MEMORANDUM

Date: August 29, 2012

From: Ellen Feigal, MD
CIRM Senior Vice President, Research and Development

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application RB4-06239

Enclosed is a petition letter from Dr. Tony Hunter of the Salk Institute, an applicant for funding under RFA 11-03, CIRM Basic Biology IV Research Awards. This letter was received at CIRM on August 27, 2012 and we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

Salk Institute for Biological Studies
La Jolla, California 92037

August 27, 2012

Jonathan Thomas, Ph.D., J.D. Chair of ICOC
Alan Trounson, Ph.D. President of CIRM

Dear Dr. Thomas, Dr. Trounson, and Distinguished Members of the ICOC

We thank the CIRM staff and grant reviewers for considering our application RB4-06239 "Generation of MILS syndrome neurons to explore therapies for mitochondrial DNA disease". Our final score from Grants Working Group (GWG) is 70, right on the border of funding. Our proposal was to generate patient-derived neurons using iPS technology in order to explore the pathological mechanisms underlying neurodegeneration caused by mitochondrial DNA mutation. Unlike nuclear gene-related disease, there is no animal model to study mutations of the mitochondrial genome. From this perspective, iPS stem cell technology represents a truly unique opportunity.

In the review summary, **the GWG reviewers also found the proposed study particularly significant**, since there are currently no effective therapies and very few experimental models for mitochondrial diseases. In the first round review**,** the reviewers thought the project was superb with **a high likelihood of success, exploring a key area with much needing to be known**, and with implications for a wide variety of diseases.

As our proposed research may have an immediate impact on evaluating therapeutic agents for mitochondria DNA disease, and no major scientific issues were found with our proposal, we are now petitioning for our application to be funded. In fact, CIRM support is essential for us to continue this research program, because of the personnel costs and the expensive experimental reagents required for stem cell studies.

In considering our petition, it is important to note that since the application was submitted, we have found that there are significant bioenergetic differences between MILS patient iPSCs and neuroprogenitor cells and contro cellsl, thus addressing a major concern raised by the GWG (details are provided in the following section). Moreover, this important discovery will greatly accelerate our progress in accomplishing the proposed studies. **In consequence, we expect to complete the work in two years, and accordingly the total funding requested would be decreased to $1,169,880**, assuming the full Salk Institute overhead rate is applied (but this would be less than $1,000,000, if a lower overhead rate were approved).

It is also important to point out that this work is being conducted in collaboration with a close colleague at the Salk Institute, Dr. Fred Gage, who is a world leader in iPSC technology and iPSC use for studying genetically-based neurological diseases. Dr. Gage is actively engaged in this project, and, indeed, this has become the thesis project of one of his graduate students. In addition, this funding would also afford the first opportunity for my group to enter the stem cell field and make contributions in regenerative medicine and thereby ameliorate human disease.

The proposed work may have a substantial impact on mitochondria DNA disease study and therapy, a contribution to both basic biology and translational research. As far as we are aware, no similar work is being funded by CIRM. We sincerely appreciate your consideration of our petition, and the commitment of CIRM and the efforts of its board members in supporting the best research in regenerative medicine.

Yours sincerely,

Tony Hunter, Ph.D.
Director, Salk Institute Cancer Center

Review Summary: The goal of the research described in this proposal is to develop a disease-in-a-dish model for an inherited mitochondrial disease, Maternally Inherited Leigh'is Syndrome (MILS). MILS causes severe neurological defects in children, and this and similar mitochondrial disorders affect 1 in 2000 individuals. The first specific aim will be to generate additional induced pluripotent stem cell (iPSC) lines and appropriate control cell lines from MILS patients. The second aim is to characterize the bioenergetics profiles of neurons undergoing differentiation from progenitors carrying MILS-mutant mitochondria. The third specific aim will be to test potential therapeutic agents and develop new approaches for drug discovery using iPSC-derived MILS neurons.

Significance and Innovation

- Reviewers found the proposed study particularly significant, since there are currently no effective therapies and very few experimental models for mitochondrial diseases. - Proposed experiments should provide greater understanding of the basic pathogenesis of MILS disease. - If successful, the project could lead to the identification and in vitro testing of novel therapeutic agents.

Comments from investigator: We appreciate the GWG for recognizing the importance and uniqueness of our work and its substantial impact on therapy. We want to bring to the attention of ICOC that the role of mitochondrial DNA (mtDNA) in human disease may be underappreciated. For example, in a recent clinical report in the *Journal of the American Medical Association,* 2/10 autistic children were found to have mtDNA deletions and 5/10 had mtDNA replication defects. Moreover, Vogelstein's group (JHU) has recently reported widespread mtDNA heterogeneity in human tissues. Thus, mtDNA mutations are not as rare as once believed. Though MILS disease on its own is a rare hereditary disease, it represents a severe form of general mitochondria dysfunction underling a much broader range of mtDNA and other metabolic disorders. The history of biomedical research has taught us that hereditary diseases almost always provide invaluable insights into more common ones.

