hope to gain a better understanding of the underlying causes of our disease of interest.
• We have identified novel aspects of skin development
• We have learned a lot more about imprinting in humans, which is different from mice.
Software/Databases

- MUMPS
- Transcriptome of human fetal and stem cell-derived RPE deposited in the Gene Expression Omnibus database
- We have a database for Lin28-regulated target genes that will benefit future research for the stem cell research community
- We have generated new software for analyzing stem cell behavior (in 1 manuscript being submitted)

Spin Off Company

- A new company was established to commercialize a patented stem cell product as a potential therapeutic.
- SIBOP (nascent)
- The spin-off company is Chondrogenics, Inc. which has contributed 1 FTE and 1 partial FTE to the CT workforce
COLLABORATION

Question 18.

Rate the importance of collaboration on your research through the CT Stem Cell Research Grant Funding

Comments:

• We have collaborated with UConn stem cell core to generate induced pluripotent stem cells.

• We collaborated with Dr. Pietro de Camilli of HHMI at Yale on elements related to Dynamin.

• We have had very successful collaboration with Yale’s human embryonic stem cell core. We also had very productive collaboration with researchers within and outside of Connecticut.

• Group program has required expertise in various areas, including immunobiology, dopamine neurochemistry and pharmacology, immunohistochemistry and electron microscopy. External collaborations with investigators from the California Institute of Regenerative Medicine have expanded the scope as well.

• The collaboration between my lab and the Pluripotent Stem Cell and Molecular Biology Lab at Pfizer has produced novel techniques.

• Collaboration at Wesleyan with Jan Naegele and Gloster Aaron, and with folks at UConn Health Center, including Ren-He Xu, Alex Lichtler, FACS facility, essential to the project and would not have happened without the stem cell initiative.

• We collaborate with Dr. Herbet Lachman, Albert Einstein College of Medicine. We use his iPSC lines derived from his schizophrenia patients.
• 1. Dr. Janine LaSalle of UC Davis, Dr. Marc Lalande of UCHC on the DNMT3B study (Martins-Taylor, et al., Epigenetics 2012).

2. Dr. Karen Montgomery of WiCell Institute, and Dr. Marc Lalande of UCHC on the copy number variation study on human iPS cells (Martins-Taylor, et al., Nat. Biotechnol. 2011).

3. Drs. Xue-Jun Li, Alexander Lichtler, and Lixia Yue of UCHC and Drs. Fangping Chen and Huixia Liu of South China University on the study of iPSC differentiation into region-specific neurons (Zeng, et al., PLoS ONE 2010).

4. Dr. Xue-Jun Li of UCHC and Dr. Fangping Chen of South China University on the study of ABCG2 expression in differentiating hESCs (Zeng, et al., Stem Cells 2009).

5. Dr. Zihai Li of UCHC on the study to use hESCs as vaccine against colon cancer (Li, et al., Stem Cells 2009).

6. Dr. James Thomson of University of Wisconsin-Madison on study of NANOG as a target of TGFβ/SMAD signaling in hESCs (Xu, et al., Cell Stem Cell 2008).

7. Dr. James Thomson of University of Wisconsin-Madison on the genome-wide mapping of SMAD target genes in hESCs (manuscript under revision).

• The stem cell grant enabled a significant collaboration between my lab and Drs. Charles Giardina and Craig Nelson in UCONN, Storrs.
Question 19.

Respond to the following statements related to collaborations on your research made available through your CT Stem Cell Research Grant Funding.

![Collaboration Statements diagram]

Comments:

- Interactions among different labs was essential
- We collaborated with a Yale group also funded by a CT stem cell grant, and this led to a major publication and press release.
Question 20.

The CT Stem Cell Research Program requires that the research be conducted in CT and within the state at an eligible academic institution. Respond to the following statements about the potential effect of that policy on collaborations outside of CT as related to your research.

