**Grant Review Appeal Letter**

November 27, 2017

CIRM ICOC Application Review Subcommittee
California Institute for Regenerative Medicine
1999 Harrison Street Suite 1650,
Oakland, CA 94612

Dear CIRM ICOC Application Review Subcommittee members:

The applicants appreciate the CIRM Grants Working Group for reviewing our application TRAN1-10422, which was submitted in response to the TRAN1 solicitation: Stem cell-based therapeutic candidate. We are grateful to hear that the reviewers value the novel cells and the potential of the cell therapy for unmet medical need. The reviewers encouragingly commented the immunologic advantages of the proposed cells and agreed on the animal models that we used to demonstrate the therapeutic efficacy as well as our selected target disease for the study. The reviewers also value the international collaboration with the institute that one step ahead of us.

On the other hand, we also received opposite opinions in the concern section. We understand different reviewers had different opinions and we apologize that our proposal might be not clear enough to gain unanimous consent with the difficulty reviewing in a short time. Here, we would like to correct some of the misunderstandings in some reviewers' comments and would like to appeal to the Application Review Subcommittee of CIRM's governing board and the Independent Citizens Oversight Committee (ICOC) to evaluate our application from the view of the mission and the aim of the TRAN1 project.

First of all, this grant application is not a regular research grant application that aims to explore a scientific interest. Some of the reviewers' comments raise very important scientific questions, however, which should be pursued as a separate research project. Our goal of the proposed project and the aim of the TRAN1 is to accelerate stem cell treatments to patients with unmet medical needs. We have proposed to isolate human primary amniotic epithelial cells, a type of placental stem cells, which possess some therapeutic efficacy for treatment of congenital metabolic disorders, under the clinically applicable GMP condition, and evaluate regulatory parameters to obtain a sufficient data set to discuss with the FDA officials at the pre-IND meeting.

We appreciate reviewers raised a total of 39 points of concerns, however, some of the items are identical. Therefore, these concerns are able to be categorized into the following four areas: (1) appropriateness of animal models, (2) immunological concerns, (3) regulatory input, and (4) feasibility to complete the project in the grant period.

First of all, the proposed stem cell therapy is targeted unmet medical needs to treat rare metabolic disorders. There are a limited number of disease models to demonstrate therapeutic efficacy. Although some reviewers suggested conducting animal studies with different species (Concerns #2, 3, 5, 7), there is no well-established animal models are available to demonstrate the therapeutic efficacy of the hAEC transplantation. We might be able to add different congenital metabolic disorder models in the future, however, we believe demonstrating therapeutic efficacy with four different models is sufficient to move forward. The reviewers suggested conducting studies with adult animals (Concerns #4, 5, 6, 7). The suggested experiment will answer an interesting scientific question from the immunological point of view. However, in clinical point of view the "early detection and treatment" is the principal of the treatment of congenital metabolic disorders. Therefore, we ventured to demonstrate the therapeutic efficacy with the neonatal models during the animal developing disease phenotypes. We are currently conducting the human AEC transplantation to adult ornithine transcarbamylase deficiency (OTC) model mice to elucidate the xenogeneic therapeutic mechanism, however, it is not a topic of this TRAN1 project. There is a clear misunderstanding about our pre-clinical studies (Concerns #1 and 3). We have conducted four different disease mouse models. Although the reviewers misunderstood these models are immunodeficient or syngeneic, three of them, the intermediate maple syrup urine disease (iMSUD), OTC and phenylketonuria (PKU) model animals, are fully immunocompetent mice, and all cell transplantation studies were in xenogeneic combination.

Regardless of these misunderstanding, all immunological concerns are not applicable to this proposal (Concerns #1, 3, 13, 14, 15). As we proposed, we will conduct HLA typing in order to provide immunotype-matched cells to each patient in the future. The immunotype-matched cell transplantation will not require life-long immunosuppression unlike organ transplantation and hepatocyte transplantation. This is one of the advantages of using the placenta stem cells, which is readily available stem cells.

We appreciate and share the concerns on the regulatory strategy. After we submitted the proposal on August 4th, the applicants held a meeting with the director, Dr. Pharm, and the members of International Center of Regulatory Science, University of Southern California. The Associate Director, Dr. Kuo agreed to join our project team and will be consulting on the all regulatory aspect including preparing the pre-pre-IND meeting package (attached please find the support letter from Dr. Kuo). Dr. Kuo and his graduate students are currently assembling the package for submitting to the FDA by the end of November. We believe this team of experts on the regulatory strategy will satisfactory convince our ability to pursue this proposed project. All concerns regarding regulatory issues will be cleared by the time when the grant will be started (Concern #16, 21, 22, 23, 24, 25, 26, 34).

