| APP # | TITLE | BUDGET REQ | FUND? | SCORE (MEDIAN) | Mean | SD | Low | High | Y | N | Resubmission | Previous CIRM Funding | Area of Impact |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DISC0-15949** | Neuroimmune interactions in the developing human brain | **$1,626,000** | **Y** | **95** | 94 | 2 | 90 | 95 | 14 | 0 | N | N | Addresses the question of how immune cells interact with neural stem cells and the impact of infection and environmental factors. |
| **DISC0-15737** | Village-based identification of human risk factors for viral neuropathogenesis | **$1,577,448** | **Y** | **92** | 91 | 5 | 75 | 93 | 13 | 1 | N | N | Creates a stem cell-based platform to identify specific genetic and molecular risk factors associated with several viruses. |
| **DISC0-15921** | Interrogating Satellite Cell and Myofiber Defects and Repair in Human DMD using Single Nuclei/Single Cell RNA Sequencing of Muscle Resident Cells | **$1,578,000** | **Y** | **90** | 91 | 2 | 90 | 95 | 13 | 0 | Y | Y | Advances the understanding of human muscle stem cells and how they contribute to disease progression in DMD. |
| **DISC0-16039** | Lewy body dementia, $\alpha$-synuclein, and cell-specific mechanisms of neurodegeneration | **$1,739,760** | **Y** | **90** | 90 | 1 | 85 | 92 | 15 | 0 | N | N | Develops a stem cell-based model for Lewis Body Dementia and studies mechanisms of $\alpha$-synuclein induced neurodegeneration. |
| **DISC0-16122** | Mapping and modeling endothelial cell fate decisions for pulmonary arterial hypertension | **$1,540,798** | **Y** | **90** | 90 | 1 | 90 | 92 | 14 | 0 | N | N | Develops a model and experimental platform to assess gene perturbations on cellular phenotypes in PAH and other conditions. |
| **DISC0-15654** | Modeling and understanding alveolar hypoplasia in Down syndrome using iPSCs-derived alveolar type II cells | **$1,524,196** | **Y** | **90** | 88 | 4 | 75 | 90 | 14 | 1 | N | N | Advances the understanding of human lung progenitor cells in health and in congenital anomaly, Trisomy 21 (Down syndrome). |
| **DISC0-15816** | Investigating the SGF29/SAGA complex in regulation of normal and cancer stem cells | **$1,647,600** | **Y** | **87** | 88 | 2 | 85 | 90 | 15 | 0 | N | N | Advances understanding of how an aberrant stem-like state is enforced by leukemia cells, using the cellular epigenetic machinery. |
| **DISC0-15774** | Modeling of GATAD2B-associated neurodevelopmental disorder and NuRDopathies: Investigation of cellular & molecular anomalies altering neurodevelopment | **$1,318,441** | **Y** | **87** | 87 | 2 | 85 | 90 | 15 | 0 | Y | N | Studies to identify and quantify cellular and molecular changes in models of corticogenesis with NuRD deficiency. |
| **DISC0-15972** | Immune cloaking of human stem cell-derived insulin-producing cells for curative cell therapy without immunosuppression | **$1,192,586** | **Y** | **85** | 85 | 4 | 75 | 90 | 8 | 6 | Y | Y | Develops an approach for creating cellular grafts that are resistant to the host immune system for a type 1 diabetes cell therapy. |
| **DISC0-15920** | Harnessing the rejuvenating capacity of pregnancy-associated factors to restore aged stem cell function | **$1,539,520** | **Y** | **85** | 84 | 4 | 75 | 88 | 10 | 5 | Y | N | Studies to understand how pregnancy-related factors might mitigate cellular senescence and enhance regeneration. |
| **DISC0-15689** | Utilizing Age-Specific Adipocyte Progenitor Cells for Cell Therapy in Older Patients | **$1,508,997** | **Y** | **85** | 83 | 4 | 72 | 86 | 8 | 6 | Y | N | Studies the potential of adipocyte progenitor cells including immunomodulatory capacity and advanatges for iPSC generation. |
| **DISC0-15755** | Microglia replacement with non-myeloablative hematopoietic stem cell transplantation for Alzheimer's disease | **$1,540,801** | **N** | **84** | 84 | 1 | 80 | 85 | 4 | 11 | N | N | |
| **DISC0-15700** | Gene-edited CD19 CAR-T cells with superior proliferation, persistence and serial-killing activity | **$1,607,994** | **N** | **83** | 82 | 4 | 72 | 85 | 3 | 12 | N | N | |
| **DISC0-15764** | Modeling Arealization to Understand Etiology of Neurodevelopment Disorders | **$1,569,312** | **N** | **82** | 83 | 3 | 75 | 90 | 4 | 11 | N | N | |
| **DISC0-15693** | Modeling Rett syndrome neurological disorder with human pluripotent stem cells to develop in cellulo screening platforms. | **$1,574,117** | **N** | **80** | 81 | 2 | 78 | 85 | 1 | 14 | N | Y | |
| **DISC0-15712** | Interrogation of tandem repeat variants contributing to neurodevelopmental and psychiatric traits using stem cell models | **$1,529,317** | **N** | **80** | 81 | 2 | 78 | 85 | 1 | 14 | N | N | |
| **DISC0-15758** | Dissecting the cellular and molecular interactions established between human embryo and maternal endometrium at implantation | **$1,540,803** | **N** | **80** | 81 | 2 | 78 | 84 | 0 | 15 | N | N | |
| **DISC0-15692** | Generation of Functional Proximal Tubules in Organoids through Gradual Developmental Mimicry | **$1,583,032** | **N** | **80** | 80 | 1 | 78 | 80 | 0 | 15 | N | N | |

