**MEMORANDUM**

**Date:** June 16, 2010

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

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

**Subject:** Extraordinary Petition for Application RM1-01731 (**LATE**)

Enclosed is a petition letter from Dr. Reed of the University of California Los Angeles, an applicant for funding under RFA 09-03, CIRM Stem Cell Transplantation Immunology Awards. This letter was received at CIRM on June 16, 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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*Elaine F. Reed, Ph.D.*
*Professor and Director*
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June 15, 2010

Robert Klein, J.D., Chair
Independent Citizens' Oversight Committee
Alan Trounson, Ph.D.
President and Chief Scientific Officer
California Institute for Regenerative Medicine

**Re: Extraordinary Petition**
**RM1-01731: Characterizing the Alloimmune Response to Human ES-derived Pancreatic Lineage Cells**
**Primary Investigator: Elaine F. Reed**
**Institution: University of California, Los Angeles**

Dear Mr. Klein, Dr. Trounson and Distinguished Members of the ICOC,

Thank you for the opportunity to submit this letter of Extraordinary Petition in regards the review of our project "Characterizing the Alloimmune Response to Human ES-Derived Pancreatic Lineage Cells"; application # RM1-01731. We appreciate the Reviewer's comments and efforts put forward in review of the proposal. However, we would like to take this opportunity to clarify some issues raised in review that might influence the outcome of our grant.

Elaine F. Reed

Elaine F. Reed, Ph.D.
Director, UCLA Immunogenetics Center
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Thank you for the opportunity to provide this letter of Extraordinary Petition in regards to the review of our project "Characterizing the Alloimmune Response to Human ES-Derived Pancreatic Lineage Cells"; application # RM1-01731. This proposal outlined studies to develop a mouse/human chimeric model, the BLT mouse, as an in vivo system to study rejection of human ES-derived pancreatic cells. The reviewers felt that the project was innovative and would likely have translational impact if successful, but were "split" regarding feasibility in several cases. We would thus like to take this opportunity to respond to the specific issues raised and clarify points that may have been misunderstood by a reviewer.

Our proposal primarily responded to the RFA request for applications that address "the development and verification of animal models to predict the human immune response to allogeneic transplantation". Mouse/human chimeric models are relatively new, and are being used more widely as they become more humanized. The BLT model is the most humanized system to date, and offers advantages that in vitro systems cannot provide, such as the potential to experimentally model human immune responses to transplanted cells. While this system to date has not been used to model hESC-derived PLC rejection, there is sufficient evidence in the literature (Tonomura et al, Xenotransplantation, 2008; cited in the application on pages 3 and 6) that this model can be successfully adapted to this purpose. The majority of our efforts are directed at developing this model, a point that may have been missed by one of the reviewers (Objective, page 3).

One concern raised in review regarded our ability to produce pancreatic lineage cells (PLC) in sufficient numbers to conduct the experiments, and that reporter expression may not be long-term in these cells in vivo. We routinely obtain 5% of ES-derived cells that produce insulin, and only require 5-10 million PLC for transplant into BLT mice. We feel that we can easily produce this many cells, as our team has extensive experience with culturing and differentiation large numbers of ES cells for subsequent transplant into chimeric mice. For our studies on hematopoiesis, we routinely culture over 1 billion ES cells prior to differentiation for each study. Regarding the concern of loss of reporter expression, in our published studies (Galic et al, PNAS 2006; Stem Cells 2008), we noted greater than 90% retention of expression of GFP (under the control of the EF1-$\alpha$ promoter) throughout hematopoietic differentiation of ES cells in vivo, and this persists for at least 2 months (page 10 and Figure 2 in the proposal). If however our reporters do not maintain expression in PLCs, we have proposed the use of other promoters as well (Goal 2, Anticipated results/alternative approaches, page 5 of the proposal). However, if no reporter expression is maintained, we can still perform our analysis of PLC load by monitoring circulating C-peptide levels, physically measuring implant size, and phenotyping cells via flow cytometry (Goal 4, page 6 of the proposal). Thus we will still be able to interpret the results of our studies. We feel that concerns regarding whether engrafted PLCs would not survive long enough to perform trafficking experiments should not be an issue as it is documented that hESC derived PLCs can engraft in immunocompromised animals and remain for over 100 days (Kroon et al, Nature Biotech, 2008) (Goal 3: Engraftment of hESC-derived PLC, page 5 of the proposal). Furthermore, it is also documented that in the BLT model porcine pancreatic islet grafts are rejected partially due to T cell infiltration, which is seen by 4 weeks (Tonomura et al, Xenotransplantation, 2008). Thus we propose to look for trafficking of human T cells to the site of our ES-derived PLCs.