Comments:

- Our research was based in CT, but investigators from outside of CT came to our center for collaboration.
- Facilitation and strengthening new collaborations have been a major strength of this program.
- Prevented the use of resources for primate studies that were available outside the state but were not available inside of Connecticut.
Question 21.

To what extent do you agree or disagree with the following statements? In the ABSENCE of future CT Stem Cell Research Grant Funding opportunities, I would:

![Bar Chart]

**In the Absence of Stem Cell Funding**

- Leave the field of stem cell research (n=86, 10 n/a)
  - 2.97

- Have more difficulty advancing my stem cell research career (n=85, 7 n/a)
  - 4.49

- Have more difficulty obtaining funding for my stem cell research (n=87, 7 n/a)
  - 4.54

- Be able to sustain my stem cell research program (n=87, 7 n/a)
  - 2.38

**PI Response with 5 equal to Strongly Agree and 1 equal to Strongly Disagree**

Comments:

- The CT stem cell grant allows us to move into new field and address important questions that have high impact and some degree of risk. NIH does not allow such research.

- I currently reside in California but believe the CT Stem Cell initiative to be highly worthwhile, well-run, and I would be supportive of it continued growth for CT investigators.

- It would be devastating for my stem cell research program without future state funding. It will be devastating for other stem cell researchers as well.

- In the current climate of federal research funding, it is extraordinarily difficult to obtain research funding. This is especially for expensive work such as hESC/iPSC stem cell work. This work is particularly costly as maintenance of the hESC/iPSC is a 7 day a week, 365 day a year task which is highly personnel-intensive and which typically consumes large quantities of expensive reagents. Moreover, in vivo studies to take the work closer towards translation involve animal models which are also costly to maintain. Stem cell work is mainly out of reach for starting investigators who do not have extensive resources to devote to their lab.

Stem cell research is particularly expensive to perform in CT where the salaries and fringe rates for personnel (at least at UConn) take up the majority of the grant dollars (see below).
As federal grant funding (ie, NIH) success is based mainly on the prior progress achieved by an investigator at the time that they submit. Without CT Stem Cell support it would be impossible for an investigator who is not yet in the stem cell field to obtain the preliminary data necessary for a competitive application, emphasizing the need for continued seed grant support. Even for investigators who already have progress in the stem cell field, the federal funding climate is insufficient to fund all meritorious proposals.

Finally, federal funding for stem cell research using hESC is always subject to potential political restrictions which would jeopardize federal funding availability, leaving researchers with few options if CT stem cell funding were not available.

CT Stem Cell Program support must continue to sustain the extraordinary progress the state and its researchers have attained through the first years of this program to date.

- The availability of research funds from the NIH is rapidly decreasing such that even the most well established investigators cannot be certain that they will be able to keep their labs going in the future. We are always working with the fear that funding will dry up and we will need to discontinue our work.

Question 22.

To what extent do you agree or disagree with the following statements? In the ABSENCE of future CT Stem Cell Research Grant Funding opportunities, I would:

![Bar chart showing responses to questions about obtaining alternative sources of funding for research. The chart indicates responses from participants in Connecticut, the United States, and internationally. The data is presented with a scale from 1 to 5, with 5 indicating strongly agree and 1 indicating strongly disagree.](chart_image)
Identify any current CT Stem Cell Research Program policy issues (obstacles/barriers/concerns) that should be considered by the state (maximum 1000 characters).

