MEMORANDUM

Date: January 21, 2011

From: Alan Trounson, PhD
CIRM President

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application RT2-01985

Enclosed is a petition letter from Dr. Martin G. Martin of UCLA, an applicant for funding under RFA 10-02, CIRM Tools and Technology II Awards. This letter was received at CIRM on January 20, 2011 and we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

Divison of Gastroenterology/Nutrition/Hepatology
Department of Pediatrics
David Geffen School of Medicine at UCLA
MDCC B2-384
10833 Le Conte Avenue
Los Angeles, CA 90095-1752
Phone number (310) 825-1460

January 19, 2011

Robert Klein, J.D., CIRM Chairman
Alan Trounson, Ph.D. CIRM President and Chief Scientific Officer

Extraordinary Petition

RT2-01985: Pluripotent and Somatic Stem Cell Models to Study Inherited Diarrheal Disorders
Primary Investigator: Martín G. Martín M.D., M.P.P.
Institution: UCLA School of Medicine

Dear Chairman Klein, President Trounson, and members of the ICOC,

Thank you for the opportunity to present this petition requesting that the ICOC support our CIRM Tools & Technology application. We also sincerely thank the reviewers for their kind comments and useful feedback regarding our application.

We carefully considered the reviewers comments and it appears that their primary concern was the lack of sufficient preliminary data in the grant application. This concern made it difficult to assess the feasibility of our proposed experiments. Though the reviewers certainly appreciated the importance of the proposed research project, we believe they simply overlooked important details that were indeed present in the application. We also would like to bring to your attention recently published data by our collaborator that fills the gap described by the reviewers.

The reviewers provided primarily the following positive feedback:

- The proposal addresses a significant bottleneck in the translation of therapies for intestinal disorders.
- Diarrheal disorders are poorly understood, understudied and associated with significant mortality, a poor quality of life that is highlighted by recurrent and prolonged hospitalization, and related health care costs. Thus any improvement in treatment options could have a large and meaningful impact in California, the country, and the world.
- The rationale for the proposal is logical and scientifically sound.
- The PI is one of the world's experts in rare inherited diarrheal disorders, is well published in the field, including articles in high impact journals that are relevant to the proposed studies, highly qualified to oversee the project, and assembled a talented group of well-qualified collaborators to carry out the proposed research.
- An enormous strength of the proposal is the PI's access to a large cohort of identified patients from which to obtain biopsies and make iPSC.
- The potential impact of the research on inherited diarrheal disorders and the strong research team.

We provide the following itemized clarifications and responses to the reviewers' comments:

#1 Reviewer Comment: The main criticism of this proposal was that there is insufficient preliminary data to predict that the specific aims will be successfully achieved.
#1 Response from Investigators: We attempted within the space provided to explain both the clinical condition under evaluation and the breath of the full range of experiments. Indeed, this grant application had four distinct but critical topics that were highlighted in order to enhance the reviewers

understanding of the project, including: 1) a review of congenital diarrheal disorders; 2) an overview of total (exome) genome sequencing; 3) growth of somatic intestinal stem cells; and 4) the development of iPS-derived intestinal epithelium.

We dedicated the third preliminary results page to briefly demonstrate our extensive experience growing primary intestinal mucosa from mice and that we dramatically improved on the recently described methods as outlined by Hans Clevers [Nature. 2009;459(7244):262-5] by recapitulating an intestinal niche by using myofibroblasts.

#2 Reviewer Comment: While the methods and tools proposed are not particularly innovative, their application to the field of gastrointestinal diseases is novel and laudable.
#2 Response from Investigators: We appreciate the reviewers comment that this work in GI disease is novel but we respectfully disagree with the suggestion that the methods and tools are not particularly innovative. We believe the proposal combines the use of three very novel cutting edge tools (intestinal somatic cells, exome sequencing, iPS derived intestines). Furthermore, as of yet, no group has described the techniques for growing somatic human primary small intestinal cells in culture, and it would certainly enhance further research studies ranging from cancer biology to nutrient absorption. We provided preliminary data (page 6 to 9) that we have successfully developed these techniques using murine somatic stem cells, including the development of an ex vivo system that allows us to grow intestines on biodegradable scaffolds. These achievements are noteworthy since they have never been reported in any species.

Most importantly, we provided preliminary data generated from iPS-derived intestinal epithelium by our collaborator at the University of Cincinnati, Dr. James Wells. Indeed, the full manuscript that describes these methods was published in last week's issue in *Nature* (Dec 12, 2010 [Epub ahead of print]), and we believe that this fantastic manuscript certainly highlights the innovative nature of the experiments proposed in this grant application.

