MEMORANDUM

Date: June 18, 2010

From: Alan Trounson, PhD
CIRM President

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application RM1-01742 (**LATE**)

Enclosed is a petition letter from Dr. Wu of Stanford University, an applicant for funding under RFA 09-03, CIRM Stem Cell Transplantation Immunology Awards. This letter was received at CIRM on June 17, 2010 after the requested deadline of 5 business days prior to the ICOC meeting, but we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

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June 16, 2010

Gilberto Sambrano, PhD
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Re: Response to Review for RM1-01742: "Understanding Immunobiology of Human Induced Pluripotent Stem Cells"

Dear Dr. Sambrano,

My colleagues and I would like to address the comments by the reviewers. In light of recent information presented at the International Society for Stem Cell Research (ISSCR) meeting, we believe our proposal warrants re-evaluation. The **major criticism** of our proposal was as follows: **"The reviewers felt the proposal's impact hinged on the critical assumption that the generation of autologous iPS cells will not be a practical clinical solution; this issue has not yet been fully evaluated, and so, the impact of allogeneic iPS cell immunity is unclear."**

We would like to point out that at the present ISSCR meeting in San Francisco, Dr. Shinya Yamanka disclosed that they have created 107 different iPS cell lines from Japanese patients. In the future, they plan to create an iPS cell bank in Japan using non-viral plasmid technique. These iPS cells will be transplanted across allogeneic barriers with optimal HLA-matching and under brief immunosuppressive protocol. After his plenary session talk, Dr. Yamanaka was congratulated by several audience members who echoed the sentiment that different countries across the globe (e.g., US, Europe, Asia, Japan) should create these iPS cell banks, fully characterize these cells, and optimize the protocols for allogeneic iPS cell transplantation.

We believe that with this new information, it is increasingly accepted among the scientific community that the immediate future of iPS cell therapy will involve *allogeneic* instead of *autologous* transplantation. The research we proposed specifically intends to develop strategies to induce immune tolerance to iPS cells during allogeneic transplantation. The information which will be gathered from our proposal will be critical to the development of effective and minimally toxic immunosuppressive therapies. As the field of iPS cell transplantation progresses, these strategies will only become all the more critical and valuable to the eventual success of the field.

The second criticism which the reviewers raised is that we propose to characterize the immunogenic profile of **partially undifferentiated cells (questionable experimental plan)**. However, we would like to clarify that we are not proposing to characterize this cell population

because we believe the cells possess any significant clinical utility. Our group already understands the problem of undifferentiated cells causing teratoma formation, which is a major obstacle for future clinical therapy. We have published several papers related to this issue based on funding from a CIRM Seed Grant (RS1-00322 *"In Vivo Imaging of hESC Derivatives and Tumorigenicity"*, 1/2008-12/2009). Rather, we wish to understand how the immune profile of a cell changes as it is reprogrammed to a state of pluripotency and then back to the differentiated state (e.g., iPS cell-derived endothelial cells). This will generate valuable information regarding which molecules on iPS cell and iPS-derived cells incite an immune response. This will help us determine which of these molecules are potential candidates to target in order to diminish the immunogenicity of iPS-derivative cells.

The third criticism was on the ability of our **humanized mouse to mount immune response ("alloreactivity in this model system has not yet been validated, and alternative plans are inadequately addressed").** We believe we have given robust evidence demonstrating the alloreactivity of this system in our preliminary data (see **Figures 10 & 11**). We would like to emphasize that these humanized mice are obtained through a collaborative research agreement from Dr. Dale Greiner (University of Massachusetts) and Dr. Leonard Shultz (Jackson Laboratory). The two in tandem are considered world's leading experts in humanized mouse models (Kumar P et al, T cell-specific siRNA delivery suppresses HIV-1 infection in humanized mice, *Cell* 2008:134:577-586; Shultz LD, Ishikawa F, Greiner DL, Humanized mice in translational biomedical research. *Nature Rev Immunol* 2007;7:118-130). More recently, their group has confirmed that these humanized mice are capable of mounting a robust allograft rejection response (Racki WJ et al, NOD-scidIL2ry^{null} mouse model of human skin transplantation and allograft rejection, *Transplantation* 2010;89(5):527-536). Thus, we are confident that this model will shed important insights into immunogenicity of human iPS cells.

