**MEMORANDUM**

**Date:** June 15, 2010

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

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

**Subject:** Extraordinary Petition for Application RM1-01717

Enclosed is a petition letter from Dr. Loring of The Scripps Research Institute, an applicant for funding under RFA 09-03, CIRM Stem Cell Transplantation Immunology Awards. This letter was received at CIRM on June 15, 2010 and we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

THE SCRIPPS RESEARCH INSTITUTE

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June 15, 2010

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

RE: **Extraordinary Petition for RM1-01717: Thymus based tolerance to stem cell therapies**

Dear Mr. Klein, Dr. Trounson, Members of the ICOC, and CIRM staff:

Thank you for the opportunity to present this petition requesting that the ICOC consider moving our CIRM application into the funding range. The Grants Working Group evaluated the scientific merit of our application and scored it at 67. During Program Review a motion was made to move this application into Tier 1. A large minority (>35%) agreed with the motion and will present their position to the ICOC.

My colleagues in Melbourne and Irvine and I have discussed the scientific review in detail. From our reading of the review, it appears that there were considerably differences in opinion among the committee members. We note that there were many positive comments from reviewers and we appreciate their enthusiasm. Here, for the most part, we will attempt to alleviate the concerns of the reviewers who were less enthusiastic about the application.

**Overview of the proposed project:**

The motivation driving this proposal was the potential to develop a means to transplant human pluripotent stem cell derivatives without rejection or immunosuppression. The idea for the approach came from conversations among the investigators; we were three scientists with very different expertise who shared the same simple goal.

I am a stem cell researcher, and I have been frustrated by the fact that the cells I am developing could only be useful for cell therapy if we can overcome the problem of rejection. My group has a large collection of hESC lines and has developed a well-characterized group of ethnically diverse iPSCs that have different haplotypes (immunologic proteins), representing Caucasians, African Americans, Africans, Filipinos, and others. These cell lines are unique and critical to the success of our project.

Richard Boyd and Ban-Hock Toh at Monash University in Melbourne are immunologists who are pioneering methods to "re-educate" the immune system in adults by rejuvenation of the thymus, which creates the immune system but paradoxically degenerates dramatically with age. They are eager to test their idea with a reality check- to see if using human pluripotent stem cells from a particular haplotype to reconstitute the thymus in a mouse would allow transplanted therapeutic human cells of the same haplotype to engraft without immunosuppression.

Tom Lane, a neuroimmunologist from UC Irvine, has developed the disease model system that we want to use for a practical test of our idea. He studies multiple sclerosis, a degenerative disease that kills myelin-forming glial cells in the spinal cord. He has shown that neural stem cells, which are a relatively easy cell type to generate from iPSCs, can migrate to areas of degeneration and alleviate the damage.

Each of us alone could not accomplish what we proposed; as a group of collaborators we have a unique combination of the tools, the expertise, and the motivation to succeed.

**Reviewers' comments:**

<u>Reviewers' evaluation of the strengths of the proposal:</u>

In general the reviewers were very positive. Reviewers *"appreciated the collaborative efforts of*

*the stem cell and thymic manipulation experts towards the accomplishment of the bulk of the first two aims"* and*…"If successful, it will have broad implications for stem cell-based therapies and organ transplantation by providing a novel approach for the induction of tolerance toward transplanted tissues."* The research team was "*judged to be very qualified to conduct the proposed studies, bringing together appropriate and recognized leaders in stem cells, immunology/thymus biology, and animal model of autoimmune disease*". In programmatic review, our project was described as having *"three unique strengths in support of recommending this application...First, the proposal makes use of three ethnically diverse hPSC lines. Second, the project involves outstanding investigators with expertise in thymic development. Third, the project proposes an advanced model for identification and isolation of thymic progenitors."*

Reviewers' concerns about the proposal:

- Cohesiveness of the project team

The reviewers were concerned that the project will "*require great effort to achieve"* and *"reviewers doubted whether the overall project could be achieved in the proposed time frame."* I understand their concerns and would like to reassure them that all three of us have succeeded in complex multi-investigator projects that require coordination of experiments. I am currently PI for a multi-investigator project that includes scientists from Australia, and have a good record of meeting milestones. I am also a co-investigator in another partnership with Richard Boyd that complements the proposed project. My collaborators and I realize that we have proposed an ambitious plan, but we all have our parts well underway and each participant is ready to launch this new project. The review commented that *"..most of the experiments were thought to be feasible,"* and *"reviewers felt the logistics for quality control and distribution of cells to different labs were straightforward and well thought out."*