Review (continued):

Feasibility and Experimental Design

- Overall, the project featured well-reasoned strategies, methods and generally feasible approaches. - Although the application included a substantial amount of preliminary data relating to aims 1 and 2, critical results demonstrating bioenergetic differences between controls and neuronal cultures from MILS patients were absent. - Potential problems due to heterogeneity in cell proliferation and the possibility that different mutations may affect cellular differentiation potential were not adequately addressed. - Reviewers expressed concern that aim 3 was significantly underdeveloped and not supported by preliminary studies.

Comments from investigator: As the reviewers pointed out, when we submitted the grant application, we had not demonstrated any bioenergetic differences between control and patient iPSCs and neural prgenitor cells. However, since submission, we have used the Seahorse XF instrument, a cellular bioenergetics analyzer, to measure the oxygen consumption rate (OCR), an indicator of mitochondrial oxidative activity, and the extracellular acidification rats (ECAR), an indicator of glycolytic activity, and have found significant bioenergetic differences between MILS patient and control cells. As seen in Fig. 1A and 1B, patient iPSCs and neuroprogenitor cells have a signficantlly lower OCR/ECAR value, indicating that glycolytic activity is upregulated to compensate for an energy deficiency due to the ATP6 mtDNA mutation. Another important finding is that mitochondria in patient cells are not able to maintain their maximum respiratory rate for as long as control cells after FCCP treatment (Fig. 1C), indicating that patient cells also have defects in their abllity to meet increased energy demand. This finding

has important implications for why MILS patients are extremely sensitive to fever. Now, we are extending the analysis to the patient-derived neurons. This remarkable difference in bioenergetics provides a readily measurable marker for patient cells, which will greatly facilitate the proposed studies in Aim 2 and 3; therefore, we expect to complete the project in two instead of three years. We agree with and appreciate the reviewers' suggestions that different mtDNA mutations may affect cellular differentiation potential. For example, dopaminergic neurons are sensitive to mitochondrial dysfunction; therefore, some mtDNA mutations may severely affect the number of dopaminergic neurons in the differentiated culture. This is indeed an exciting question we will pursue. Aim 3 was a proposal to test potential therapeutic agents, but since we are still in the initial stages of characterizing the cellular model, we have not yet initiated these tests, and, in consequence, we do not have any preliminary data.

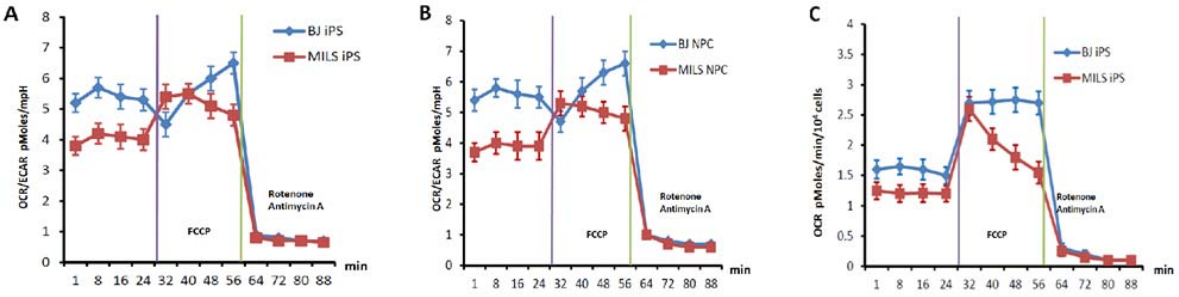

Fig. 1. Oxygen consumption rate (OCR), an indicator of mitochondrial oxidative activity, and extracellular acidification rate (ECAR), an indicator of glycolytic activity, were determined by Seahorse XF analysis. The three mitochondrial inhibitors were sequentially injected after measurement points of basal OCR and ECAR. FCCP is a mitochondrial uncoupler that dissipates the proton gradient which allows the measurement of maximum respiratory rate. Rotenone and Antimycin A are complex I and III inhibitors which block the mitochondria respiratory activity. In all these experiments, three independent MILS patient and control iPSC (**A**) and NPC (**B**) lines were tested. MILS patient iPS cells and neuroprogenitor cells have a lower basal OCR/ECAR (points before the purple line); (**C**) The OCR plot shows that MILS iPS cells are not able to maintain maximum respiratory rate (between the purple and green lines).

Review (continued): Principal Investigator (PI) and Research Team - PI is an outstanding scientist, extremely prolific researcher and leader in the study of signal transduction pathways that regulate the cell cycle. - Reviewers expressed some concerns that the PI and research team has limited expertise in research on either mitochondria or stem cells. - Composition of the research team appears generally adequate to carry out the proposed studies. - An exceptional group of collaborators are associated with the project; however they are not directly involved as key personnel and their level of commitment is unclear.

Responsiveness to the RFA - The proposal is entirely responsive to the RFA

Comments from investigator: The proposed work needs expertise and knowledge in metabolism, neurobiology, stem cell biology and signal transduction, which are beyond any single lab and PIs. As the reviewers pointed out, we have assembled an exceptional group of experts from Salk in these areas. We meet regularly to discuss the project. Each lab has a designated person to coordinate the collaboration and specific experiments. The key personnel, Dr. Xinde, a postdoc in my group, supported by a CIRM training grant, has been intensively trained in stem cell and neurobiology in Dr. Fred Gage's lab in the last three years. In summary, our project is novel, important and feasible; and it fits the spirit of the CIRM initiative to explore the basic science of human disease and conquer them.