#3 Reviewer Comment: The reviewers' noted that that the research plan relies heavily on techniques that are as yet unpublished or have not been established in the applicant's laboratory.
#3 Response from Investigators: Dr. Wells was kind enough to let me use some of his preliminary results for my application but of course I was not able to provide the full details of the methods that were used to generate iPS-derived intestines prior to publication. We did provide a letter from Dr. Wells that he is willing to train our lab on these techniques. As outlined in Wells' *Nature* article, the methods are routine and involve a short period of priming with activinA, and subsequent exposure to Wnt3a and FGF4. These cells are then transferred to a 3-D culture system that we have extensive experience using as outlined in the grant application.

We also mentioned in the grant that Dr. James Byrne, our UCLA collaborator, has extensive experience with ES/iPS techniques, including a letter that emphasizes his commitment to the project. We believe that given our own extensive experience with 3-D culture system, and the various collaborators with experience with ES/iPS technique, that we would be able to apply these various methods in our UCLA laboratory.

The reviewers were correct in suggesting that our lab had not yet established this novel iPS/ES system in our laboratory, but we were prohibited by UCLA from beginning these experiments until we received the approval of the ESCRO committee and obtained IRB approval. We finally received approval in the last several weeks, and we are currently growing the cells for this specific project.

#4 Reviewer Comment: No preliminary data suggesting that they will be able to obtain a sufficient number of crypts from small human biopsy samples to grow gut epithelium in culture.
#4 Response from Investigators: The reviewers are correct that we did not provide data that we are able to obtain a sufficient number of crypts to perform these studies. However, our application does

indicate that we would also attempt to grow somatic cells from human surgical samples (page 10, 13, 14). We suggested that we would utilize our experience with murine samples to develop and refine the conditions and reagents required to generate similar gut organoids obtained from human subjects. Indeed, over the last several months we were able to maintain and propagate human mucosa in an in vitro setting and we will be refining these methods further and developing an ex vivo method as well.

#5 Reviewer Comment: Even if this tissue is grown, there is little acknowledgement of the difficulties associated with using these primary cultures to perform the detailed and technologically complex experiments proposed.
#5 Response from Investigators: Our laboratory has extensive experience performing the various output assessments. These assays include standard immunohistological and nutrient uptake assessments that we have successfully used on mouse and human cells.

#6 Reviewer Comment: The applicant plans to transduce cultured gut organoids with lentiviral vectors, which the reviewers suggested could be extremely difficult.
#6 Response from Investigators: Our application notes our success in transducing the unit organoid niches of murine samples. Due to space limitations, we chose not to reference that the human iPS derived cells are transducible using the lentivirus/adenovirus as outlined in the *Nature* paper, thus making the over-expression and attenuating experiments feasible.

Programmatic Review: No programmatic reason to fund the application was suggested.
Investigators comments on Programmatic Review: We believe that this grant proposal fills an important programmatic need in the area of inherited disorders that effects the underserved pediatric population. Our project is clearly relevant to the CIRM mission to move research to the pediatric clinic, and is therefore a very translational project. The grant will focus on the generation and use of disease-specific iPS cells as described by our collaborators in a recently published *Nature* article. We hope to use these techniques to uncover the molecular basis of several severe diarrheal disorders and that may one day be managed using therapies that target stem cells. On a human note, we emphasize that most children with these disorders have few medical options with the exception of home intravenous nutrition or intestinal transplantation. The reviewers also highlighted, the PI is the world's experts in rare inherited diarrheal disorders and highly qualified to oversee the project.

We have spent the last year obtaining all of the required training and documentations including ESCRO and IRB approval to isolate stem cells for the proposed study. Indeed, just this week we consented our first two subjects and have obtained already isolated fibroblasts from one of them. We have many more very eager families that are looking for answers to their children’s debilitating disorder.

We thank you for considering our comments. We hope that our responses are helpful in your review of this application, which addresses the needs of a truly underserved patient population. Please do not hesitate to contact me if I may be of any additional assistance.

Sincerely,

Martín G. Martín M.D., M.P.P.
David Geffen School of Medicine at UCLA
Professor of Pediatric Gastroenterology and Nutrition
650 Charles E. Young Dr. S.; CHS Rm B2-376; Los Angeles, CA 90095-1752
Phone: 310-794-5532; Email: mmartin@mednet.ucla.edu