| APP # | TITLE | BUDGET REQ | FUND? | SCORE (MEDIAN) | Mean | SD | Low | High | Y | N | Resubmission | Previous CIRM Funding | Area of Impact |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DISC0-15902** | Regulatory map for guiding the generation of transplantable human hematopoietic stem cells | **$1,576,043** | **N** | **80** | 80 | 4 | 70 | 84 | 0 | 15 | N | Y | |
| **DISC0-15763** | Understanding the mechanisms and developing therapeutics for the neurodegenerative Parkinson's disease using human iPSCs derived models | **$1,593,011** | **N** | **80** | 79 | 3 | 70 | 84 | 0 | 15 | Y | N | |
| **DISC0-15916** | Exploring Potential Drugs to Enhance Neural Recovery in Combination with Neural Stem Cells after Spinal Cord Injury (SCI) | **$1,583,998** | **N** | **80** | 77 | 5 | 65 | 84 | 0 | 15 | N | N | |
| **DISC0-15855** | The functional impact of psychedelics on human brain organoids | **$1,584,000** | **N** | **78** | 78 | 4 | 70 | 85 | 1 | 14 | N | N | |
| **DISC0-15770** | Ultrasound Controllable CAR T Cell Therapy for Pediatric Glioblastoma | **$1,200,000** | **N** | **77** | 77 | 3 | 70 | 84 | 0 | 14 | N | N | |
| **DISC0-15688** | Using Human Pluripotent Stem Cells for Biomedical Innovation Aimed at Improving the Health of Women and Girls. | **$1,564,889** | **N** | **75** | 76 | 4 | 70 | 84 | 0 | 14 | N | N | |
| **DISC0-15903** | Instruction of Hematopoietic Stem Cell Fate from Human Pluripotent Precursors | **$1,529,743** | **N** | **75** | 74 | 8 | 50 | 86 | 2 | 13 | N | N | |
| **DISC0-15793** | Development of a humanized swine model for translational studies of CNS-targeting cell therapies | **$999,124** | **N** | **75** | 74 | 2 | 70 | 75 | 0 | 15 | N | Y | |
| **DISC0-15943** | Investigating epigenetic reprogramming and cell extrinsic signaling events in the specification and maturation of human primordial germ cells | **$1,492,031** | **N** | **75** | 74 | 3 | 70 | 80 | 0 | 14 | Y | N | |
| **DISC0-15890** | Immune exhaustion as a mediator of hepatic stem cell expansion in pediatric acute liver failure | **$1,509,999** | **N** | **75** | 73 | 2 | 70 | 75 | 0 | 14 | N | N | |
| **DISC0-15713** | An iPSC-derived approach to studying how NOD2 affects Paneth cells in Crohn's disease | **$1,503,866** | **N** | **75** | 72 | 5 | 65 | 80 | 0 | 15 | N | N | |
| **DISC0-15808** | The influence of human neural stem cells on autoimmune and regenerative function in mouse models of multiple sclerosis | **$1,506,309** | **N** | **70** | 71 | 2 | 70 | 75 | 0 | 15 | Y | Y | |
| **DISC0-15886** | Evolutionarily Conserved Mechanisms that Control Stem Cell Aging and Rejuvenation | **$1,141,490** | **N** | **70** | 71 | 5 | 60 | 75 | 0 | 15 | N | N | |
| **DISC0-15904** | Neural Stem Cell Aging and Neurodegeneration | **$1,610,930** | **N** | **70** | 71 | 4 | 65 | 80 | 0 | 15 | Y | N | |
| **DISC0-16005** | Regenerative medicine meets oncology: hiPSCs crafting the future of immune competent human skin cancer models | **$1,540,193** | **N** | **70** | 70 | 5 | 65 | 85 | 1 | 14 | N | N | |
| **DISC0-16038** | Making of Geometry: Mapping the mechanics that shapes the human neural tube | **$1,478,078** | **N** | **70** | 70 | 4 | 65 | 80 | 0 | 15 | Y | N | |
| **DISC0-15944** | Using an iPSC Derived Human Lung Airway Model to Determine the Antiviral Effects of Airway Surfactants | **$1,392,506** | **N** | **70** | 69 | 2 | 65 | 70 | 0 | 15 | N | N | |
| **DISC0-15925** | Mechanisms of synaptic neurotransmitter dysregulation in human Alzheimer's disease neurons | **$1,435,016** | **N** | **70** | 68 | 3 | 60 | 70 | 0 | 14 | N | N | |

