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March 11, 2016
To: CIRM ICOC members
From: Jeanne Loring and Andrés Bratt-Leal
The Scripps Research Institute

Regarding: Significant new information not available at time of grants working group review on **February 11th, 2016**: **TRAN1-08468**

Dear ICOC Members:

We are writing to inform the board of significant new information that we believe would have affected the recommendation by the grants working group (Appendix A, attached) had it been available at the time of review of our application on **February 11th**.

Our team submitted a TRAN1 application to develop an autologous cell therapy for Parkinson's disease using induced pluripotent stem cells. The application was submitted on **November 20th, 2015**. Between November and February, we generated new information that was not available to the GWG. Importantly, we also received guidance from the FDA that alleviates the major concerns of the reviewers.

We summarize the new information below, and we provide a detailed point-by-point list of new information (Appendix B, attached) that would have alleviated the reviewers' concerns if the information had been available to them.

Publication and grant approval. A serious concern raised by the GWG was that we would not be able to obtain useful information from our quality control assessment using whole genome sequencing. This concern has been addressed by a recent peer reviewed publication published by the Loring lab in Nature Communications (available online **February 19th, 2016**), entitled, "Whole-genome mutational burden analysis of three pluripotency induction methods"; Kunal Bhutani, Kristopher L. Nazor, Roy Williams, Ha Tran, Heng Dai, Željko Džakula, Edward H. Cho, Andy W. C. Pang, Mahendra Rao, Han Cao, Nicholas J. Schork & Jeanne F. Loring.

This publication, which was recently featured by the CIRM Blog ("*CIRM-funded study suggests methods to make pluripotent stem cells are safe*" posted February 22nd, 2016) used comparative genomic analysis of nine iPSC lines generated using three reprogramming methods, including the Sendai method that we are using.

Had we been able to cite this paper, we believe that the GWG would not have been concerned about the use of sequencing as part of our cell line assessment. In a brief scan of reviews of other applications, we noticed that this issue came up several times. Our publication provides the methods and code we used for the analysis, and since it is publicly available, other applicants will be able to adopt our analysis methodology for their own whole genome sequencing studies.

In addition to this publication, on **February 24th**, we received notice that our **Center of Excellence in Stem Cell Genomics** (CESCG) (Call 2) Collaborative Research Project application was approved. Our CESCG award is specifically focused on using whole genome sequencing and RNA sequencing of the

Parkinson's disease patients for quality control in our study. The RNA sequencing data will be used to inform quality release criteria for the differentiated cells to be used for transplantation.

Several other concerns of the GWG have been addressed by two other events that occurred after the submission of our application:

1) On **February 15th** we completed a **6-month study in which dopaminergic neurons from three patients were transplanted to an immunodeficient (nude) rat model of Parkinson's disease.** The cells from the 3 patients all reversed the parkinsonian phenotype in the rats at the same rate. Examination of the rat brains showed healthy transplanted cells and extensive outgrowth of fibers. The study provides evidence of the following: we are producing functional dopaminergic neurons which engraft long term and maintain their phenotype with significant outgrowth.
2) On **February 17th**, we completed our Pre-Pre IND meeting with the FDA, which addresses several concerns of the GWG. The committee advised us that we were on track to proceed to the Pre-IND meeting and recommended only a few changes to our plans.

One GWG concern was that our safety study was designed with too few animals. Our Pre-Pre IND briefing document described our plans for pilot safety studies. The **FDA advised us that the number of animals in our proposed safety study is appropriate, pending the outcome of our pilot studies.**

Another concern raised by the GWG was the possibility that cells produced from different patient cell lines would be variable. This concern was not shared by the FDA because the feedback we received was that **our study design was sufficient to determine the amount of variability among cell lines and to inform the design of our IND-enabling studies**.

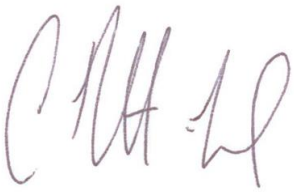
The completed animal study, as well as our ongoing development of quality control gene expression profiling analyses for cells to be used for therapy ("NeuroTest"), supported by a CIRM Tools and Technologies grant awarded in October, 2015 (**CIRM RT3-07655: *User-friendly predictive molecular diagnostic assays for quality control of stem cell derivatives for transplantation and drug discovery*),** demonstrates our successful strategy to reproducibly produce therapeutically effective cells from iPSCs from different patients.