Reviewer summary statement: "In summary, although the PI, the research team, and the project's innovation are excellent, the proposal's potential impact is limited by unclear rationale, a questionable experimental plan, and lack of appropriate alternative plans."
We hope we have satisfactorily addressed the 3 major issues that the reviewers referred to above. Given that this field is moving so fast, in retrospect, it would not have been possible for the reviewers to have predicted with any certainty that iPS cell therapy in the future would involve an allogeneic and not an autologous transplantation scenario. However, as there is now evidence that this is the likely situation, we believe the contribution which our proposal will make to the field of iPS cell therapy is significantly increased. If the stem cell researchers in Japan are looking at the immunogenicity of allogeneic iPS cell transplantation, we feel that the stem cell researchers in this joint California-Germany possessing significant expertise in the areas of stem cells, immunology, and non-viral iPS cell reprogramming should **not** be penalized for the perceived **"unclear rationale"** in our proposal **"Understanding Immunobiology of Human Induced Pluripotent Stem Cells".** In summary, we believe the "unclear rationale" is actually a "clear rationale" and is being adopted by iPS cell pioneers such as Shinja Yamanaka and his whole team in Japan. Hence, we urge the CIRM scientific and patient advocate committee to take time and review our appeal. A summary of our rationale and impact of the grant is attached below. Thank you for your time and consideration.

Sincerely,

Joseph C. Wu, MD, PhD

Rationale and Impact (up to 1 page. Excess pages will be discarded):

In recent years, there is much interest in using embryonic stem (ES) cells to regenerate tissues and organs. In contrast to adult stem cells, ES cells possess unlimited self-renewal and pluripotency [1]. The ability to differentiate into different cell types has stimulated research in generating neurons, cardiac muscle, hematopoietic progenitor cells, hepatocytes, pancreatic beta cells, and other cell types for potential clinical applications [2]. However, despite the excitement surrounding ES cell research, important issues surrounding immunogenicity have not been fully addressed and strategies to avoid rejection remain largely untested. These are fundamental challenges that must be met before stem cell therapy can become a reality. Previous studies from our group and others have clearly demonstrated that ES cells express major histocompatibility (MHC) antigens and therefore are at risk for rejection [3-5]. We are excited that our recent publication in *PNAS* has shown that the immunological response against human ES cells can be dampened by immunosuppressive drugs, even in a *xenogeneic* mouse model [5].

An alternative approach to avoid ES cell immunogenicity is to derive patient-specific induced pluripotent stem (iPS) cells by transduction or transfection of pluripotency genes or proteins [6-12]. In theory, iPS cells would not face the same histocompatibility barriers as ES cells because they are derived and transplanted into the same person. However, to date no study has investigated whether iPS cells will indeed evade immune recognition if transplanted autologously. Perhaps another more practical concern is whether "personalized iPS cell therapy" will be economically feasible to the population at large. Although the upstream *derivation* process will undoubtedly improve with time, the downstream *validation* process will continue to be both *time consuming* and *costly*. For example, over the past decade, Geron has worked extensively on their licensed human ES line from Wicell (H1, H7, and H9). Yet their latest application involving human ES cell-based product (GRNOPC1) for treatment of acute spinal cord injury is still on hold by the FDA due to concerns of cyst formation (www.geron.com). Since each patient-specific iPS cell line will encounter the same (*if not greater* due to a heterogeneous pool of donors) set of challenges of achieving proper differentiation without teratoma formation, the economics of any commercial company interested in producing different iPS cells for individual patient is a question that may ultimately decide the fate of iPS-based therapies. Furthermore, for acute diseases such as myocardial infarction, liver failure, or stroke, it would be more effective if off-the-shelf products (e.g., ES/iPS cell derived cardiac, hepatic, and neuronal cells) can be administered in a timely fashion (i.e., within a short critical window of time after the injury). One way to meet some of these challenges would be to create an iPS cell "bank" encompassing the most common HLA types along with agents that can modulate the donor cell population and host immune system.

Our proposal addresses **three issues critical to the understanding of immunogenicity of iPS cells** and the development of strategies to induce long-term engraftment of transplanted cells. ***First***, to understand the immunogenicity of iPS cells, we will profile the expression of numerous immunogenic genes and surface proteins at various stages throughout the reprogramming and differentiation process and also compare the immunogenicity of viral vs. non-viral vs. protein-based techniques. ***Second***, we will develop a "humanized" mouse model which will allow us to study how the *in vivo* environment affects the expression of the same immunogenic markers assayed prior to transplantation while concurrently monitoring the allogeneic *in vivo* immune response towards iPS cells. ***Third***, to induce long-term tolerance and engraftment, we will target both the transplanted iPS cells as well as the host immune system. We will decrease the immunogenic potential of iPS cells by decreasing the MHC-I expression and increasing the expression of T-cell apoptosis inducing ligand CD95 (FasL). In addition, we will induce host immunological tolerance to the iPS cells by treatment with costimulatory molecule blocking antibodies. Successful completion of this "forward-thinking" proposal will produce important contributions to both the field of stem cells and transplantation biology.