There is another misunderstanding about establishing small-scale biobank. We proposed to establish a small-scale biobank with hAECs from 50 different placentae to demonstrate the feasibility and obtain the data to estimate the HLA coverage in the southern California population. We did not propose to establish cell lines like establishing cancer cell lines from clinical samples. This misunderstanding might lead the reviewers to the concerns on feasibility to complete the project in the grant period (Concerns #35, 36, 37, 38). One of the advantages of the hAEC is the abundant cell number. An average of 200 million cells can be isolated from one placenta. Therefore, it does not require expanding cells (mass production) to establish a biobank. In this proposal, we will establish a small-scale primary hAEC biobank (Concerns #21, 31, 32). Also, this misunderstanding led the reviewers to the concern on the order of HLA typing and cell banking. Since we cryopreserve all primary hAEC immediately after the cell isolation, the reviewers' concerns on the order of procedure are not applicable (Concerns #27, 28, 29, 30). The information from the small-scale biobank with HLA typing is essential to determine the diversity of the accessible southern California population for our project in order to estimate the HLA coverage for establishing a clinical grade biobank network. Therefore, the clinical impact of this small-scale biobank is significant (Concern #28).

The proposed project is designed to meet the CIRM required the TRAN1 timeline. According to our preliminary research with the clinicians at the maternal hospital, the availability of healthy non-pathogenic placenta is not a concern. The manpower will be the limiting factor to complete the project in the proposed timeline. Therefore, we requested a budget to assemble the cell procurement team who can isolate at least one case per week. Based on the safe estimate, we are able to process at least 100 cases (placentae) in 2 years. We estimated the half of the cases will be characterized by their HLA and pathological profile. Therefore, we are confident with the feasibility of the proposed project.

Followings are the provided reviewers' concerns and our brief answers in blue font.

**Concerns**

1. Some animal testing needs to be done in an immune competent model
    - Three of mouse models that we used to demonstrate the therapeutic efficacy in our pre-clinical studies are immunocompetent model.
    - The immunological concern does not apply to the proposed project. Because we proposed to conduct HLA typing to provide immunotype matched hAEC to each patient.

2. Work in additional species would be helpful
    - There are no congenital metabolic disease model animals except rodents.
    - It is not clear what information will be gained from the studies with additional species.

3. All studies are in rodent models; often in immunodeficient or syngeneic animals
    - Three of mouse models that we used to demonstrate the therapeutic efficacy in our pre-clinical studies are immunocompetent model. All studies were done in xenogeneic combination.

4. Would like to see more details of the animal studies; testing in older animals closer to what may occur in humans would be helpful
    - The applicants appreciate the reviewer's scientific interests. The preliminary data section of this proposal focused on demonstrating therapeutic efficacy of hAEC in pre-clinical models. The research data provides insight of the therapeutic mechanisms will be published soon from our lab.
    - The dynamic human cell behavior in human liver structure may be learned only from the previous human hepatocyte cell transplantation studies due to the species differences.

5. Treatment in immature mice with immature livers may not translate well to people; other models should be tested with more mature livers, similar to patients
    - Limited animal models for these rare disease

- We are currently conducting the cell transplantation in adult mice. However, none of animal models can be exactly simulate the patients' liver due to the species difference in the structure, anatomy, and lifespan.

6. It is not clear if cells will engraft the in the same numbers in older animals
    - One of the advantages of cell replacement therapy is that the treatment (cell transplantation) can be repeated with minimally invasive procedure until the therapeutic efficacy is observed.

7. Animal study data was weak, another animal model should be used in proof of concept testing to demonstrate cell function in older animals
    - As described above this concern is not applicable.

8. Very little "own" preliminary data (most of the animal data generated by applicant is not published)
    - Two out of four disease models are already published. A manuscript about study data with PKU model is under revision. A manuscript describes OTC mouse model study is in preparation.
    - Three animal model studies (Hurler, iMSUD, OTC) were supported with previous CIRM funding. The report is available in the CIRM website.

9. Applicant needs to define clearly the expectation for engraftment and required frequency of treatments to maintain an effective dose
    - As described in the application, the frequency of treatment will be varied depend on the patients' enzyme activity as previous clinical hepatocyte transplantation demonstrated. However, the effective dose will be estimated from the data that will be obtained from this proposed study as one of the data sets for the pre-IND meeting package.

10. There is little discussion of risks, including the longevity of the engrafted cells
    - We have shown long-term disease phenotype improvement in Hurler, OTC models (up to 21 weeks).
    - No tumorigenic characteristic of hAEC has been shown by many different researchers, therefore the longevity of the engrafted cells is not considered as a risk factor.

11. The durability of the treatment appears unknown
    - We agree with the reviewer's question. We have shown long-term disease phenotype improvement in Hurler, OTC models (up to 21 weeks). However, this is a common limitation of pre-clinical studies for any type of biological materials.

12. There are concerns related to the durability of treatment in humans
    - We agree with the reviewer's question. We have shown long-term disease phenotype improvement in Hurler, OTC models (up to 21 weeks). However, this is a common limitation of pre-clinical studies for any type of biological materials.
    - Please consider that this is an approach for "unmet medical needs". There is no alternative therapy for these diseases.