- I am disappointed by the quality of many of the reviews for stem cell grants that have been awarded and not awarded.
- Very frustrated that grants are not judged by the objectives outlined in the RFP announcements. It appears that award made by the CT stem cell committee is to individuals they know rather than grants that obtained the highest external award score. In addition, hype was valued more than an honest presentation of data, problems and solutions. The committee should know that so much of the stem cell field is filled with over promising and over interpretation of in vitro data. They need to reward grants that focus on objective assessment of in vivo outcome of hES derived progenitor/tissue repair strategies.
- Selection of grants to be funded should be done by scientists on a basis of scientific merit.
- It would be helpful if this program could set up the NIH salary cap limit for senior collaborators salary support. Senior collaborator 5% effort could cost significant grant support.
- Stem cell research is, by nature, interdisciplinary. In particular, the study of neural stem cells must incorporate knowledge from neurobiology, genetics, molecular biology, bioinformatics, and pharmacology, among others. State funding should foster and encourage the need for interdisciplinary collaborations.
- A substantial obstacle to stem cell research is that very important decisions that directly impact stem cell research (for example, lab space, appointment and promotions for junior faculty, and assignment of core departmental resources among others) are under the control of the individual Departments, which typically have rigid criteria for assigning and administering such resources. The existing departmental structure, arranged by individual disciplines, is ill suited to foster interdisciplinary research.
- I agree that the program should emphasize stem cell translation. I do think the funding needs a balance between basic research and application.
- The policies are working well.
- I am not aware of any barriers.
- The State of Connecticut has done a commendable accomplishment in setting up this stem cell research program. One concern however might be the involvement of too low a number of peer reviewers which might lead to undesirable results in awarding procedure. It also might be wise to include philanthropy into the fund raising procedure. This might allow for including inflationary adjustments into the fund allocation without having to resort to cuts in the number of grants awarded. This might increase the competiveness of the research in Connecticut compared to national or international research efforts.
- One obstacle was obtaining approvals from multiple institutional committees that
are not coordinated with each other. They use paper-based applications that are processed sequentially, which takes a lot of time since each committees meets only once a month. A possible solution would be to create a universal application for all of them. The PI could submit the universal application electronically to all relevant institutional committees simultaneously, with the funded grant application serving as the accepted protocol of record for them all. The application could be processed by all the committees in parallel, with committee members assigned to help the PI achieve final approvals within three months, while maintaining the scientific integrity of the research.

- The main obstacle to success by stem cell researchers in the state is the lack of funding. If CT wants to keep its place among the leaders in stem cell research world-wide, it must maintain its stem cell program.
- The state of CT has special challenges due to the set wage and fringe rates for technical personnel. This is not to say that technical personnel are paid too much or should not receive appropriate benefits; to the contrary, they are paid fairly and deserve the benefits they have. However the high fringe rates (~50%) places an enormous burden on grant dollars and puts CT scientists at a disadvantage to other investigators in other states. Assistance in the form of cost sharing of fringe rates by the state would be a tremendous help for CT researchers in stem cell research.
- There has been some question on the Stem Cell Research Advisory Committee regarding whether the research cores at Yale and UConn should continue to receive funding. I feel strongly that they should. In some ways, funding of the cores is the only funding that is guaranteed to succeed. Whenever research grants are funded, some percentage of them will not succeed. In contrast, the cores will succeed in providing expertise, training, and facilities for all CT stem cell researchers.
- The major problem is restrictions of the work to within the State. It may appear to make sense economically, but it handicaps the quality of the science. It may also increase costs.
- Bureaucracy is extreme. Lots of time required to fill out forms such as this one, progress reporting requirements tedious, time-consuming compared to federal and other grants.
- The research program has been shifted toward translational and clinical research for the last few cycles. Although the impact of many basic researches may not be necessarily obvious, these studies are vital for translational and clinical researches.
- Holding scientists to performing the experiments described in their proposals is actually a bad policy. Science moves quickly, and often, multiple people have the same brilliant idea. This means that in the time between writing the grant and getting the money, another group may have performed and published the experiments described in the grant. So, if the newly funded individual has to perform the experiments she or he described, despite the fact that they have already been done, no new information will be gained and it hurts the career of the individual because they are wasting time on unpublishable results. It’s best to require that an individual sticks to the general idea of the grant, but not the specific idea.
- For the fund awarding process, the peer review score rank should be considered a heavy weight. The stem cell committee had too much power to decide the funding.
They decided not to fund my grant even though I had a very good score. Many of them have conflict of interest and biased views. And the committee should be changed every year.

