



MEMORANDUM

Date: April 28th, 2009

From: Alan Trounson, PhD
CIRM President

To: Independent Citizen's Oversight Committee

Subject: Extraordinary Petition for Application TR1-01229

Enclosed is a letter from Dr. Dieter C. Gruenert of the California Pacific Medical Center Research Institute, an applicant for funding under RFA 08-05, CIRM Early Translational Research Awards. This letter was received at CIRM at least five working days prior to the April ICOC meeting, and we are forwarding it pursuant to the ICOC Policy Governing Extraordinary Petitions for ICOC Consideration of Applications for Funding.

As required by that policy, I have reviewed the petition (referencing reviewer comments and the submitted application as necessary) in consultation with Dr. Csete and the scientific staff, and concluded that the petition does not present compelling evidence that should alter the recommendation or score of the Grants Working Group (GWG).

This proposal to "develop stem cell-based therapy for patients with sickle cell disease and beta-thalassemia" was evaluated by the reviewers as a "focused application addressing an unmet medical need with a good chance of success". The reviewers were concerned that the "approach may not be adequate to overcome some substantial technical obstacles".

The applicant acknowledges the legitimacy of the reviewers concerns regarding the technical obstacle but suggests that emphasis placed on these concerns was too great. We believe the concerns of the reviewers are justified and agree with the conclusion that the technical issues in the proposal make it less feasible during the time frame of the award, and, therefore, less competitive. The applicant also provides data that was not available at the time of review. We do not consider and do not think that the ICOC should consider the scientific impact of information or data that was not before the GWG. Under our system of expert scientific review, it is essential that the ICOC have the opportunity to hear the GWG's assessment of scientific propositions asserted by applicants. The Early Translational program will be offered again in 2010.

CIRM staff will be prepared to provide further analysis should that be requested by any member of the committee.

Redactions, if any, have been made pursuant to the policy in consultation with the author(s) of the letter. An unredacted version will be available for review in closed session.

The enclosed letter represents the views of its author(s). CIRM assumes no responsibility for its accuracy.

In addition, a copy of the CIRM Review Summary for this application is provided for reference.



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A Sutter Health Affiliate

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April 19, 2009

Robert Klein, JD; Chairman, Independent Citizen's Oversight Committee
Alan Trounson, PhD, President,
Marie Ceste, MD, PhD, Chief Scientific Officer
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RE: Extraordinary Petition for ICOC consideration of funding application TR1-01229,
"Treatment of Sickle Cell and Thalassemia"

Dear Sirs and Madame:

We are submitting this Extraordinary Petition for your consideration and support of our Early Translational Research Award, RFA 08-05 entitled "Treatment of Sickle Cell and Thalassemia". In this Petition, it is our intent to address the concerns of the reviewers as well as emphasize the importance of the work proposed in the context that it represents a population of individuals that have been underserved by CIRM funding.

Since we are addressing a very significant population that has been underserved by CIRM funding (there appears to be only one grant, Disease Team Planning Award in 2007 to Dr Donald Kohn at CHLA on sickle cell disease), our proposed project directed at the development a therapeutic intervention for sickle cell and thalassemia would appear to be both relevant and appropriate. The large African American, Latino, Mediterranean and Southeast Asian populations in California who suffer from these diseases could substantially benefit from greater and more diverse efforts to develop therapies.

In our application we have proposed several alternative technologies to circumvent the points raised by the reviewers. We are aware of the point raised concerning the potential, albeit small, for integration of the adenovirus vectors. To decrease the potential for rare adenovirus integrants, we therefore propose to use a "gutless" vector that is polycistronic and can readily express 4 distinct reprogramming genes in the context of 2A peptide linkers, instead of 4 individual vectors. In addition, we proposed an alternative plasmid based approach that has been shown to be effective for generating iPS cells without apparent vector integrations. For both approaches, the iPS cells will be screened for the rare, unwanted integrated vector sequences. Only cell lines without integrants would be used/ for further study. There was also a concern about the extensive screening required to identify cell lines without integrants. Such rigorous screening is inherent in selecting any genetically modified cell line used therapeutically. Furthermore, recent studies have indicated that episomal and transposon based systems might also provide a means to generate iPS cells without significant integration or long-term expression. Finally, the RNAa approach, pioneered by Dr Li offers an alternative novel technology that we have already shown to augment expression of several reprogramming genes.

The concern about the classical homologous recombination strategy has been addressed by our SFHR gene targeting data – as the review indicates. While the review summary indicates that there is a key concern about the difficulty in achieving homologous recombination in human cells, and that this process might affect the stability of the iPS cells, in the proposal we have, in

addition to the SFHR strategy, indicated that this approach has been previously used in hESCs and have proposed to modify the somatic iPS cell precursors as well as the directly modifying the iPS cells.

Finally, the review indicates that we will be modulating the Hox B4 gene to achieve hematopoietic lineage directed differentiation and that this presents a risk for leukemia. We (Dr Kan) now have data, which was unfortunately not available at the time this proposal was submitted, that shows erythroid differentiation of human iPS cells by modulating the culture conditions without the direct modulation of Hox B4.