13. Concerns about the loss of immune modulation after engraftment/differentiation were not addressed in application
14. Additional data is needed to support lack of immune response of final differentiated cell product
15. It is unclear if the immunologic advantages are still present when the cells mature in vivo
    - These concerns are not applicable because we proposed to transplant patients' immunotype matched cells.
    - New report will be published soon from our collaborators in regard to the immunological characteristics of hAEC. However, it is not applicable to this proposal.

16. A more rigorous risk assessment is needed
    - We will consult with the FDA as a pre-pre-IND meeting for the requirement from the FDA.

17. Perhaps too ambitious a project: focus on one metabolic disease may be helpful
18. Focusing on the metabolic disorders that don't have currently available therapies is recommended; the probability of success is relatively higher with the greatest unmet medical need and should also assist w/ reasonable timeline

- At the next stage of our project to translate this stem cell therapy, we will decide to one metabolic disease as the target disease and focus on the therapy for the disease. In this proposal, we will focus on collecting GMP grade hAEC characteristics.

19. Regulatory strategy should be reviewed; targeting multiple diseases is not a good idea and the team should focus on a single indication and review strategy for the U.S.
20. The application would benefit from a stronger regulatory strategy; for example, it is not clear what the target indication is for the pre-IND meeting
21. A clear understanding of the regulatory requirements for manufacturing scale-up was not demonstrated
22. Need stronger input from someone with experience with regulatory bodies
23. Regulatory input was weaker than expected
24. Applicant would benefit from a pre-pre-IND in order to obtain additional regulatory advice
25. Applicant should consider additional studies to support a pre-IND meeting
26. Donor eligibility criteria did not appear to be met for US FDA regulations (21CFR 1271)
    - We have assembled a regulatory strategy team with experts.
    - The pre-pre-IND meeting package will be submitted to the FDA by the end of November.
    - The input from the pre-pre-IND meeting will be integrated to the proposed TRAN1 project.

27. Human leukocyte antigen (HLA) matching strategy was not well thought out - are making 50 cell lines and then doing HLA typing
28. It was not clear if the 50 HLA typed hAECs would have a meaningful clinical impact
29. HLA coverage of the cells banks should be addressed
30. HLA matching issue: should do before banking
    - The proposed small-scale biobank with 50 different primary hAECs will be established.
    - Primary hAECs will be cryopreserved immediately after the cell isolation. We do not establish “cell lines”.
    - The HLA typed 50 cells will provide diversity information to estimate the HLA coverage in the southern California area.

31. The ability to manufacture large quantities of cells is unproven
32. Unproven track record in mass production of cells
    - Primary hAECs will be cryopreserved immediately after the cell isolation. We do not expand cells.
    - The abundant hAEC does not require “mass production”.

33. Transferring the process to the U.S. and manufacturing all of the proposed cell banks is a big concern
    - We will use the EU regulatory information as a reference to prepare a FDA acceptable pre-IND package. Any cells or reagents will not be transferred.

34. Quality control testing not adequately addressed
    - We will follow the suggestion from the FDA.

35. Timeline is likely not reasonable for establishing 50 master cell banks as cell bank testing is going to be pretty significant - but should discuss with the FDA
36. Too many cell lines planned; may not be feasible
37. Tight and unrealistic timeline
38. Very aggressive timeline for this approach
39. Several practical issues reduce feasibility
    - Primary hAECs will be cryopreserved immediately after the cell isolation. We do not establish “cell lines”.
    - We have estimated the number of cells based on the clinical record from the maternal hospital and designed the project to meet the two-year time frame of the TRAN1 application.

USC School of Pharmacy

**International Center for Regulatory Science**
*Eunjoo Pacifici, Pharm.D., Ph.D.*
*Director*

September 5, 2017

Toshio Miki, M.D., Ph.D.
Assistant Professor of Research
Department of Surgery and Biochemistry & Molecular Medicine
Keck School of Medicine of USC

Dear Dr. Miki,

Thank you for inviting us to be part of your proposal "cGMP-grade placental stem cell production and characterization for treatment of congenital metabolic disorders." We are fully committed to support and participate in the execution of FDA regulatory aspects of your project and to provide relevant services.

Putting a laboratory discovery into clinical use is seldom a straightforward process. It requires understanding of the regulatory process and a dedicated team of research scientists, regulatory scientists, clinicians, and intellectual property experts to come together in order to achieve FDA approval. As the Associate Director of Consulting for the International Center for Regulatory Science at USC and a certified Regulatory Affairs Professional, I, along with Dr. Eunjoo Pacifici, can help the team obtain the knowledge that will be needed to navigate this process and provide consultation throughout.

In particular, I will advise on preparing a questionnaire for a pre-pre-IND meeting, and facilitate the communication with the FDA before you start the proposed project. Once your project has launched, I will advise on preparing a package for the pre-IND meeting with data that you will obtain from this proposed project. The scope and details of service and billing will be covered in a separate document.

I wish you best of luck, and look forward to working with you.

Sincerely,

C. Benson Kuo, Ph.D. RAC, Patent Agent
Associate Director, Consulting Service
International Center of Regulatory Science
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