CALIFORNIA INSTITUTE FOR REGENERATIVE MEDICINE

**MEMORANDUM**

**Date:** January 21, 2011

**From:** Alan Trounson, PhD
CIRM President

**To:** Independent Citizen's Oversight Committee

**Subject:** Extraordinary Petition for Application RT2-01972

Enclosed is a petition letter from Dr. Craig Levin of Stanford University, an applicant for funding under RFA 10-02, CIRM Tools and Technology II Awards. This letter was received at CIRM on January 19, 2011 and we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

STANFORD UNIVERSITY SCHOOL OF MEDICINE

DEPARTMENT OF RADIOLOGY

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January 19, 2011

To: Chairman of the ICOC and to the President and the Chief Scientific Officer of CIRM
From: Craig S. Levin, PhD, Professor of Radiology, Stanford University School of Medicine
Re: Extraordinary Petition for CIRM RFA 10-02 Application **RT2-01972**: Novel PET/MRI Tools to Increase In Vivo Detection Sensitivity of Transplanted Stem Cells

Dear Chairman of the ICOC and the President and Chief Scientific Officer of CIRM:

We are petitioning for the following information to be considered in support of the above application at the upcoming January 27, 2011 meeting of the Independent Citizens Oversight Committee (ICOC). This application was recently reviewed by the Grants Working Group with a decision not to fund. However, we contend that the stated main reason for not funding in the Review Summary, the concern of "clinical applicability" of the ideas, was actually the focus of our proposal and addressed in multiple sections, but somehow was overlooked by one key reviewer. Please may we ask you to carefully consider our point-by-point response below and re-consider funding this important work. But first, for your convenience, in a few short paragraphs we summarize key sections of the Review Summary:

**Overall Summary of the Application (taken directly out of the Review Summary):**
In summary, the applicants propose to develop a technology to resolve the in vivo cell-tracking bottleneck to clinical translation. Reviewers were supportive of the technology overall and felt the applicants could likely achieve the proposed aims. However, since the proposal only described an instrument suitable for small animals and did not consider the technical and safety issues of translating the method to humans, they were left skeptical of the clinical utility of this method. Therefore the application was not recommended for funding.

**Summary of Positive Review Points (taken directly out of the Review Summary):**
Reviewers agreed the lack of clinically applicable, long term tracking methods to determine the fate of transplanted cells poses a significant translational bottleneck to the development of cell therapies. If successful, the applicants' novel and innovative technology could enable sensitive, simultaneous detection of a smaller number of cells, their location and viability in vivo. This technology could have a broad and major impact on the preclinical development of multiple cell therapies.

The presented preliminary data support feasibility of the proposed project. The panel found the overall plan complex and aggressive, yet achievable, and noted extensive coordination across multiple groups would be required for implementation.

The principal investigator (PI) is an expert in imaging physics and has assembled an excellent multidisciplinary team with respected records in imaging stem cells with reporter genes and stem cell biology. The team is strengthened by their clinical experience with a PET reporter gene method. Facilities are state of the art and the budget appropriate.

**Point by Point Response to Reviewer Critiques:**
**Critique #1:** The applicants will in parallel fabricate a combination high sensitivity PET, high spatial resolution, small animal MRI instrument.

