MEMORANDUM

Date: October 14, 2010

From: Alan Trounson, PhD
CIRM President

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application TR2-01768 **(LATE SUBMISSION)**

Enclosed is a petition letter from Dr. Deng of the University of California Los Angeles, an applicant for funding under RFA 10-01, CIRM Early Translational II Awards. This letter was received at CIRM on October 14, 2010 and we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

Extraordinary Petition

TR2-01768: Regeneration of Functional Human Corneal Epithelial Progenitor Cells
Principle Investigator: Sophie Deng, MD, PhD
Institute: Jules Stein Eye Institute, University of California, Los Angeles

To the ICOC Chairman and to the CIRM President and Chief Scientific Officer.

Thank you for the opportunity to submit a CIRM Development Candidate Feasibility (DCF) proposal under the RFA 10-01. With due respect for the peer review process, I would like to bring to your attention that there are multiple criticisms that reflect flawed understanding of limbal stem cell deficiency and the underlying stem cell biology which have led to under appreciation of a few key points and impacts of our proposal by the review panel. Our proposal aims to regenerate autologous human limbal stem cells, or corneal epithelial stem cells for transplantation. The specific aim one is to establish a xenobiotic-free culture system to grow human limbal stem cells by replacing the mouse 3T3 cells with human feeder cells. This will allow for immediate initiation of phase I-II clinical trial to start transplantation of autologous limbal stem cells expanded in culture, and this aim alone will achieve the goal of this RFA. We then go beyond this and propose to further improve the growth efficiency, and to regenerate human limbal stem cells via transdifferentiation from skin stem cells by modulating the Wnt and/or Notch signaling pathway using small molecules. The design of our studies has taken careful considerations of the translational purpose of the research. We like to emphasize that we incorporate the testing of the efficacy and toxicity of each small molecule into the screening protocol. We specifically choose small molecules because they can easily be made cGMP-compatible and they do not have long term unwanted effects once removed from the culture. The findings from specific aim two and three will also be readily translated into clinical application. Therefore, success of <u>any one of the three specific aims</u> in our proposal can lead to clinical application and meets the mission of this DCF, making this a high-yield proposal.

1. *Reviewers questioned the applicant's assertion that an alternative cell source was needed to treat bilateral disease, since a recent study described treatment of a patient with bilateral disease using autologous cells grown from a spared limbal region*.

This comment clearly reflects a misunderstanding of the clinical presentation and pathophysiology of limbal stem cell deficiency. The study referred to by the reviewers is likely 'Limbal stem-cell therapy and long-term corneal regeneration' by Rama et al. (NEJM, PMID: 20573916). The case would be patient #24 who had partial limbal stem cell deficiency. In partial limbal stem cell deficiency, there are still surviving stem cells to be biopsied and expanded in culture for transplantation. Total limbal stem cell deficiency means that no limbal stem cells exist on the ocular surface and an alternative cell source is absolutely necessary. The commentary on this particular paper by Dr. Elaine Fuchs in Nature, 'Regenerative medicine: an eye to treating blindness' clearly states the need to find an alternative cell source for bilateral disease. In fact, <u>Dr. Fuchs has proposed skin stem cells as an alternative cell source.</u> The reviewers might not have realized that patient #24 was 1 out of the 112, or 0.9% of the patients treated in that clinical series. Indeed, many of the patients who have been referred to me for consultation of limbal stem cell deficiency do not fall into this category at the UCLA Jules Stein Eye Institute. This indicates that expansion of autologous limbal stem cells in bilateral disease can only treat those patients who still have sufficient residual stem cells for expansion. This again underscores the need to look for alternative cell source to treat total limbal stem cells with bilateral involvement.

2 *Reviewers acknowledged that the proposed project could address a medical need but suggested that the project's impact would be only incremental and, although aimed broadly at corneal disease, would likely only be appropriate for a limited number of corneal disease patients.*

As clearly explained in the proposal, the mainstay treatment in our country is transplantation of non-HLA matched limbal tissues from either living-related donor or from cadaver, which subjects the patients to immunosuppression therapy that carries risks of life-threatening systemic side effects. This form of transplant has an overall 70% survival rate in 5 years. This means that a safe and effective therapy is NOT available in our country. Our proposal could lead to the very first phase I-II clinical trial that could result in a safer, more effective, and patient specific stem cell-based treatment to restore vision without immunosuppression and the associated risks. The proposed study in our application is the most critical step in the bench-to-bedside translation. Without this step, the fruits of stem cell research will be meaningless in patient care. The ability to offer such a patient specific stem cell-based treatment is groundbreaking. The potential impact of our proposal to achieve patient specific stem cell-based therapy is substantial and fits in the ultimate mission of CIRM. Our novel cell-engineering method could set a new model for establishing treatment approach in other diseases.