In summary, based on our new data, our DNA sequencing publication, the recent approvals of two of our quality control-focused CIRM grants, and feedback from our meeting with the FDA, we believe that we are ready to proceed on our pilot studies to inform our IND-enabling studies.

Some of the GWG concerns conflict with the guidance given by the FDA, and had the GWG been aware of the feedback we had received from the FDA, many of their concerns would have been addressed.

Please see detailed responses to the reviewers' concerns. We welcome questions and are happy to clarify any issues that may concern the ICOC.

With best regards,



Jeanne F. Loring, PhD

Andrés Bratt-Leal, PhD

Appendix A: Summary of GWG review, as provided by CIRM staff

Application #	**TRAN1-08468**
Title (as written by the applicant)	Autologous cell therapy for Parkinson's disease using iPSC-derived DA neurons
Translational Candidate (as written by the applicant)	Autologous dopaminergic neurons derived from patient-specific induced pluripotent stem cells
Area of Impact (as written by the applicant)	Parkinson's disease
Mechanism of Action (as written by the applicant)	The proposed candidate is intended to replace the lost dopaminergic (DA) neurons in the brains of Parkinson's disease patients. It is estimated that by a time patients are diagnosed with Parkinson's disease, they have already lost over 50% of their DA neurons in their brains. Earlier studies using fetal tissue demonstrated proof of principle for cell replacement therapy. We will use highly qualified patient-specific DA neurons to eliminate the need for immunosuppression.
Unmet Medical Need (as written by the applicant)	Currently, there is no treatment for Parkinson's disease that can stop the progressive degeneration or replace lost neurons. Current treatments, including pharmacological intervention and deep brain stimulation only provide limited relief and decline in efficacy with time.
Project Objective (as written by the applicant)	A well-prepared pre-IND meeting.
Major Proposed Activities (as written by the applicant)	• Assess in vivo behavior with a dosing study, combination tumor/biodistribution/toxicity study and cell delivery using a large animal model. • Characterize comparability between patient cell lines, determine final product and develop in process and release testing. • Transfer technologies, protocols and cells to a cGMP facility for banking and cell production under cGMP conditions.
Statement of Benefit to California (as written by the applicant)	Thousands of Californians suffer from the degenerative effects of Parkinson's disease, a disease for which there is no cure. There is hope, however, that stem cells could provide the key to providing long-term relief. Our study seeks to treat patients with cells derived from their own stem cells, a process which could be applied to other diseases such as diabetes and heart disease and could potentially be used to the benefit of many of the citizens of California.

Funds Requested	$7,971,025
GWG Recommendation	***Tier 2 – Not recommended for funding.***

TRAN1-08468

TRANSLATIONAL

Final Score: 70

Up to 15 scientific members of the GWG score each application. The final score for an application is the average of the individual member scores. Additional parameters related to the score are shown below.

Median	70
Standard Deviation	5
Highest	80
Lowest	60
Count	14
Tier 1 (85-100): Exceptional merit and warrants funding, if funds are available.	0
Tier 2 (1-84): Not recommended for funding.	14

Score Influences

Proposals were evaluated and scored based on the criteria shown below, which are also described in the RFA. The scientific members of the GWG were asked to indicate how their evaluation of the proposal against each criterion influenced their overall score. The total number of reviewers indicating a positive, negative, or neutral influence for each criterion is shown.

Criterion	Positive Influence	Negative Influence	Neutral Influence
Does the proposal have a potential for impact?	8	2	4
Is the rationale sound?	7	3	4
Is the proposal well planned and designed?	2	9	3
Is the proposal feasible?	1	6	7

Reviewer Comments

The following is a compilation of comments provided by multiple reviewers following the panel's discussion and scoring of the application. All reviewers were asked to provide brief bullets on key strengths, concerns, or recommendations related to the proposal that CIRM compiled and edited for clarity.