| APP # | TITLE | BUDGET REQ | FUND? | SCORE (MEDIAN) | Mean | SD | Low | High | Y | N | Resubmission | Previous CIRM Funding | Area of Impact |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **DISC0-15847** | Transplantation of hiPSC-neurons to treat inflammation-based cognitive deficits in CNS injury | **$1,540,499** | **N** | **70** | 57 | 21 | 1 | 70 | 0 | 14 | N | Y | |
| **DISC0-15827** | Addressing Fundamental Neurobiology of Autism with brain organoid models | **$1,196,603** | **N** | **65** | 67 | 4 | 60 | 75 | 0 | 14 | N | N | |
| **DISC0-15937** | Hematopoietic stem and progenitor cells serve as central hubs during perinatal liver inflammation | **$1,612,940** | **N** | **60** | 60 | 0 | 60 | 60 | 0 | 15 | N | N | |
| **DISC0-15657** | Targeting neurodevelopmental and genetic mechanisms of cerebral palsy | **$1,573,659** | **N** | **/** | / | / | / | / | 0 | 14 | N | N | |
| **DISC0-15974** | MADR transgenic and somatic transgenic manipulation of human iPSCs and emergent lineages | **$1,536,599** | **N** | **/** | / | / | / | / | 0 | 15 | N | N | |
| **DISC0-15893** | Expanding in vitro fertilization technologies via direct meiosis induction | **$1,335,834** | **N** | **/** | / | / | / | / | 0 | 14 | N | N | |
| **DISC0-15674** | Identification of metabolic pathways that mitigates hyperglycemic insult to fetal heart | **$1,522,702** | **N** | **/** | / | / | / | / | 0 | 13 | N | N | |

