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Keck School of Medicine
University of Southern California

January 20, 2013

Re: **RB5-07512 "**Mechanism of Direct Lineage Conversion into Induced Motor Neurons"
PI: Justin Ichida, Ph.D.

Dear CIRM ICOC,

I am writing to express my enthusiasm for my Basic Biology V proposal, "Mechanism of Direct Lineage Conversion into Induced Motor Neurons" and outline the reasons why I believe it is a good candidate for programmatic consideration for funding by the ICOC.

A major goal of CIRM is to harness the potential of stem cells and reprogramming to enable the development of therapeutics where there is unmet medical need. There are various approaches; one of the most promising utilizes patient-specific somatic cells to understand, model, and treat human disease. A view shared by many leading scientists is these patient-directed disease systems will more accurately recapitulate disease processes than either their rodent counterparts, or the transformed human cell lines used to date.

Clearly, the utility of this approach is seated on the fundamental premise that *in vitro*-derived, patient-specific cell types accurately mimic the molecular and functional properties of the same cell types in the patient. The central nervous system (CNS) can be used to illustrate the importance of this point.

The CNS is assembled from a diverse collection of neurons, each with unique properties. These discrete characteristics underlie the proper integration and function of each neuron within the brain and spinal cord circuitry. However, their individual qualities also render particular neurons either resistant or sensitive to distinct degenerative stimuli. Thus for each neurodegenerative disease, a specific set of neuronal subtypes is destroyed, causing the hallmark presentation of that condition. Therefore, to study a given neural disease, we must be able to make relevant neuronal subtypes *in vitro* that possess the unique properties of the cells affected in each disease.

Whereas a large effort has compared iPS cells to ES cells, the relationship between adult cell types derived from iPS cells, or through direct lineage reprogramming, to their natural counterparts has not been extensively explored. This important goal is the focus of our study. The target is a critical cell-type of biomedical importance: the spinal motor neuron, which degenerates in diseases such as amyotrophic lateral sclerosis and spinal muscular atrophy.

In our first aim, we will address the question of how well *in vitro*-generated cells mimic their natural counterparts. To do this, we will perform a quantitative genomic comparison of motor neurons, comparing *bona fide* human spinal cord neurons to those generated directly from human fibroblasts by lineage reprogramming or through directed differentiation of human iPSCs.

I would like to emphasize that motor neurons are one of the only cell types for which we can perform this type of analysis because we can produce them by both lineage reprogramming and stem cell differentiation. Importantly, and unlike other lineage reprogramming-based studies using unspecified induced neurons, the induced motor neurons produced by our protocols have a specific counterpart in the human nervous system, which makes this critical comparison possible.

Our study will have important implications for the field of *in vitro* disease modeling. It will either validate the accuracy with which lineage reprogrammed or stem cell-derived motor neurons mimic *bona fide* motor neurons, or highlight ways in which they differ. For any differences between *in vitro* and natural motor neurons that may impact disease studies, we will determine if they arise from incomplete epigenetic reprogramming or by aberrant modifications added during reprogramming/differentiation. Overall, this study will provide the first rigorous molecular evaluation of reprogramming and stem cell-derived cells, which is the cornerstone of using these approaches for translational applications.

The second critical issue addressed by my proposal is the need for a way to increase the efficiency of direct lineage conversion. Producing neurons and other cell types by direct lineage conversion from fibroblasts provides an important complement to the iPSC-based approach because it is 3-4 times faster, skipping the iPSC step. This allows the rapid identification of patients whose cells exhibit disease phenotypes *in vitro*, allows the study of large numbers of patients to increase statistical confidence in phenotypic differences, and could enable drug testing on an array of different patients in parallel.

Though fast, direct lineage reprogramming is inefficient. Our goal is to increase efficiency by an order of magnitude to make the approach fully practicable. To this end, our second aim optimizes the use of a small molecule we recently identified that leads to a 10-fold increase in the direct conversion of human fibroblasts into motor neurons. It is important to note that the use of this compound allows the efficient production of functional motor neurons from adult human fibroblasts, which normally reprogram inefficiently compared to neonatal fibroblasts. The inefficiency of lineage reprogramming has prevented the adoption of this approach for translational studies, and this discovery, which we already have in hand, provides a means to finally overcome this barrier. By understanding the mechanism by which this chemical acts, we will determine the precise timing and duration needed to maximize neuron production. In addition, our preliminary data suggest that this molecule can increase the production of other neuronal subtypes by direct lineage conversion, and we will determine in aim 2 if this is a general result. Thus, we anticipate our second aim will enable the development of a strategy for the effective use of direct lineage conversion in neuronal disease studies.

Identifying the transcription factor cocktails that generate specific neuronal subtypes is central to the broader application of our approach. Based on our previous studies, our hypothesis is that transcription factors broadly expressed in neurons can be used to convert fibroblasts to a neuronal ground state that can then be further patterned using subtype-specific factors. Our third aim will test this hypothesis. Our ultimate goal is not simply an improvement in lineage reprogramming of neuronal subtypes for our own studies but a

roadmap for the generation of new neuronal subtypes to explore broadly neural disease.

In light of the above, I would like to address some concerns raised by the reviewers. In the Feasibility and Experimental Design section of the review summary, a reviewer states, "- Some reviewers were not convinced that there would be enough cells to carry out the ChIP-Seq in human iMNs in Aim 3." I agree that acquiring enough iMNs for ChIP-Seq would be a difficult task, but that is not the designed experiment. We intend to perform ChIP-Seq on *fibroblasts* expressing the iMN reprogramming factors, not on iMNs. Because fibroblasts can be expanded to large quantities, we do not anticipate that it will be problematic to acquire enough cells for ChIP-Seq. This same approach, performing ChIP-seq on fibroblasts overexpressing neuron reprogramming factors, has recently been used successfully to identify binding sites for induced neuron reprogramming factors (Wapinski *et al*., Cell, 2013). This published data shows that fibroblasts can be obtained in large enough quantities to perform ChIP-seq, and that ChIP-seq on fibroblasts overexpressing the reprogramming transcription factors is sensitive enough to identify the transcription factor binding sites. Given this clarification and supporting evidence, we anticipate that our proposed ChIP-Seq studies will be feasible. Although I felt that this misunderstanding of the experimental approach could have merited an appeal based on a material dispute of fact, I chose not to appeal to ensure that my application would be eligible for programmatic consideration on the Jan 29th meeting of the ICOC.

A second concern was whether the low efficiency of reprogramming would lead to heterogeneity in the induced motor neuron population, complicating interpretation of our results. This is an important consideration. To address this concern, we assessed heterogeneity in the induced motor neuron pool through immunocytochemistry and single cell qPCR. The results suggest a reassuringly high level of homogeneity in the induced motor neuron population with respect to key molecular markers. The small molecule enhancer of motor neuron reprogramming will likely mitigate against these concerns by increasing the reprogramming efficiency. Therefore, we anticipate that our studies will be feasible and successful.

Finally, I would like to stress the impact this award could have on my career development. As a young investigator, this funding and opportunity to carry out the proposed work would allow me to establish an independent research program and contribute to this exciting area of biomedical research for the foreseeable future. I hope that the importance of the scientific issues addressed by our study and the clarification of the feasibility of the proposed experiments will encourage the ICOC to consider my application for funding.

Sincerely,

Justin Ichida
Assistant Professor of Stem Cell Biology
and Regenerative Medicine
Eli and Edythe Broad CIRM Center
1425 San Pablo St. BCC 307
Los Angeles, CA 90033
ichida@usc.edu, (323) 442-0063