One reviewer was concerned that the immune responses seen in this humanized mouse model has not been validated as being truly human. On the contrary, these NOD-SCID common gamma chain negative mice cannot mount specific murine responses as they are truly immunodeficient, completely lacking murine T cells, B cells, NK cells and having very few dendritic cells. In the BLT system, human progenitor cells differentiate in the context of a human thymic implant, thus they learn that the human cells from which they are made are "self". In addition it has been published by others that human HLA-restricted T cell responses to EBV infection are established in these mice (Melkus et al, Nature Med, 2009), thus human antigen presenting cells are functional. Human immunoglobulin responses are also seen, and B and T cell-mediated rejection of transplanted pancreatic cells has been documented (see above). We have noted human dendritic cells, macrophages and B cells in these mice, all of which could be potential antigen presenting cells (Figure 1, page 9 of the proposal). We agree with this reviewer that it would be important to determine which cell type is presenting antigen, and we will incorporate this question into our studies.

In response to the comment that Aim 2 in particular applies a battery of immunological tests rather than taking a hypothesis-driven approach, we would like to reiterate the stated hypothesis of specific aim 2 (page 4 of the grant application):
Our hypothesis is that transplantation with HLA incompatible hESC-derived pancreatic tissue in hu-BLT mice will induce T cell activation via the indirect allorecognition pathway and generate T effector cells and humoral immune responses against the mismatched HLA antigens leading to rejection. We then describe in detail the methods and assays that we will use to study this hypothesis. The role of the direct and indirect recognition pathways in renal transplant has been studied both in animals and in transplant patients. We and others have studied the role of the direct and indirect recognition in post transplant patients, and have established functional assays to assess the presence, in the peripheral blood, of allo specific and peptide specific memory T cells.

In response to the comment made that the emphasis on direct versus indirect antigen presentation was described as contrived and lacking in vivo correlation, we would like to emphasize that it has been known for over 25 years that there are two major mechanisms by which the adaptive immune system can recognize these alloantigens, namely the direct and indirect pathways of the alloresponse. In the direct pathway, recipient T cells recognize intact donor MHC molecules on the surface of donor antigen-presenting cells (APCs); the indirect pathway involves the recognition of donor antigen which has been processed and presented in peptide form in the context of self-MHC on the host's own APCs. The direct pathway has long been known to be a potent means of eliciting acute allograft rejection, which is partly owing to the remarkably high frequency of T cells with direct allospecificity. In recent years, however, much attention has focused on the role of the indirect pathway in transplantation. While donor APCs within a transplanted organ gradually deplete with time, the indirect pathway continues to present alloantigen to the host immune system for as long as the graft is present. There is now compelling evidence connecting this ongoing indirect allorecognition with chronic rejection, both in experimental models and in the clinical setting. Chronic rejection remains a major cause of loss of transplanted organs, and understanding and overcoming it is therefore a crucial clinical challenge. Finally, our goal was to develop an in vivo model in response to your RFA. Once this model is fully developed, clearly a series of additional interesting mechanistic studies could be performed.

Regarding the effort of collaborators on this proposal, it was felt that the 5% effort of the individual overseeing the generation of BLT mice was too little, and raised an issue regarding commitment to this project. At UCLA we have established a highly successful core facility that makes mouse/human chimeric models on a recharge basis. Thus the technical effort to make these animals is taken into account by costs in the “animals” portion of the budget. The 5% effort requested to cover efforts by the Director of this core facility was needed to help analyze and interpret data, not to generate these mice. We feel that the requested effort is sufficient for this purpose and does not overburden our project with unnecessary salary costs.

We thank the committee for evaluating this response, and regardless of the outcome of our request wish to thank CIRM for their efforts on behalf of the stem cell field.