| Application # | DISC0-15949 |
|---|---|
| **Title**<br>(as written by the applicant) | Neuroimmune interactions in the developing human brain |
| **Research Objective**<br>(as written by the applicant) | This project will identify mechanisms by which immune cells regulate human brain development, and use stem cell derived models to identify pathological changes induced by congenital virus infection. |
| **Impact**<br>(as written by the applicant) | Neurodevelopmental disorders caused by immune cell perturbations will be impacted by the fundamental understanding of how immune system controls normal development of the brain. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Generation of spatiotemporal map of neuroimmune interactions<br>• Generation of stem cell resources for modeling neuroimmune interactions in developing brain<br>• Studying the consequences and mechanisms of congenital viral infection<br>• Generation of reference datasets for human neuroimmune interactions |
| **Statement of Benefit to California**<br>(as written by the applicant) | Residents of the State of California have voted to support research enabled by the CIRM, with specific focus on neurological disorders. Congenital viral infections substantially increase the risk for neurological and psychiatric disorders. Incidence of congenital viral infections has increased dramatically over the last decade in California. This project will identify the molecular pathways through which these environmental risk factors lead to neurodevelopmental phenotypes. |
| **Funds Requested** | $1,626,000 |
| **GWG Recommendation** | (85-100): Exceptional merit and warrants funding, if funds are available |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 95

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

| | |
|---|---|
| **Mean** | 94 |
| **Median** | 95 |
| **Standard Deviation** | 2 |
| **Highest** | 95 |
| **Lowest** | 90 |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 14 |
| **(1-84): Not recommended for funding** | 0 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 13<br>**No:** 0 | • The goal is to use more complete organoids to elucidate molecular mechanisms underlying neuro-immune interactions during human brain development and their vulnerability to environmental perturbations.<br>• The proposal addresses an important limitation to brain organoids as a research tool. The applicants developed chimeric brain organoids with incorporated microglia. |

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| | • The application is focused on the study of neuro-immune interactions, specifically how microglia interact with neural cells (and neural stem cells in particular). The cellular and molecular pathways underlying such interactions are largely unresolved. This proposal will test the hypothesis that microglia secreted factors regulate neurogenesis in the developing human brain.<br>• Another goal is to understand the consequences of rubella virus (RV) infections on brain development. No tractable system exists to date and the approach addresses this bottleneck. RV affects 1.7 per 1000 live births in California and infects microglial cells. Studies in organoids that do not contain microglia are thus limited in understanding synaptic processes and neuronal maturation. RV lacks tropism for the developing mouse brain, necessitating studies in a human system.<br>• To achieve both goals, they will use (i) chimeric organoid models they developed by supplementing stem cell derived organoids with stem cell derived microglia, as well as (ii) primary human brain slices in culture.<br>• Current stem cell derived brain organoids lack microglia, the immune resident cell type of the brain. Thus the applicant's chimeric organoids are a great system for the study of neuroimmune interactions in development and disease.<br>• The project will yield molecular and mechanistic insight into the cellular programs that are regulated by microglia-neural stem cell interactions during human brain development, and how these programs may be altered in the context of infection. If successful, it may nominate candidate targets for therapeutic intervention to mitigate the consequences of prenatal viral infections during pregnancy. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 13<br>**No:** 0 | • Published and preliminary data have shown that microglia derived factors have critical roles for various processes during early brain development. In addition, maternal immune activation has been shown to be a risk factor for the development of intellectual disability in the offspring.<br>• The need to study microglia-neuronal interactions is well justified. The choice of the model system in well justified.<br>• Mice cannot fully recapitulate the microglia populations in humans as some transcriptional states are not conserved.<br>• The chimeric organoid model has been published and validated. Extensive preliminary data is presented to support the spatial transcriptomics experiments, which will be performed to map out microglial states across different developmental stages. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 13<br>**No:** 0 | • Aim 1 will extend the applicant's spatial transcriptomic analysis to include a time line - this is a critical step for understanding developmental processes and also offers a unique research tool for the field. The generation of KO lines to test the impact of mutant microglia on organoid development is interesting. Preliminary data show a role for the knocked-out genes in proliferation.<br>• Aim 2 is highly translational and will use single cell genomics (transcriptomics and epigenomics) to determine the time course of molecular changes induced by rubella virus (RV) infection in the developing human brain. The applicant has developed a GFP reporter RV that will be used to perform high-throughput CRISPR activation screening for genes required for RV infection of microglia in prenatally developing human brain.<br>• The representation of organoid cells in the single cell RNA sequencing data is not clear - how many cells will be captured?<br>• Minimal / no pitfalls and alternative approaches are discussed for Aim 1. This is backed by the claim that they have already demonstrated successful data generation for other projects using the same workflows.<br>Pitfalls and alternative approaches are discussed for Aim 2. If the organoid slices are problematic, they will use organotypic slices instead.<br>• The results of the experiments will be informative to the field, regardless of the outcomes.<br>• Pitfalls are discussed but alternative approaches are scant. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 13<br>**No:** 0 | • The applicant performed preliminary studies to identify the spatial temporal dynamics of microglia distribution in developing brain organoids.<br>• The applicant shows feasibility of transplanting microglia into organoids purified from mid-gestation tissue or human IPS derived cells.<br>• Microglial cells transplanted become undetectable six weeks after transplantation, suggesting that trophic factors are needed to sustain microglia survival in organoids. The applicant shows that addition of such factors is sufficient to maintain microglia. |