- Concern that applicants who are not funded do not get reviews to assist in future applications.
- People who sit on the Stem Cell Advisory Committee should not be allowed to compete for funding. We have a paradoxical situation where researchers award money to themselves. Half a million or more. Why not create two lists of names? On one page list current and past members of the Advisory Committee. On the other page list the names of grant winners and dollar amounts. Check if the same names appear on both lists.
- The reporting duties were too complicated. The delay in the release of funds between each year of funding created major administrative problems.
- The advisory committee has too much power to overturn the peer reviewers’ suggestions.
- Funding has been strongly biased toward human pluripotent stem cells, away from adult somatic cells. These cells however, contribute directly to human disease and tissue regeneration. The drawback with this strong bias is forcing the proposals to forge a connection between their existing research interest/expertise to human ES cells, which sometimes falls quite superficial. Stem cells biology encompass a much larger conceptual frame work - limiting this line of exploration to ESCs in the long run could be harmful for stem cell research as a whole. I’d hope to see more emphasis to be placed on adult tissue stem cells.

Question 24.
How would you suggest the CT Stem Cell Research Program be improved (maximum 1,000 character maximum)?

- There is a lack of understanding of the rules for administering the funds at Yale, so that many expenses that CT stem cell should have covered were not approved at the University level. There should be training and transparency on budget preparation and administration from the investigators on up the chain through business offices to the state.
- Larger grants would cover a more realistic portion of the project cost.
- The emphasis on clinical and translational research, at the expense of basic research, is premature for the stem cell field and results in lower quality science being funded by the program.
- Improve the review process. It needs to be more responsive to scientific issues.
- The CT Stem Cell Research Program would benefit from a small change in the review process, which would be to change the scoring system so that there are not so many grants that receive the same score.
- I would suggest continued funding for CT stem cell research. One improvement might
be to assist researchers in using CT stem cell grant results to apply for federal funding.

- Less administration. Obtaining ESCRO first time required a major effort and a lot of time.
- CT stem cell research program has been a significant support for our research.
- Promote original funding initiatives/mechanisms to directly support collaborative research and interchanges across disciplines.
- Start-up funds dedicated to hiring new personnel in stem cell research that would cross departmental boundaries (i.e., a computer scientist in a biomedical department)
- Money for technical personnel and equipment dedicated stem cell research that would not normally be supported by NIH, to free investigators from expensive core resources
- Give money for travel to learn new techniques and approached, or to go to meetings, especially for junior investigators
- Promote original funding initiatives/mechanisms to directly support collaborative research and interchanges across disciplines.
- The program has been run quite well in my opinion. Certainly some clarity as to future support would improve the program and encourage CT investigators to work in this important area. I also feel that partnerships between academic institutions and private companies should be encouraged.
- Possibly more workshops and access to core facilities
- The size and duration of the awards should be extended. The relatively short duration to bring in a new methodology is a challenge.
- It is an excellent program. I also think the program should promote the inter-institutional collaborations.
- 1. Diversify the projects. Currently, there are too many projects that aim to develop protocols for stem cell differentiation. It is better to have more projects related to either basic mechanistic study (genetic, proteomic, microenvironment, etc) or disease-oriented research.
- 2. Increase the support on collaborative projects. It is collaboration, especially across multiple disciplines that often leads to breakthroughs.
- The CT Stem Cell Research Program has stimulated great interest and discovery in the stem cell field in the state of CT. The only improvement that would be necessary to continue this momentum in stem cell research and discovery in our state is to ensure a renewed mandate for many years to come.
- I found the CT Stem Cell Research Program to be highly efficient and supportive for myself and other investigators who were involved. In comparison, I have noted or have learned of significant inadequacies in other state-run programs related to stem cell research in other states besides CT.
- As an investigator, more opportunities for funding would be my recommendation, in the form of leadership awards, transition to independence, early career, etc.
• California has been able to recruit a great deal of top flight stem cell scientists whereas CT has not been as successful.

• If more funding can be made available by the state, it will be a major boost on an already excellent program.

