



MEMORANDUM

Date: August 31, 2012

From: Alan Trounson, PhD
CIRM President

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application RB4-05816 (LATE)

Enclosed is a petition letter from Dr. Wange Lu of the University of Southern California, 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.



Keck School of Medicine
University of Southern California

Oct 31, 2012

Eli and Edythe Broad
Center for Regenerative
Medicine and Stem Cell
Research at USC

To the Chair of the ICOC and the President of CIRM,

We are submitting this extraordinary petition to re-evaluate our submission to the CIRM Basic Biology IV. After studying the reviewers' comments, we felt that the significance and innovative aspects of the grant were undervalued. In addition, some of the specific criticisms are biased away from the current state of the art in the literature. We have compiled a detailed response to reviewers' comments. The proposed project is clearly one of the future directions of the stem cell field. Such studies will significantly impact our understanding of pluripotent stem cells. We hope CIRM will reconsider and award funding for our proposal.

Review Comment #1: *While studying the chromosome architecture characteristic of pluripotency is useful, reviewers were not convinced that the proposed studies would have a major impact on understanding pluripotency.*

Response #1: The study of higher-order chromosome structure is important for the understanding of pluripotency. We have stated in the grant that nuclear architecture in different cell types are different. During somatic cell nuclear transfer, the nuclear architecture changed significantly within a short period of time. Much evidence has suggested there are must be a pluripotency-specific nuclear architecture and this architecture must plays a role in pluripotency in pluripotent stem cells.

One of the most important areas in the stem cell field is epigenetics changes in somatic cell and pluripotent cells. Much effort has been focused on the epigenome changes such as DNA methylation and histone modification. Very little has been done on nuclear architecture due to technical constraints. Our proposed studies will be a pioneering effort in the stem cell field and will have a huge impact to the understanding of the basic biology of stem cells.

Review Comment #2: *Reviewers were unclear of the significance that the proposed studies would have on developing improved methods of reprogramming, especially given that the efficiency of generating induced pluripotent stem cell lines is already improving with integration-free methods.*

Response #2: While we appreciate the reviewers' point of view, we firmly believe that this proposal is significant. Reprogramming methods have indeed been improved in the past few years with integration-free methods. However, further modifications of this method are still required for its clinical application. The time for iPS cell induction is long and its efficiency is still too low. This may account for the genome abnormality of iPS cells. Using the Oct4 distal enhancer as bait and circular chromosome conformation capture (4C) approach, we have identified novel genes required for pluripotency in mouse ES cells. This approach can be applied to human ES cells to identify novel pluripotency-related genes that can be potentially used to improve reprogramming efficiency.

Review Comment #3: *The research plan was not viewed as highly innovative.*

Response #3: The studies of nuclear architecture is an emerging area. Such studies are made possible now because of newly developed techniques. This grant proposal uses these cutting-edge approaches to determine the nuclear architecture and relate this to pluripotency. Such studies are highly innovative.

Review Comment #4: *Preliminary data provided are sound; however the pluripotency readouts are not adequate, as they do not include an analysis of differentiation potential.*

Response #4: We included analysis of Alkaline Phosphatase staining, and showed that upon Grb7 gene knockdown AP positive cell colonies are significantly reduced. In the grant proposal, we clearly stated the differentiation potential after knockdown of candidate genes were compromised (as data not shown) because of space limitations.

Review Comment #5: *Reviewers felt that Aim 3 was vague and lacking important experimental detail, although conceptually*

interesting.

Response #5: Aim 3 addresses how higher-order chromosome structure is established in pluripotent stem cells. We discovered that CTCF and Rad21 binding were enriched in the Oct4 interactome. Both proteins were chromosome looping proteins and we will test their roles in the establishment of nuclear architecture and in somatic cell reprogramming. Experimental details were similar to what is described in Aim1 and 2, which we clearly indicated in the proposal.

Review Comment #6: *Reviewers were unclear on the rationale for the selection of the cell types that will be compared with pluripotent stem cells.*

Response #6: There are so many somatic cell types that we simply select a few of the most commonly used in the field.

Review Comment #7: *There was some concern expressed that the PI does not have a strong publication record in the particular area of this proposal.*

Response #7: We have two manuscripts under review that are not listed on our biosketch due to CIRM regulations. As we previously discussed, this is a new area of research and limited studies have been performed with 4C and pluripotent cells. We feel that we have an excellent level of expertise in this area.

In summary, the studies of higher-order chromosome structure represent an exciting and important research area in stem cell biology. Our proposal will tremendously impact our understanding of human stem cells and their application in cell replacement therapy.

Sincerely,



Wange Lu, PhD

Associate Professor

Broad Center for Regenerative medicine and Stem Cell Research
USC Keck School of Medicine