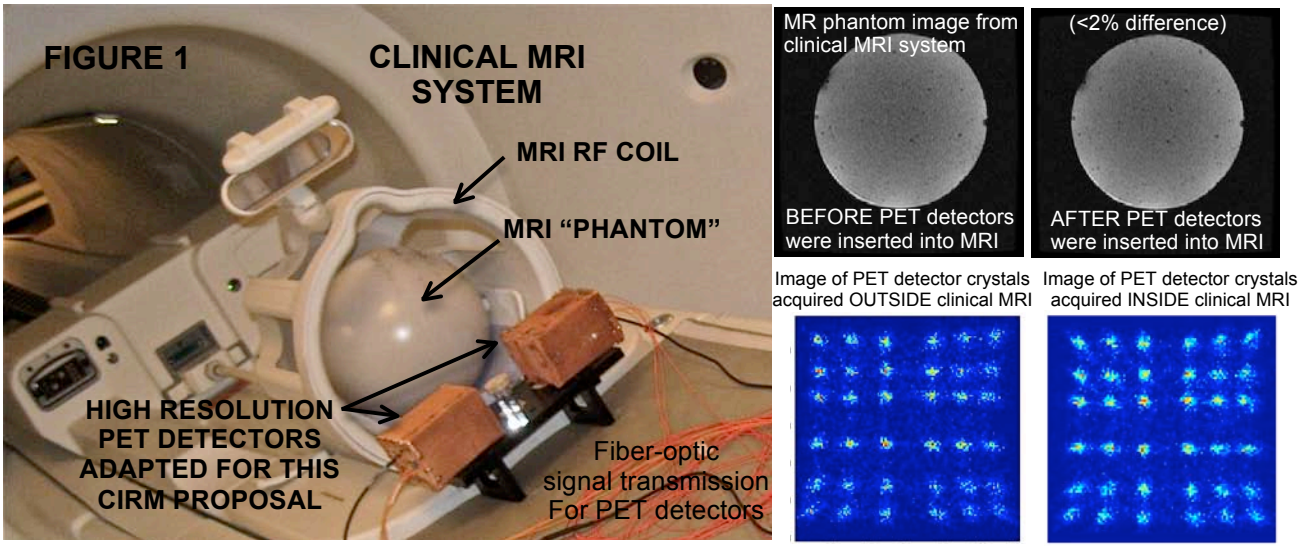
**Response:** It appears there may have been a misunderstanding on this proposed "parallel" development: a high sensitivity, high resolution PET system will be created that can be inserted into an existing small animal MRI scanner. That is, a novel PET system will be developed for reporter gene stem cell imaging, and it will be inserted into an existing MRI system for high resolution anatomical co-localization of the PET reporter gene signal from stem cells in living subjects.

**Critique #2:** Although some aspects of this technology are already being used in clinical studies, one reviewer expressed concern that both the proposed PET/MRI instrument and studies were limited to small animals, leaving open the question whether and how this technology can be translated to larger animals and to humans.

**Response:** Regarding the stem cell imaging assay goals of the proposal, our application proposes to study novel and substantial changes to the current methodology of stem cell imaging using the PET reporter gene strategy. These changes focus on both signal amplification so that 10-fold fewer cells can be detected, as well as the fundamental manner in which the reporter gene is inserted into the cells, with the goal of promoting safety, i.e. reducing or eliminating several perceived risks of introducing genetically modified cells into humans. That is, the whole focus of that work IS on clinical translation into humans. However, the proposed novel PET reporter gene strategies must be tested in animals first before translation in the clinic, which is the focus of the current proposal. This clinical translation motivation/applicability for the work was stated in multiple sections of the proposal, but the reviewer must have missed it.

Regarding the new PET instrumentation goals, the application emphasized that the high resolution, high sensitivity small animal PET system is built from a "shovel ready" technology that is being borrowed from another project we are currently pursuing to build a CLINICAL PET insert for an MRI system. The novel MR-compatible PET detector technology has been tested in a clinical MRI system demonstrating immeasurable interference between the two imaging modalities (**see Figure 1**). For the CIRM proposal we adapt this proven clinical technology to enable the production of a high resolution, high sensitivity MR-compatible small animal PET system capable of visualizing and quantifying greater than 10-fold fewer stem cells in living subjects. This clinical applicability of the instrument technology proposed was stated in multiple sections of the proposal, but the reviewer must have missed it.

Thus, in summary, the proposal is precisely focusing on clinical utility of the proposed technologies to resolve the in vivo cell-tracking bottleneck, and is focusing on advancing imaging assay and instrument **concepts that already are directed toward or have already made their way into the clinic**.




**Critique #3:** The review group highlighted potential technical challenges in the transition from mESC to hESC, including generation and validation of reporter lines from four hESC lines. A discussant noted the entire application depends upon successful non-viral reporter gene integration, yet the proposal lacks alternative plans in case this fails. The review group would have appreciated a more detailed description of in vivo functional testing of the reporter cell lines. Finally, the lack of in vitro/vivo safety testing of the hESC lines with integrated reporter constructs was considered a critical omission for the program as this could affect translatability of the proposed technology.

