CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE

MEMORANDUM

Date: August 31, 2012

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

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application RB4-06277 (**LATE**)

Enclosed is a petition letter from Dr. Yanhong Shi of the Beckman Research Institute of City of Hope, an applicant for funding under RFA 11-03, CIRM Basic Biology IV Research Awards. This letter was received at CIRM on August 31, 2012 (less than 5 business days prior to the ICOC meeting) and we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

August 30, 2012

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

Extraordinary petition for RB4-06277 in response to RFA 11-03: Basic Biology Awards IV Modeling Alexander disease using patient-specific induced pluripotent stem cells

Dear Drs. Thomas, Trounson, and Distinguished members of the ICOC:

My proposal "Modeling Alexander disease using patient-specific induced pluripotent stem cells" received a score of 70, which is 2 points below the initial funding line, and was not recommended for funding. I am writing this extraordinary petition to ask you to reconsider my proposal for funding for three main reasons.

1. In CIRM's portfolio, there are not many applications focusing on fatal diseases of central nervous system (CNS) that shorten the life of children. We propose to study Alexander disease (AxD), a rare, but often fatal neurological disorder. It occurs in diverse ethnic and racial groups with about 12% US cases in California. Symptoms include mental retardation, seizures, spasticity, and psychomotor developmental delay. The most common form of AxD occurs during the first two years of life, and these patients usually die by the age of six. There is no cure or standard treatment for AxD so far; studies on AxD mechanisms and approaches to new therapies can be greatly advanced by using the recently developed induced pluripotent stem cell (iPSC) technology, which is proposed in our research.

2. We now have a powerful tool to uncover the pathological mechanisms of AxD using patient-specific iPSCs. AxD is due to a genetic disorder which primarily affects astrocytes, the most abundant cells in the brain, and is the only known example where astrocyte dysfunction is the sole, underlying cause of the neurological disease. Due to the inaccessibility of primary patient astrocytes, mechanistic studies on AxD have been limited to using rodent astrocytes, human immortal astrocytes, or astrocytoma cells, which are not optimal for disease modeling. Recent development of methods to generate iPSCs from somatic cells makes it possible, for the first time, to generate patient-specific cells for disease modeling. We propose to derive iPSCs from AxD patient skin or blood samples, differentiate these iPSCs into astrocytes, and establish a cellular model for AxD using these patient astrocytes. This study will generate the first human iPSC-based cellular system for AxD disease modeling and drug discovery, and to develop effective therapies for this so far incurable disease.

3. The iPSC-based AxD cellular model will also allow us to elucidate the importance of specific astrocyte functions in the CNS, an understudied research area, and to uncover key pathological mechanisms of astrocyte dysfunction, which should be applicable to many other, more complex neurodegenerative diseases, such as Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis and amyotrophic lateral sclerosis, that also involve astrocyte dysfunction.

I would like to respond to specific points made in the review of this application, regarding (I) Significance and Innovation, (II) Feasibility and Experimental Design, and (III) Principal Investigator and Research Team.

I. Significance and Innovation

1) As stated in the review, "reviewers noted that AxD is a very rare disorder; however, the potential disease mechanisms uncovered by this program may inform more common human neurodegenerative diseases involving protein aggregation and astrocyte dysfunction."

We appreciate the fact that the reviewers saw the potential of our study. It is worth noting that AxD is the only known example of a human neurological disorder where astrocyte dysfunction is the sole, underlying cause of the neurological disease. Therefore, although AxD is a rare disorder, it offers the unique opportunity to develop strategies to restore impaired astrocyte functions in diseased cells. As the reviewers noted, this knowledge will be applicable to the study and treatment of many more common neurological diseases that also have astrocyte abnormalities. In addition, AxD is also a disease of protein aggregation. Understanding AxD pathology could also advance our understanding of other neurological diseases involving protein aggregation. Thus, there is considerable potential impact for this proposal by providing an unprecedented resource to study disease mechanisms and to develop novel therapies for this so far incurable neurological disorder and many other more common neurological diseases that also involve astrocyte dysfunction and protein aggregation.

2) As stated in the review, “while some reviewers did not find the proposed experiments highly innovative, others argued the proposal’s focus on critical yet understudied astrocytes is innovative and of major importance.”

We agree with the reviewers who have recognized the proposal’s focus on astrocytes and the need for this study. The proposed study of developing astrocytes as a cellular model for a neurological disorder addresses an important yet understudied question, therefore could advance the field substantially. This proposal, if funded, will be the first attempt made to characterize human astrocytes in neurological diseases, representing a novel topic of research. We also appreciate that “all reviewers agreed that if successful, the project could yield important results that would contribute to the field.”