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| | ● All the proposed experiments are reasonable, with a high likelihood of success.<br>● The team is appropriately staffed with the right expertise. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 13<br>**No:** 0 | ● Both primary tissue specimens and pluripotent stem cells are from diverse backgrounds, Both sexes are represented.<br>● Samples from XX and XY individuals and diverse genetic ancestries are included. |

| Application # | DISC0-15737 |
|---|---|
| **Title**<br>(as written by the applicant) | Village-based identification of human risk factors for viral neuropathogenesis |
| **Research Objective**<br>(as written by the applicant) | We will identify the risk factors underlying viral infections of the fetal brain using a novel human stem cell-based platform that has the potential to accelerate basic and translational discoveries. |
| **Impact**<br>(as written by the applicant) | Our work will identify the biological factors that influence inter-individual differences in susceptibility and immune response to neurotropic viruses, which could inform future antiviral drug design |
| **Major Proposed Activities**<br>(as written by the applicant) | • Build a 150-donor village that captures the immense diversity of California citizens<br>• Identify risk factors for Zika, Measles, Dengue, and Cytomegalovirus infection<br>• Understand the biological contributors to differences in immune response magnitude across individuals<br>• Characterize differential responses to inflammatory environments across donors |
| **Statement of Benefit to California**<br>(as written by the applicant) | Viral infections are a common cause of illness, and there are considerable differences across people in their susceptibility and response to infection. Increased presence of mosquito vectors and overall decreases in vaccination rates are putting more and more Californians at risk for viral-mediated diseases. Here, we will study viruses that are existing or emerging threats to California using human stem cell lines that represent the ancestral backgrounds of nearly 80% of Californian residents. |
| **Funds Requested** | $1,577,448 |
| **GWG Recommendation** | (85-100): Exceptional merit and warrants funding, if funds are available |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 92

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

| | |
|---|---|
| **Mean** | 91 |
| **Median** | 92 |
| **Standard Deviation** | 5 |
| **Highest** | 93 |
| **Lowest** | 75 |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 13 |
| **(1-84): Not recommended for funding** | 1 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 14 | • The applicant seeks to understand how genetic variation impacts susceptibility of neural progenitors to viral infections (Zika, Dengue, CMV, Measles). Taking the recent pandemic as |