**Response:** My co-PI Dr. Joseph Wu has extensive experience with creating human ESCs and human iPSC lines that express the reporter genes proposed. This is quite evident by his prolific publication record and by him being generally regarded as a thought leader in the area of stem cell imaging. Here I list some of his recent publications in the past year related to human ESC and human iPSC biology:

1. Sun N, xx, Wu JC. Long term non-invasive imaging of embryonic stem cells using reporter genes. Nat Protocol 2009;4:1192-201.
2. Wilson KD, xx, Wu JC. Dynamic microRNA expression programs during cardiac differentiation of human embryonic stem cells: role for miR-499. Circulation Cardiov Genet 2010;3(5):426-35.
3. Jia F, xx, Wu JC. A nonviral minicircle vector for deriving human iPS cells. Nature Methods 2010;7(3):197-9.
4. Narsinh KH, xx, Wu JC. Increased heterogeneity of human induced pluripotent stem cells revealed by single cell transcriptional profiling. Journal Clinical Investigation 2011; in press.
5. Pearl JI xx, Wu JC. Short-term blockade of leukocyte costimulatory molecules promotes engraftment of embryonic and induced pluripotent stem cells. Cell Stem Cell 2011; in press.

Because of the space limitation in the CIRM proposal, it was *not* possible for us to list *every* alternative approach for every proposed experiment. The idea of using non-viral reporter gene integration with the site-specific φC31 integrase system is quite sound and we already have generated human cardiac progenitor cells and human induced pluripotent stem cells expressing reporter genes using this technique, and already included references for this work in the proposal. In addition, our collaborator Dr. Michele Calos has used the same φC31 integrase system to create iPSCs. Note: Dr. Calos also has a funded CIRM grant, RL1-00634-1, entitled: "Safe Efficient Creation of Human Induced Pluripotent Stem Cells without the Use of Retroviruses" where the goal is similar, except our focus is to create cell lines with reporter genes and hers is to make iPSC lines with site specific integration. Hence we really feel that the criticism of "not including alternative plans" in the proposal should not be deemed as a major problem of the proposal given the considerable experience of our collaborator, the prolific publication record related to stem cell imaging by my co-PI, and the space limitations of the proposal.

**Critique #4:** The very limited 1-2% commitment of multiple key co-investigators was noted as a potential obstacle to successful execution of this complex project.
**Response:** The small % effort for co-investigators was a product of a relatively low funding level of this CIRM award mechanism. However, as evidenced by their support letter, those PI's are dedicated to the success of their particular contributions to this project. Please note that the corresponding post-doctoral fellows assigned from each of those PI laboratories were allocated relatively large % efforts in order to assure that the proposed goals of each lab's contributions will be successfully achieved.

**Critique #5:** Further, reviewers felt it unlikely this large, diverse team would be able to meet weekly as planned. They noted coordination would likely rely on the communication and collaboration of postdocs from the PI and co-investigators' labs.
**Response:** In our program, we have many multi-PI program projects (P50, U54, U01, R01, etc.) that already require weekly, semi-weekly, monthly, and quarterly, meetings of investigators and their staff, in addition to the individual research staff collaborating on a daily basis as needed. So we are very used to having frequent programmatic meetings and so please be assured that the proposed meetings that are necessary for success will take place.

**In summary**, we appreciate the input provided by the Grants Working Group. However, we feel quite strongly that the 2 main criticisms (the clinical translatability of our PET/MRI system and lack of alternative approaches for creating human ESC line with non-viral integration) are to a certain extent unfounded. We have provided significant preliminary data as well as strong publication track record to justify that our CIRM grant is feasible and clinically translatable. We hope the ICOC panel will reconsider the evaluation of our proposal as it represents critical work that needs to be done in order to resolve the bottleneck to clinical translation of in vivo stem cell-tracking with reporter genes. Thank you.

Sincerely,

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