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March 23rd, 2023

Dear CIRM Program Officers,

We thank the CIRM Grants Review Working Group for their consideration and insightful review of our application, entitled *Overcoming barriers for airway stem cell gene therapy for cystic fibrosis*, for the Discovery Stage Foundation Award (DISC0) program. Our team was delighted to learn that our proposed project resonated with the reviewers and received unanimous, positive reviews about the strengths of our proposal and a score of 85, which is in the fundable range. With this letter we wanted to address the four points raised by the reviewers on specific issues. We are able to address all of these points as described below:

1. "No discussion is provided on how sustained the proposed therapy is expected to be. While the cells are long-lived, the therapeutic effect from the gene transfer might not to be permanent. A discussion of potential clinical scenarios would be useful for assessing the scientific rationale of the project."

The proposed gene therapy strategy is targeted at the basal stem cells in the airway, which are the long-term, self-renewing source of the differentiated cells that express CFTR. In this clinical scenario, there is site-specific gene insertion into the genomes of the basal stem cells for CF patients. These genetically modified basal stem cells should persist long-term. However, as we are proposing an airway delivery strategy, redosing should be possible without limiting immune responses, if it is needed in case patients lose expression of CFTR after months to years.

2. "To address the sustainability of the therapy, the investigators could possibly take advantage of a functional Ussing Chamber assay that measures the short-circuit current as an indicator of net ion transport taking place across an epithelium proposed in Aim 3. For example, they could carry out a time course of the epithelial ion transport in transfected CF patient cells differentiated into air-liquid interphase cultures."

This is an excellent suggestion. We will incorporate Ussing chamber CFTR current measurements into our analysis of CF-patient airway basal stem cell (ABSC) air-liquid interface cultures in a time-course to assess the duration of therapy.

3. "However, all the proposed work will be conducted in vitro in reductionist model systems. This is justified, given an early stage of study. However, with an eye for future clinical applications, the absence of an in vivo model makes the project risky. There is a concern about the proposed model, which is far from reproducing what happens in the lungs of CF patients. It is very likely that the proposed experiments will work in this model, but in patients with an increase in mucus viscosity and extensive biofilms, it is a high risk."

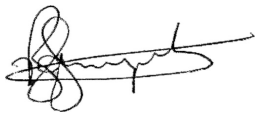
Beyond the scope of this Discovery Stage Foundation Award, we do plan to use the CF rat and ferret models to recapitulate the in vivo CF airway with the appropriate bacteria and biofilms. To inform these studies, the foundational research proposed in the present project

in Aim 3.3 uses highly representative air-liquid interface models of the CF airway from patient derived CFTR-null ABSCs. This stem cell system can produce mucus with the same viscoelasticity present in the CF airway, mimicking the CF pathophysiology.

4. "Off-target effects should be better proaled (sic) with therapeutically relevant doses".
   We will assess off target effects with the higher doses needed for therapeutic effect.

We appreciate these suggestions of the reviewers and are confident that we will successfully complete the aims of this project to advance towards clinical applications. Drs. Jonas and Kohn will be available to join the upcoming ICOC call on March 28th to address any questions during the discussion of our project. We hope that the overall enthusiasm, positive evaluation, and discussion of our proposed research will enable further consideration for funding moving forward.

Sincerely,



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