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| **No:** 0 | one example, there is a clear need to understand how differences in host genetics can impact viral responses.<br>• The application identifies scalability — population-scale functional genomics — as currently challenging or unachievable and standard (array-based) phenotyping as subject to noise that can wipe out signal. The proposal uses an innovative pooled screen ('cell villages' with Census-seq) to overcome these limitations and achieve large-scale (150 donors of iPSCs/NPCs).<br>• Yes, this project will have a strong impact on functional genomics, virology, gene expression, and statistical genetics/GWAS fields. Moreover, the 'cell village' model, which was pioneered by the applicant, is a paradigm-shifting approach to testing many natural variants at scale.<br>• The project proposes an ambitious screen to give insight into how genetic variation affects human responses to four viruses, influences inflammatory responses, and modulates immune signaling. The project has the potential to generate a large amount of valuable phenotypic data.<br>• The proposal utilizes a clever multiplexing approach called 'cell villages' to screen large numbers of individual genetic lineages simultaneously - this could have implications for human disease.<br>• The project is likely to identify single gene variants with large effect sizes. In one sense this is "low hanging fruit" as compared to more subtle gene interactions. However, this represents a concerted effort to simultaneously define genetic effects in a very efficient manner.<br>• Methods exploration is limited in the project. Can the applicant achieve results with 1,000 cell lines? What are the factors that help these lines to live together in culture? For the eQTL's that are least reproduced, how might they be related to culture conditions? All of these questions are very relevant to practical issues in utilizing IPSC's in a scalable manner for the study of many diseases.<br>• This is the perfect bottleneck-busting project, as represents high throughput extraction of information from a large number of cell lines.<br>• The project will definitely have impact, but how much compared to the applicant's already published work? |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 14<br>**No:** 0 | • The rationale is sound and derived from multiple well-established protocols and areas of investigation. The authors have successfully performed this analysis for Zika and identified an important SNP in an innate immune gene. This suggests they are likely to be successful in identifying additional variants of this type. The project is highly relevant to human disease, investigating neurotropic viruses in human organoid cultures.<br>• Looking at difference in infectivity as a function of genetic background in a scalable system - makes sense.<br>• The main point is the applicant is one of very few people to get this working (for reasons that are not clear) but that's basically the main requirement, which they check.<br>• Very relevant. My concern is for all the promise the need for extensive optimization (which this project doesn't touch) means the methods will remain a gem in the hands of a single lab.<br>• There is sound rationale. The applicant mentions that one of the pathogens (Dengue) was detected recently (October 2023) for the first time in California, making this a fitting project for CIRM. The applicant also mentions many other viral diseases (including COVID-19, which has claimed 7M lives worldwide).<br>• I like the idea of finding the "super-responders" — that is, cells whose genetics may endow them with enhanced resistance to viral pathogenesis. This is an innovative idea that the Cell Villages approach is ideally suited for making discoveries.<br>• Previously, this applicant discovered a large-effect SNP in the IFITM3 gene that accounts for 60% of differential infectivity between individuals in a Cell Village. The allele is highly represented in European populations but is less prevalent elsewhere. This work was published recently in Cell Stem Cell.<br>• Yes, viral pathogenesis impacts every individual and this project has the potential to discover new genes and therapeutic targets to prevent severe disease. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 14<br>**No:** 0 | • The project is well designed and powered to give results. Pitfalls and alternative approaches are considered and well-described. The plan is quite ambitious - completing just two of the three aims would be a major success.<br>• The applicant should undertake further methods development; if this is the only lab able to run this system, the project loses much of its impact. Certainly the specific results they seek are interesting, but they will not achieve global clarity without making the method and its caveats clear.<br>• Note that the applicant's past work is substantial. They have already made 'cell villages' and completed eQTL studies with Zika. However, this plan is commensurate with urgent needs. |