- Choice of cell types for animal model transplants

The reviewers *"faulted the rationale behind its choice of cells for transplantation",* and went on to say that "*the supplied preliminary data suggested that mouse oligodendrocyte precursor cells (OPC) can remyelinate neurons in the disease model, but that preliminary data does not support a rationale for this proposal, which plans to transplant an entirely different cell type, neural stem cells."* This misunderstanding was entirely our fault. It is our intention to use hNSCs rather than hOPCs to promote remyelination in the MS model. Although we indicated within the Preliminary Data section that engraftment of syngeneic mouse NSCs promotes remyelination, the legend title for Figure 1 states that GFP-OPCs promote remyelination. The figure should have indicated that when NSCs were engrafted they subsequently differentiated to mature oligodendrocytes/OPCs. Dr. Lane has recently published a paper (June 1, 2010) on the migration of NSCs in his MS model: *Carbajal KS et al. Migration of engrafted neural stem cells is mediated by CXCL12 signaling through CXCR4 in a viral model of multiple sclerosis. Proc Natl Acad Sci U S A.* The mechanism by which NSCs ameliorate pathology in the MS model is unclear; while a reviewer indicated that NSCs *"most likely act through a distinct immunomodulatory mechanism,"* Dr. Lane uses an intraspinal transplantation method rather than intravenous injection, and his evidence points to direct remyelination by the donor NSCs rather than immunomodulation. Also supporting this view is the fact that Dr. Lane has reported that immunologically mismatched NSCs are rapidly rejected.

- Immunogencity of stem cells and methods for generating chimeric thymus.

The reviewers were concerned about specific aspects of the generation and characterization of chimeric mice and offered insightful suggestions. The reviewers stated that *"no description was provided regarding the immuno-competence of the specific animal model proposed. This left reviewers unclear as to whether the transplantation study data pertained to translationally relevant allogeneic responses or to xenogeneic responses."* We understand the confusion, but

we did state in our application that “immune competent C57Bl/6 mice will be used” for these studies, and we will initially inject both human and mouse stem cells. We recognize that the xenogeneic and allogeneic responses differ but in light of previous claims that NSCs are not immunogenic, we have to first determine that ***any*** immune response is detectable.

The fundamental problem of rejuvenating thymus function is a unique feature of this application and one in which our Australian collaborators are recognized world leaders. The reviewers’ concerns arise in part from the challenge we had in explaining the multiple aspects of this approach succinctly enough in the application. Many studies have shown that the thymus establishes self-tolerance through presentation of self-antigens to developing T cells, those which are potentially autoreactive being either deleted by apoptosis or functionally “silenced” by the generation of regulatory T cells (“Treg”). Disturbance of this thymus-based tolerance pre-disposes to autoimmune diseases. We proposed to extend these tolerance mechanisms so that the donor cells are recognized as “self” and not rejected. Tolerance is best facilitated by establishing donor-self chimeric thymus, since tolerance is based on the long-term (permanent) existence of donor antigens in the thymus. Reviewers were concerned that the project to *“differentiate thymic epithelial progenitors from hESCs for construction of a thymic organoid…will require longer than anticipated and may not be achievable within the time frame of the grant”.* We recognize that this is an ambitious goal, and did not make clear that the aim to differentiate thymic progenitors is to be carried out as a collaboration between the Australian lab and mine. My lab has considerable experience in developing robust methods for directing hESC/iPSC differentiation, including endodermal lineages. Because of our other collaborations with Australian scientists, we have already met the practical challenges such as shipping of cells between the sites and having frequent teleconferences in disparate time zones.

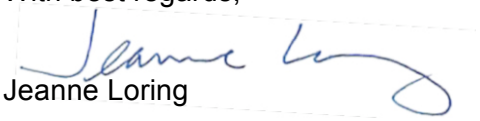
The reviewers had concerns about the details of our methods, including that the transcription factor we plan to use to detect differentiation is not specific to thymic epithelial progenitor cells. We chose to use (GFP) FOXN1 as a marker because this transcription factor is critical to formation of thymic epithelium that has the capacity to create the complete thymus microenvironment necessary for T cell formation. The sharing of markers among multiple lineages is a common event in development, and we are aware of the potential for misidentification. FOXN1 is also expressed in the skin, but we can distinguish the two lineages because thymus is endodermal and skin is ectodermal. Because we are driving the cells through an endodermal pathway (for which we have additional markers) we can safely use the expression of FOXN1 as a marker. We will thoroughly characterize the cells (including molecular profiling) to be sure we have the right phenotype.

Further concerns about the details of our methods can be ameliorated by our Australian colleague’s progress. Since submitting the grant, by modifying the induction conditions they have greatly improved the efficiency of generating putative endodermal hTESC from hESCs. In a single experiment they can generate millions of putative hTESC that have been used to create “organoids” *in vitro* with re-aggregation with pediatric mesenchyme. These organoids are being characterized by transplantation under the kidney capsule of immunodeficient mice.

In summary, our group of collaborators has already made considerable progress toward the goals of our application even in the short time since the application was submitted. We hope that we have alleviated the concerns of the reviewers and will be able to move forward with the support of CIRM and the Victorian government.

Please contact me if you have further questions or concerns.

With best regards,



Jeanne Loring