• I would not have been performing human stem cell research without the state funding, and many other researchers that I talked to had a similar feeling. The state funding made Connecticut scientists’ stem cell research progress at a much faster pace and made our work visible to the national and international stem cell community.

• If there is additional funding or new mechanisms of funding stem cell research, I foresee that Connecticut will become the leader in stem cell research on a larger and broader scale. And more importantly, new findings will be possible to advance human health.

• Fund more grants, limit amounts to $500,000.

• In many cases the time period of two years for seed grants is somewhat on the shorter side. Seed grants may suffer more from start-up issues than established investigator grants where research support is already established through an existing laboratory. Therefore the time frame should either be extended (especially in the case of start-up/postdoctoral recipients) with an equivalent funding increase or seed grants should only be available for high risk research in established laboratories.

• Restrictions on the use of funds for attending stem cell conferences outside of the US such as the International Society of Stem Cell Research meetings should be lifted.

• This is an excellent program as it is. Thank you so much for being a pioneer for state-sponsored stem cell research. So many other states have followed our lead.

• Continued funding for next 10 years will really strengthen the program with much more potential for payout.

• Increase funding level and fund more researchers. This approach in the long run would benefit tax payers more, as failure is part of the scientific enterprise, especially novel (out of the box) scientific ideas. But novel ideas can lead to far better therapeutic measures to benefit patients. Funding more novel ideas is the best way for success.

• 1. Outreach and education to the state could be enhanced in order to increase public support for the program. More public media releases would help the public understand the importance of stem cell research for the state, especially hESC research, in order to balance out some of the political and theoretical rhetoric.

• 2. Funding of seed grants should be a major emphasis. Seed grant funding is essential not only to recruit and maintain new investigators in stem cell research in the state, but also to allow established stem cell research programs to leverage their knowledge towards a new direction still within stem cell research, thus keeping the momentum we have gained for stem cell research in the state.

• 3. The maximum number of years for non-seed grants should be 3 years, and the max level of funding could be reduced. This would enable funds to be spread out more widely, broadening the base of stem cell research in the state and strengthening existing
stem cell research programs so that they can pursue other sources of funding more competitively.

- The CT stem cell research program has been phenomenal for me on multiple levels as indicated below. The biggest change I would recommend is that when the stem cell funding is once again approved for another 10 year term, that the CT legislature considers investing more than $10 million per year. I recommend $25 million per year, which would allow for investment in clinical trials.

- Benefits to me of the CT Stem Cell Research Funds:
  - I have come to know, respect and collaborate with stem cell researchers from outside of my institution.
  - I have obtained funding for novel ideas that have led to high impact publications and to data that allowed me to obtain a new NIH grant.
  - The research environment around me has blossomed due to the CT stem cell research funding. Basically, the entire Yale Stem Cell Center owes its existence to the CT Stem Cell research Program, which gave the Yale administration confidence that their investment in building the program would yield excellent research that would receive support from CT stem cell funds. This includes the hiring of Haifan Lin, a true international leader in stem cell research, and the recruitment of at least 10 amazing new faculty members who do stem cell research including: Caihong Qiu, Natalia Ivanova, Jun Lu, Andrew Xiao, In-hyun Park, Shangqin Guo, Matthew Rodeheffer, Valerie Horsley, Yibing Qyang, and Valentina Greco. In addition, extraordinary Yale faculty who were not previously performing stem cell research are now doing so including: Martin Garcia-Castro, Jeffery Kocsis, Richard Flavell, Lawreece Rizzolo, Kevan Herold, Anita Huttner, Eugene Redmond, Erik Shapiro, Tian Xu, Weimin Zhong, Yingqun Huang, Micael Snyder (not at Stanford), Dianqing Wu, Valerie Reinke, Sandra Wolin, Richard Sutton, and Flora Vaccarino, amongst others.
  - So far, I have been pretty pleased with the whole program and how it is run. There have been some areas of concern. One is that the review process has led to confusion and often consternation, as review scores are hard to understand by the applicants because the evaluation comments are so brief and uninformative. I would like to see at least a full paragraph devoted to the report on the review of each proposal. That way people would know how to improve them or at least what the issues were. Also, I think it’s important to try to spend the money as wisely as possible, by funding the most exciting and promising projects, without regard to balancing between institutions. Some young applicants are very good and deserve seed grants, but some of these may be wasted if used by the principal investigators simply as additional sources of funding for the lab.
  - Continue investing in critical shared core facilities. Allow activities that are justified to be supported (cheaper or better elsewhere), but not investigator support. Link up with other states which provide similar funding. Provide more aggressive resources for evaluating and filing patents on discoveries.
  - Eliminate restrictions that limit the pool of qualified reviewers for the grant applications. When reviewer quality is low, applications are funded in an essentially random manner. The entire program hinges on funding the best proposals, therefore
every effort should be made to have experts in the stem cell field review the proposals.