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| | • The major novelty ('cell villages') has been thoroughly de-risked in prior publications. This work expands on the prior work with additional pathogenic viruses.<br>• There is a thoughtful section on analytic methods for eQTL and related analyses. Also, the feasibility statement indicates that much of the leg work has already been accomplished.<br>• Pitfalls are nicely listed, including addressing the major limitation of the approach: non-cell autonomous effects. The limitation of any pooled screen approach could cause NPCs from one donor to influence the responses of NPCs from a different donor. However, the authors use prior data from Zika to show that this has not been the case.<br>• Another pitfall — effect size detection — is also nicely addressed. They cite their prior work showing that even 5-fold smaller 'cell villages' are well powered to detect variants. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 14<br>**No:** 0 | • The project is feasible based on the preliminary data, if a bit ambitious. The team is highly qualified and involves an impressive collaborative network.<br>• The environment is excellent; the budget is appropriate.<br>• As they've completed all aspects of this project prior, this seems feasible.<br>• The team leading this project is probably the only one to trust.<br>• The applicant institution is a great environment with excellent human genetics and stem cell biology.<br>• The budget is appropriate. |
| | *none* |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 14<br>**No:** 0 | • The project is especially relevant to DEI as it directly addresses sources of human variation. The experimental design carefully considers inclusion of underrepresented communities. The DEI plan is appropriate and thorough.<br>• Diversity of gender and ethnicity is at the heart of the pooled screening approach proposed.<br>• The project is truly aimed at helping underserved populations, and seems likely to deliver.<br>• Since the applicant uses 100 lines they can adequately represent many groups.<br>• The applicant has hosted summer students from outreach-oriented research programs and is a founding member of the institution's DEI initiatives.<br>• The applicant has extensive prior DEI involvement. |

| Application # | DISC0-15921 |
|---|---|
| **Title**<br>(as written by the applicant) | Interrogating Satellite Cell and Myofiber Defects and Repair in Human DMD using Single Nuclei/Single Cell RNA Sequencing of Muscle Resident Cells |
| **Research Objective**<br>(as written by the applicant) | We will describe, for the first time, human muscle satellite cell, myofiber and immune cell dynamics due to dystrophin deficiency and AAV gene therapy in human muscle at single nuclei resolution. |
| **Impact**<br>(as written by the applicant) | These studies will elucidate satellite stem cell and myofiber defects in Duchenne and Becker Muscular Dystrophy and determine efficacy, mechanism and toxicities of exon skipping and AAV-gene therapy. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Continue bioinformatic analysis of existing DMD snseq/scRNAseq dataset.<br>• Perform biopsies and prepare and bank new muscle tissue, PBMC and expanded satellite and T cell populations in culture and determine dystrophin expression.<br>• Extract nuclei for snRNAeq and cluster analyze newly acquired BMD/DMD biopsy snRNAseq in the context of our large healthy and DMD snRNAseq data reference set.<br>• Perform pilot experiments involving TNC/EGFR competitor interaction to follow up snRNAseq data identifying increases in TNC expression in DMD versus healthy muscle.<br>• Collect and snRNA/scRNA sequence and analyze GT biopsies relative to snRNAseq reference set.<br>• Clone dominant TCRs and screen for specificity to dystrophin micro-dystrophin or exon skipped dystrophin peptide antigens. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Duchenne Muscular Dystrophy leads to significant disability and premature death due to progressive muscle weakness, imposing significant physical and financial consequences on patients and their families. Approximately, 1500 California families are affected by DMD. There is a large unmet need as there are no curative treatments. Studies proposed may lead to novel targets for drug discovery, therapeutic strategies for targeting satellite muscle stem cells, and better gene therapies for DMD. |
| **Funds Requested** | $1,578,000 |
| **GWG Recommendation** | (85-100): Exceptional merit and warrants funding, if funds are available |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 90

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

| | |
|---|---|
| **Mean** | 91 |
| **Median** | 90 |
| **Standard Deviation** | 2 |
| **Highest** | 95 |
| **Lowest** | 90 |
| **Count** | 13 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 13 |
| **(1-84): Not recommended for funding** | 0 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in