Strengths

- The overall plan of producing autologous iPSC-derived A9 dopaminergic neurons is supported by data and discussions that have been on-going in the Parkinson's Disease (PD) research community. An autologous approach is expected to be feasible in PD patients.

- The current team is well-suited for the cell production component of the study.

- The project has significance and has sufficient impact.

Concerns

- Although the strategy is promising, this proposal is not yet ready for translation. It is missing key methodological details. For example, the development plan and logistics have major flaws. There is also a lack of preliminary efficacy data.

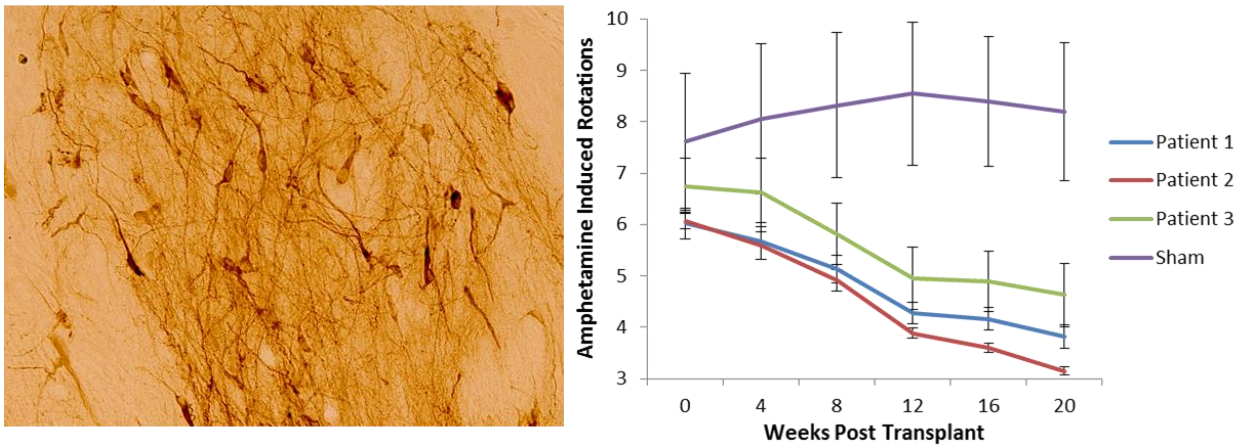
Appendix B: Detailed responses to reviewers' concerns.

Concern:

- "Although the strategy is promising, this proposal is not yet ready for translation. It is missing key methodological details. For example, the development plan and logistics have major flaws. There is also a lack of preliminary efficacy data".

Response: additional information.

New Results: Animal study in collaboration with Curt Freed – In our application, we included preliminary data from an animal study performed by Curt Freed, Ph.D. at the University of Colorado through 12 weeks. We now have data 20 weeks post implantation, which conclusively demonstrates **very consistent results among iPSC-derived dopaminergic neurons from three different patients.** The recoveries were comparable in animals transplanted with patient cells. Further, this recovery was sustained through 20 weeks. **Supplemental Figure 1** shows that there was substantial outgrowth of TH+ dopamine neurons in the rat brain. The behavioral recovery results from dopamine release from the transplanted neurons, confirming that the neurons are functional.



Supplemental Figure 1. Tyrosine hydroxylase positive neurons are present in the putamen of rats transplanted with patient-derived DA neurons after 6 months (left). Rats treated with 3 different patient lines demonstrated consistent and sustained recovery of motor behavior (right) n=7.

Concern:

- ***"***There are questions about the nine iPSC lines that have been derived to date. The program intends to use these lines to establish cGMP banks for future DA neuron production. However, the reprogramming method raises concerns about the impact on suitability of these cells for clinical use. The upcoming meeting with the FDA should provide more insight on the project direction if the applicant provided enough information to allow the FDA to make a clear assessment".

Response: additional information.