- More funding would be better.
- Expand the number of reviewers
- The research program could have a new category to solicit proposals from new or established researchers outside CT and recruit them to relocate to CT.
- The peer review and funding decision process is honestly not as good as it could be. Better effort to identify experts for reviewing grants could be made—perhaps scientists not applying for a grant in the current cycle could help identify appropriate reviewers. The funding decisions should then follow the peer review scores.
- By continuing the ongoing research commitment, and extending it beyond the initial 10 years commitment period, to further the advances made through the ongoing support.
- Increase funding, extend seed funding term from 2 year to at least 3 year. Two years is not enough.
- Improve the grant review process. For example, a scientific group discussion may be helpful for solving the following issues: the scores for the same grant are too far away; or too many grants end up with the same final score.
- Some concern that pressure to fund translational/clinical work will be pushed at the expense of funding for basic studies, may not be ready in all fields for this transition.
- More money
- Not a single award should exceed $400,000.
- Never, absolutely never, should CT Innovation award $1,000,000 to a stem cell researcher, not even $500,000. Giving >$500,000 to one person is a decision based on the political influence and the competing centers of power; not on the reasonable plan to boost stem cell research in Connecticut.
- Give the entire funds and require us to do a short reporting each year and a final reporting at the end. Do not delay the start of the second or third year of funding. I have had to re-budget multiple times to keep the people in my lab employed during the time that the state delayed the release of funds and use funds from other non-stem cell grants.
- More opportunities for funding of non-tenure track Faculties
- To avoid conflicts of interests, active research scientists from Connecticut should not be members of the State Stem Cell Research Advisory Committee.
- It will be really beneficial to keep the future stem cell research funding.
- Reduce the bias toward human pluripotent stem cells.
- Encourage stem cell research using other systems.
- Let Connecticut be the center of stem cell biology, not just human ESC biology.
- The program is working well from my perspective. I would like to see it continued.
• Create more flexibility in shifting funds among budget categories to best take advantage of changing opportunities.

• Since research can be variable, more flexibility in the funding (moving expenses between categories and changing personnel) would facilitate our research.

• I think that individuals who have successfully used their State funding (i.e. generating manuscripts and publishing their data in peer-reviewed journals) should be given priority for subsequent funding. Some investigators that have not been successful in this regard have been awarded additional funding. Investigators who have used their money for the research that they proposed and have been able to publish it in a timely manner should be given priority. This is the best investment for the money.

• I would like to suggest that the CT Stem Cell Research Program rewards the most productive investigators by providing one or two $200,000 research prizes each year.

• The CT Stem Cell Research Program is an outstanding achievement. It has brought many prominent researchers to Connecticut, and also invigorated the work of established researchers such as myself. It has also allowed existing researchers in Connecticut to continue to be productive, contributing scientists, to make important contributions to our knowledge stem cell biology and to provide jobs for Connecticut residents at a time when NIH funding has become increasingly difficult to sustain. Moreover, the funds provided have allowed many scientists, including myself, to obtain additional funding to support research here in Connecticut.