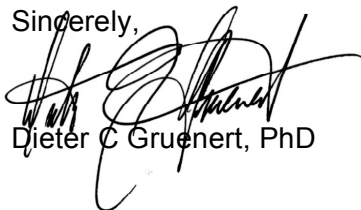
The review also indicates that there is concern that the mouse model proposed is not adequate for translation into the clinic. We are aware of the limitations of any animal model; however, the NOD/SCID IL2RG KO mouse is considered to be one of the better models for recapitulating the human hematopoietic system and sickle cell disease. Furthermore, there are multiple sickle cell models to which we have access. Moreover, we can perform a secondary transplant of the human cells from the original transplantation to determine whether the cells have long-term viability and efficacy.

Overall, the review indicated, on one hand that there was a good chance for success, but, on the other hand, that there were technical issues that needed to be overcome. We have addressed these issues in this petition, and feel confident that we will be able to use our experience and our ability to innovate to overcome technological issues that might arise. These studies require that we are able to generate patient derived corrected clones of autologous cells that can reconstitute the hematopoietic system. The studies proposed are designed to enhance the probability that this goal will be achieved.

This is an opportunity to support an effort that has a high likelihood for success and could be as translatable into the clinic as any preclinical study. In addition, our collaborative group brings together well-established and new investigators that have pioneered a number of techniques and systems outlined in the proposal. Three of the investigators would be new to CIRM funding, while one (Dr Li) has received a New Cell Lines grant, thereby increasing the umbrella of CIRM support and bringing in both new investigators with a broad knowledge and technological base as well as a research institute (CPMCRI) that is a part of a major medical complex/facility with multiple clinical and therapeutic avenues. We are confident that our collaborative efforts will enhance CIRM's overall mission and benefit not only the African American, Latino, Mediterranean and Southeast Asian populations in California, but also have an impact nationally and internationally.

Thank you for your consideration and support.

Sincerely,



Dieter C Gruenert, PhD

TR1 - 1229: Recommended if funds available (70)

EXECUTIVE SUMMARY

The project proposes to develop stem cell-based therapy for patients with sickle cell disease and beta-thalassemia. To this end, the applicant will reprogram somatic cells from each patient to induce pluripotent stem cells (iPSC) that will eventually be converted to hematopoietic stem cells and used to reconstitute the patient's hematopoietic system. Induced pluripotent stem cells will be derived using either non-integrating adenovirus vectors encoding key reprogramming genes or through the use of activating double-stranded RNA to transiently enhance expression of these genes. Once iPSCs have been created, the disease-causing mutations will be corrected by gene targeting, either through classical homologous recombination or oligonucleotide-based homologous replacement. Investigators will test conditions to direct the corrected cells along the hematopoietic lineage to generate multipotent hematopoietic stem cells (HSC). As proof of concept, these corrected, iPSC derived HSC will be tested for their capacity to both engraft and reconstitute the hematopoietic system in myeloablated, immunocompromised mice.

Sickle cell anemia and beta thalassemia are the most commonly inherited genetic diseases worldwide. Current treatment options, including HSC transplantation from HLA identical siblings, unrelated donors, and cord blood transplants, have been curative. However, transplants are associated with morbidity and occasional mortality. Development of autologous therapies for these diseases would avoid the problem of rejection. The PI has a solid publication record in homologous gene transfer technology (SFHR), and has assembled a team that includes a pioneer in hemoglobinopathies as well as experts in homologous recombination and RNAi techniques. This application has achievable goals, but reservations regarding the experimental plan, in particular the animal model, tempered enthusiasm. The applicant proposed adenovirus as a non-integrating tool for the generation of iPSC; however, low rates of integration can occur with these vectors, necessitating extensive testing of the resultant lines. Mutant globin genes will be corrected in the patient cell lines. The difficulty of achieving homologous recombination in human cells was a key concern, and if successful, this process might alter iPSC genetic stability and phenotype. An alternative proposed gene correction strategy, SFHR, avoids this process, and submitted preliminary data has demonstrated SFHR successfully introduced mutations in the beta globin gene of human HSC. The final phase of the study, the conversion of corrected iPSC into reconstituting hematopoietic stem cells, remains a formidable hurdle. The applicant is aware that introducing HoxB4 cDNA, while effective in this conversion, has been complicated by the development of HoxB4-associated leukemia; therefore a transient, endogenous HoxB4 gene activation strategy was proposed. In order for transplants to be curative, long-term reconstitution must be achieved. Transplantation into myeloablated, immunodeficient mice is the gold standard test for reconstitution. Unfortunately, the short mouse lifetime limits the demand on transplanted HSC and therefore limits the ability to predict long-term results. Reviewers felt that a more relevant clinical model may be required for clinical translation.

In summary, this is a focused application addressing an unmet medical need with a good chance of success. The group has extensive expertise and they are using the current state-of-the-art techniques. However, this approach may not be adequate to overcome some substantial technical obstacles.