FDA Pre-pre-IND meeting outcome (February 17th): Because the iPSCs were generated with Sendai virus, the FDA indicated that the iPSCs should be tested for avian viruses. The FDA confirmed that the plans to demonstrate that the virus is not retained in the cells were appropriate. The FDA requested that we obtain all possible information about the preparation of the virus and to test our cells for any contaminants that might be present in the virus preparation. In addition, the CMC reviewer agreed to review the complete reagent list and testing plan in a separate briefing document. The FDA indicated that with a suitable testing plan that mitigates the risk of the various reagent exposures, the use of the non-GMP iPSC seed banks for a Phase 1 clinical trial would be feasible. We have developed a plan to

address these issues and to present the information at our Pre-IND meeting.

Concern:

- "There was not sufficient data on cell line to cell line variability. Only two iPSC lines were taken forwarding into the ongoing proof-of-concept studies. More data must be gathered to understand key variability (mutations, off-target cell types, residual iPSCs) and the potential impact on safety".

Response: additional information.

New results: Our application was submitted using **3 iPSC lines for proof-of-concept** studies (see **Supplemental Figure 1** and **Figure 14 of the application**). We agree that the major challenge of a patient-specific cell therapy is to develop methods for reproducible cell preparations, and our major focus is to define release criteria that will be applied to each cell preparation from each patient.

Concern:

- "The designs for the animal studies do not match the attached protocols. The large animal pilot delivery study in Table 10 is a large study and should not be conducted until the product is defined and a delivery device chosen. This is a definitive IND enabling study. The proposed protocol using the large animal model is appropriate".

Response: additional information.

FDA Pre-pre-IND meeting outcome (February 17th): This is a pilot study using a small number of animals. In our discussions with the FDA, the committee indicated that this study would provide enough useful data to prove worthwhile as a pilot study. Because the brain is a sensitive area in which to deliver cells, and because the cells cannot be removed once transplanted (as the approach used by Viacyte in their diabetes trial), this pilot study is needed to demonstrate our ability to deliver cells to the appropriate region in a large brain with minimal collateral damage to the cortical regions of the brain.

Concern:

- "The pilot tumorigenicity study has too few animals for meaningful interpretation. There is no description of what is considered to be a tumor in the safety studies. What markers will be used? If there is slow proliferation or low grade proliferation, will that be considered safe? What is the cut off?"

Response: additional information.

Progress: Because of the length of time required for these studies, we have initiated the pilot tumorigenicity study described in our proposal.

FDA Pre-pre-IND meeting outcome (February 17th): The FDA committee considered our pilot study to be adequate, and reminded us that larger scale studies will be necessary for IND-enabling studies. The protocols for assaying for tumorigenicity have been developed for other pluripotent stem cell–derived cell therapies, and we will use these as a guide. The studies will be performed by a skilled CRO.

Concern:

- "The applicant should have provided more information on how the whole genome sequencing data would be analyzed to determine whether mutations represent a safety risk. This is a big challenge given that there are likely a significant number of background mutations that arise from the culture".

Response: additional information.

Research published February 19th. Our whole genome sequence analysis and methods were published on February 19 in Nature Communications. This publication describes exactly the analysis we proposed to perform for the patient cell lines, specifically addressing the challenge of determining

whether mutations represent a safety risk. A link to the Nature Communications article and the abstract are provided below.

Grant application approved. On **February 24th**, we received notice that our application to our Center of Excellence in Stem Cell Genomics (CESCG) (Call 2) Collaborative Research Project application was approved. Our CESCG award is specifically focused on using whole genome sequencing and RNA sequencing of the Parkinson's disease patients as a quality control assessment in our study.

FDA Pre-pre-IND meeting outcome (February 17th): The FDA does not require these analyses, but was positive about our proposed studies. We believe that genomic analysis will be an important quality control tool and will inform our release criteria.