3. *Clear endpoints and go/no go criteria for many activities were not adequately defined. For example, the selection criteria for establishing human feeder cell lines are poorly defined, and there was no discussion of alternatives if appropriate lines were not identified.*

The reviewers might not have fully appreciated the three parameters clearly stated in the proposal to define the selection criteria compared to the standard mouse feeder 3T3 cells in vitro: 1) proliferation potential by colony forming efficiency (CFE) and Ki67 expression; 2) self-renewal determined by a minimum of 3 serial passages; and 3) maintenance of the corneal epithelial stem/progenitor phenotype based on expression of putative stem cell markers by immunohistochemistry and/or immunoblotting as well as qRT-PCR. We also have clearly specified in the proposal all the putative stem cell markers and maturation markers that will be used in this screening process. The reviewers might not realize that a set value of all of the parameters does not exist because we are working on primary human cells that are isolated from different donors. The value varies among different donors and the number of healthy limbal stem cells. The final endpoint is a level comparable to that obtained using the 3T3 feeder cells, which is clearly stated in the proposal.

In specific aim one, we have proposed to test three, not just one potential human feeder cell line that have been reported to support the growth of limbal stem cells. This implies that if one human feeder candidate fails to match the efficiency of mouse 3T3 cells, there are two alternatives. In addition, we have also proposed to use an alternative culturing method, limbal tissue explant that might support better growth because the tissue contains limbal stem cell niche. We believe that we have proposed sufficient alternative strategies in the event of a candidate failure.

4. *Reviewers felt that the proposal lacked compelling preliminary data in support of the use of Wnt and Notch modulation to regulate LSC differentiation.*

We have provided extensive data generated in my laboratory to show the specific expression of Wnt2, Wnt6, Wnt11 and Wnt16b in the human limbal progenitor cells. Furthermore, activation of Wnt/β –catenin leads to proliferation but does not increase the differentiation of limbal stem cells in primary culture. These findings strongly indicate that Wnt signaling plays an important role in the proliferation of limbal stem cells. In addition, my laboratory and others have shown that activation of Notch 1 is detected in the limbal region. Notch signaling has been reported to

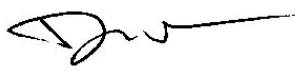
regulate proliferation and differentiation of human corneal epithelial cells. Specifically, Jagged1 induces activation of Notch signaling, stimulates proliferation, and decreases K12 expression (Ma et al. PMID 17652726). Taken together, these data strongly support critical roles of Wnt and Notch in regulation of limbal stem cells proliferation and differentiation. It is entirely feasible to identify Wnt molecule(s) and Notch modulator(s) that could increase the efficacy of limbal stem cells growth without inducing differentiation.

As a board-certified ophthalmologist specialized in limbal stem cell deficiency, corneal transplantation, and ocular surface reconstruction, I have established the first keratolimbal allograft transplantation co-management protocol in collaboration with the Kidney Transplant team at UCLA aiming to improve the long term survival of the allograft. I have pioneered the development of a novel non-invasive test to detect and classify limbal stem cell deficiency using in vivo laser scanning confocal microscopy. I have been awarded a Prevent Blindness America Investigator Award to carry out a clinical study to further test this new technique. In addition, in collaboration with Dr. Jian Yu Rao, Chief of Cytology at UCLA I have established impression cytology as a standard clinical test to confirm the diagnosis of limbal stem cells at UCLA and we are in the process of developing a new diagnostic method that has a higher specificity and sensitivity of detecting limbal stem cell deficiency. These new developments in patient care have made our Corneal Service as a major referral center for patients with limbal stem cell deficiency.

As a clinician-scientist, I have successfully established independent research on corneal epithelial stem cell biology and my laboratory is one of the first to investigate the niche factors and the regulation of limbal stem cells at the molecular level. We are the first to show the roles of Wnt signaling pathway in human limbal stem cells. In collaboration with Dr. Lou Lu at Harbor-UCLA medical center we also start to elucidate the impact of oxidative stress on limbal stem cell survival. Because of my extensive clinical experience in limbal stem cell deficiency and research experience in limbal stem cell biology, I have a clear understanding of the current and future clinical needs in treating limbal stem cell deficiency, and this unique vision has guided the design of this translational proposal to develop patient specific stem cell-based therapy for limbal stem cell deficiency. To achieve this goal I have chosen Drs. Geraldine Weinmaster, William Lowry, Eric Wexler, and Wange Lu as co-investigators who will provide their expertise in Notch signaling regulation, skin epidermal stem cells, human ES cell proliferation and differentiation, and Wnt signaling regulation in neural stem cells, respectively. This unique collaboration across different inter-disciplinary areas in stem cells will ensure the success of the proposed research.

In summary, the specific aim one alone will enable the initiation of phase I-II clinical to start patient specific stem cell-based therapy for limbal stem cell deficiency. Both specific aim two and aim three have the potential to develop a novel and more efficient bio-engineering methods to regenerate limbal stem cells for transplantation. We have the team, the expertise in stem cell biology, clinical and translational research, the reagents, and the environment necessary to carry out our projects. We sincerely appreciate the commitment of CIRM and the Members of the ICOC to support the most promising and advanced translational research to clinical application. Thank you very much for your consideration of our petition for funding.

Sincerely,



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