II. Feasibility and Experimental Design

1) As stated in the review, “the panel did not find the preliminary data convincing that all techniques required to successfully execute the program were established in the PI’s laboratory.”

We disagree with the reviewers in this regard. Not only have we established key techniques required for this project, including establishing AxD patient iPSCs and wild type iPSC controls, but also we have successfully differentiated both wild type and AxD patient iPSCs into neural rosettes. Since the submission of this proposal, we have derived astroglial progenitors from both wild type and AxD iPSCs and are now in the process of further differentiating these cells into mature astrocytes.

To ensure all the techniques required for this project are available to us, we have formed a collaborative team by bringing together several areas of expertise. This team includes Dr. Juan Carlos Belmonte from the Salk Institute, an expert in patient iPSC derivation and disease modeling, Dr. Su-Chun Zhang from University of Wisconsin-Madison, who has pioneered in establishing the technique for differentiation of human pluripotent stem cells into functional astrocytes, and Dr. Albee Messing, who has been working on AxD for more than a decade and made the exciting discovery that AxD is due to mutations in the GFAP gene in astrocytes. The combined expertise of the team ensures that we have all the techniques required for this project.

2) As stated in the review, “reviewers generally agreed that the proposal is well-structured and follows a logical rationale; however, the interdependence of the specific aims could jeopardize the success of the program.”

We would like to point out that because of the progress we have made to date with the AxD iPSCs and their differentiation, none of the specific aims would appear to be in jeopardy. We

have proposed three Aims for this project. Aim 1: To generate AxD iPSCs; Aim 2: To derive astrocytes from AxD iPSCs; and Aim 3: To model AxD using astrocytes derived from AxD iPSCs. The reviewers may have been concerned that if we are unable to finish Aims 1 and 2, we would not be able to achieve Aim 3. However, we have already established several AxD iPSC lines and have differentiated these iPSCs into astroglial progenitors. As soon as we obtain mature astrocytes from these progenitors, we can proceed with Aim 3 to model AxD, the key part of the proposal. Therefore, the preliminary results we have generated warrant the likelihood for success of this program.

3) As stated in the review, "the narrow pathway focus of the mechanistic studies was judged to be high risk; this risk could be mitigated by including additional signaling pathways."

Our laboratory specializes in studying signaling pathways in the nervous system. We have considered several molecular pathways involved in astrocyte dysfunction and protein aggregation and decided to focus on the AKT/mTOR-glutamate transporter 1 (GLT-1) signaling pathway, because decreased expression of GLT-1 was among the most notable changes in astrocytes from both mouse models and AxD patients. Furthermore, testing whether reduced GLT-1 expression in AxD patient astrocytes could lead to oligodendrocyte death via glutamate excitotoxity would be a novel approach to explaining the pathogenesis of AxD, which could not be recapitulated in animal models. Therefore, this focused approach is the most logical starting point in understanding the pathological mechanism of AxD. As we gather more data in our studies, we may explore additional pathways as discussed in our proposal.

III. Principal Investigator (PI) and Research Team

1) As stated in the review, "the PI has an established track record of studying neural stem cells in mammalian brains and has published in respectable but not top tier journals. She has a more limited tract record in the iPSC field." "Although the PI's lack of experience in astrocyte derivation raised concerns, reviewers agreed that the investigator has an established collaborator who is experienced in astrocyte differentiation."

While my track record is mainly in the study of neural stem cells in mammalian brains, my laboratory has built an expertise in the iPSC field, as evidenced by the funding from both NIH and CIRM. My laboratory has developed a protocol to derive human iPSCs from somatic cells with substantially enhanced reprogramming efficiency under a NIH RC1 grant and has established techniques for patient iPSC derivation and neural differentiation with the support of a CIRM Early Translational III Award. Several manuscripts on iPSC study from my laboratory are in preparation and submission.

Realizing the fact that the iPSC field is rapidly moving, I have recruited two internationally renowned stem cell scientists, Drs. Juan Carlos Belmonte and Su-Chun Zhang, to our research team. As the reviewers agreed, our collaborator, Dr. Su-Chun Zhang, is experienced in astrocyte differentiation. He just published a detailed protocol describing the procedure of differentiating human iPSCs into functional astrocytes in Nature Protocol 2011 and will provide us with the expertise in astrocyte differentiation for the proposed project.

Thank you for considering this petition for funding.

Sincerely,

Yanhong Shi, Ph.D.
Associate Professor of Neurosciences
Beckman Research Institute of City of Hope