Whole-genome mutational burden analysis of three pluripotency induction methods
Kunal Bhutani, Kristopher L. Nazor, Roy Williams, Ha Tran, Heng Dai, Željko Džakula, Edward H. Cho, Andy W. C. Pang, Mahendra Rao, Han Cao, Nicholas J. Schork & Jeanne F. Loring
Abstract: There is concern that the stresses of inducing pluripotency may lead to deleterious DNA mutations in induced pluripotent stem cell (iPSC) lines, which would compromise their use for cell therapies. Here we report comparative genomic analysis of nine isogenic iPSC lines generated using three reprogramming methods: integrating retroviral vectors, non-integrating Sendai virus and synthetic mRNAs. We used whole-genome sequencing and *de novo* genome mapping to identify single-nucleotide variants, insertions and deletions, and structural variants. Our results show a moderate number of variants in the iPSCs that were not evident in the parental fibroblasts, which may result from reprogramming. There were only small differences in the total numbers and types of variants among different reprogramming methods. Most importantly, a thorough genomic analysis showed that the variants were generally benign. We conclude that the process of reprogramming is unlikely to introduce variants that would make the cells inappropriate for therapy.
http://www.nature.com/ncomms/2016/160219/ncomms10536/abs/ncomms10536.html

Concern:
• "There is no evidence that the produced cells can be functional, as expected an in vivo test to show demonstrated production of dopamine or a proposed test would strengthen this proposal".

Response:
We have established a collaboration with a TSRI colleague, Dr. Loren Parsons, to use HPLC to determine the extent of dopamine release in our cultures, as well as identification of any other released neurotransmitters. Although we have planned to do this analysis, we did not make HPLC analysis a priority in our grant application because the recovery of function in the hemiparkinsonian rats provides conclusive evidence of dopamine release by the transplanted cells.

The FDA committee (February 17th) did not consider in vitro analysis of DA release to be a critical release criteria for production of the cell product for a Phase 1 trial. Also, it should be noted that our electrophysiology experiments (noted in the application) show that the neurons are immature at day 25, which is important for their survival after transplantation. After a total of 60-80 days in vitro, the cells show electrophysiological characteristics of fully mature A9 DA neurons.

Concern:
• "The team needs added expertise for the behavioral analysis study".

Response:
We have considerable expertise within our group. In addition to our collaborator, Dr. Curt Freed, a long-time colleague who carried out one of the two NIH-supported human trials using fetal tissue to treat PD

patients, we have enlisted Dr. Jeff Kordower, another long-time colleague who is one of the most respected experts on behavioral analysis of Parkinson's disease model systems. In addition, the PI has direct experience in behavioral analysis of hemiparkinsonian rats transplanted with fetal dopamine neurons (Loring et al, 1989). She and colleagues developed the model based on advice from Dr. Anders Björklund, who was a scientific advisor to Hana Biologics, where she performed these studies.

Concern:

• "The regulatory strategy appears to be incomplete in its design. The product design must be locked in before proceeding with the FDA".

Response: additional information.

FDA Pre-pre-IND meeting outcome (February 17th): The reviewer's concerns are not supported by our feedback from the FDA meeting. The FDA committee advised us that our strategy was sound and that we should proceed with the studies we proposed in support of our Pre-IND meeting.

Additional Comments

Comment

• "The investigators should add one outcome that is a measure of DA levels in cultures cells and in the medium under basal and stimulated release conditions".

Response:

Although the FDA committee did not indicate such studies are necessary to prove function, since the animal model requires dopamine release, we have arranged a collaboration with our TSRI colleague, Dr. Loren Parsons, to analyze neurotransmitter release during differentiation of our neurons in culture.

Comment

• "Preliminary data that shows the cells can provide meaningful outgrowth would strengthen the proposal".

Response:

New results: We have now completed a six-month behavioral study in nude rats, in collaboration with Dr. Curt Freed. **Supplemental Figure 1** shows that there is extensive outgrowth of the cells in the striatum of hemiparkinsonian rats.

FDA Pre-pre-IND meeting outcome (February 17th): The FDA committee agreed that if sufficient outgrowth can be demonstrated from our rodent studies, primate studies will not be necessary.

Comment

• "Reviewers suggested that convincing data from an immunocompromised or humanized animal model showing maintenance of phenotype and differentiation could improve the proposal".

Response: additional information.

New results: We have now completed a six-month behavioral study in nude rats, in collaboration with Dr. Curt Freed. The animals demonstrated sustained recovery through 6 months and histological analysis indicates the presence of TH^+ neurons in the transplantation site (Supplemental Figure 1), without any evidence of tumor formation.