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Dear ICOC Application Review Subcommittee,

The development of iPSC technology has been transformational for biomedical research and has led to incredible promise toward the realization of precision medicine ideals. We believe that undifferentiated iPSCs have been overlooked as an informative cellular model. This assertion is based on our pilot data showing that functional assessment of patient-derived undifferentiated iPSCs can model individual-level genetic risk for nonalcoholic fatty liver disease (NAFLD), a very common and highly underdiagnosed condition that disproportionally impacts the LatinX community (now the most prevalent group within California). Thus, our proposal, **DISC2-12358 entitled, "iPSCs as a screening tool to predict risk of nonalcoholic fatty liver disease"** seeks to develop an iPSC-based risk score to predict patient-specific future disease risk.

There was unanimous consensus that our proposal was significant and served the needs of underserved communities with reviewers noting that "*NAFLD is a major unmet clinical need*," and that, "*this proposal pioneers the frontier and is very innovative*." Early identification of high-risk individuals (such as adolescents with a parent newly diagnosed with severe disease), can encourage more aggressive clinical efforts towards disease prevention. This point is particularly salient for NAFLD as there are no targeted therapeutics, no universal disease screening, and the disease can be reversed in early stages through diet and lifestyle interventions. As one Reviewer noted, "*a functional representation of the genome is achieved without having to wait for an individual to grow to adulthood*". This enthusiasm led to an overall score of 80 $\pm$ 7 and the inclusion of a minority report with 5 out of 14 reviewers supporting funding.

The primary difference between the majority vs. minority opinion was a disagreement regarding our use of undifferentiated iPSCs where the majority believed that "*differentiated cells should be used*". We agree that most applications of iPSCs in biomedical research are benefited by using cells that most authentically phenocopy the physiologically relevant cell type (e.g. a hepatocyte in the case of NAFLD). However, a diagnostic test requires only that the assay is highly robust and reproducible, straightforward to implement across different laboratories, relatively inexpensive, and most importantly, informative enough to be clinically meaningful. Currently protocols to generate iPSC-derived hepatocyte-like cells are premature for this purpose as they yield highly variable cultures, have a relatively high failure rate even in the hands of experts, are 5-10x more expensive and take 4-6 weeks longer to generate compared to undifferentiated iPSCs, and ultimately may not be more informative than undifferentiated cells based on our pilot data.

Thus, while we appreciate that there are limitations with the undifferentiated iPSC model, we believe that their potential benefits in terms of ease of culture, relatively low variability, and economy while remaining informative of individual level genetic risk outweigh these concerns. This sentiment was expressed within the minority report as reviewers were "*convinced by the strong preliminary data of the proposal*." We envision our iPSC-based assay as a first-generation test, demonstrating a paradigm changing proof of concept that patient-derived iPSCs can be used to clinically diagnosis future disease

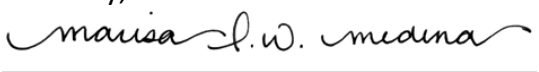
risk. Indeed, reviewers noted that "*such assays are currently an unmet promise of stem cell science, and this application directly addresses CIRM's mission."*

One minor concern was that *"inflammation and fibrosis…will not be modeled."* From an academic perspective we agree that such information may yield a more precise risk score. However, we were concerned that creation of a score that was dependent on multiple functional assays would be overly complex and not viable from a commercial perspective. As stem cell technologies mature, we anticipate that next generation tests may utilize differentiated cells and may incorporate more complex cellular phenotypes that model risk of disease progression.

We thank the committee for the opportunity to respond to critiques and respectfully request reconsideration for funding. Of note, the Medina laboratory has utilized this project as the basis for mentorship of six high school students through the CIRM SPARK program (including Sheila Teker and Christian Castillo from the summer 2021 cohort).

Thank you in advance for your time and consideration.

Sincerely,



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