the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 12 **No:** 0 | • The proposal investigates an important topic that is highly relevant to human disease. Available single cell data from human patients are currently limited. The investigators have acquired snRNAseq data, which is an invaluable source of information to begin to understand disease severity in DMD patients.<br>• A panelist valued the dataset that will become available from this study to associate disease progression with molecular signatures of human satellite cells in untreated and repaired (AAV-treated and/or exon-skipping treated) samples.<br>• The scientific environment is excellent, and the team has all the necessary resources to conduct the proposed experiments.<br>• Better understanding of gene expression in DMD is needed.<br>• A broad recruitment plan is in place for the collection of samples. |
| | *none* |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 12 **No:** 0 | • Preliminary data are very good. A large part of the proposal is a continuation of what the team is currently doing.<br>• Access in human samples from untreated and treated patients is a significant advantage in the field of DMD that will enable collection of data to enable single profile of different cell populations.<br>• The team have expertise in human muscle biopsies. They collected 10 additional biopsies since the previous submission (1 year).<br>• The preliminary data are strong and supportive of the proposed studies.<br>• The project directly investigates a human disease. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 12 **No:** 0 | • It is somewhat unclear what the next steps will be, but the data generated will be of huge benefit to the field.<br>• Aim 1 is a continuation of previous experiments and will obtain additional data on DMD muscle resident satellite and niche cell gene expression.<br>• The project's plan, design and analysis are well described.<br>• Pitfall and alternative approaches are briefly mentioned but not well described. Still, this research is important to be conducted. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 12 **No:** 0 | • Outstanding team that is currently working together.<br>• The team has generated extensive data.<br>• The project contains a comprehensive proposed data analysis, and it is likely to be achieved in the proposed timeline.<br>• The proposal is conceptually good and the investigators have implemented well-validated methods. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 12 **No:** 0 | • The proposal will include a diversity of biopsies from a wide range of mutations, ages, disease progressions, and ethnic and socio-economic backgrounds.<br>• The team will employ a full-time community liaison.<br>• The investigators have taken all appropriate measures (including a full-time community liaison) to provide patient outreach and expand to new patients via community activities and charity groups |

| Application # | DISC0-16039 |
|---|---|
| **Title**<br>(as written by the applicant) | Lewy body dementia, $\alpha$-synuclein, and cell-specific mechanisms of neurodegeneration |
| **Research Objective**<br>(as written by the applicant) | We will generate insights about mechanisms by which $\alpha$-synuclein leads to neurodegeneration of the forebrain and substantia nigra, regions affected in Lewy body dementias and Parkinson disease. |
| **Impact**<br>(as written by the applicant) | If the proposed studies are successfully achieved, they will impact our understanding of Lewy body dementias specifically and $\alpha$-synucleinopathies and neurodegeneration more broadly. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Differentiate iPSCs from affected, unaffected and gene-corrected individuals into forebrain and dopaminergic neurons.<br>• Transduce neurons with biosensors to visualize biological pathways relevant to hypothesized mechanisms of neurodegeneration.<br>• In some cultures, perform genetic perturbations designed to investigate the cause-or-effect relationships of differentially expressed genes discovered by scRNAseq analysis of these models.<br>• Perform automated imaging and longitudinal single cell analysis to further characterize the temporal dynamics of putative mechanisms of neurodegeneration and to evaluate the effects of perturbations.<br>• Analyze the images with computational tools including machine learning / deep learning / artificial intelligence to discover and quantify disease phenotypes and effects of perturbations. |
| **Statement of Benefit to California**<br>(as written by the applicant) | As the largest state in the US, California is the most affected by Lewy body dementias and Parkinson disease, which inflict tremendous suffering and financial burden on patients and their families. Our project will benefit Californians by pioneering new discoveries that may lead to therapies to alleviate this suffering. This project will also and generate intellectual property for California and employ Californians. The PI has a track record of leveraging CIRM funding to attract federal funding. |
| **Funds Requested** | $1,739,760 |
| **GWG Recommendation** | (85-100): Exceptional merit and warrants funding, if funds are available |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 90

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

| | |
|---|---|
| **Mean** | 90 |
| **Median** | 90 |
| **Standard Deviation** | 1 |
| **Highest** | 92 |
| **Lowest** | 85 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 15 |
| **(1-84): Not recommended for funding** | 0 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 14 **No:** 0 | • The project aims at developing a new cell based model of Lewy body Dementia (LBD) which is much needed in the field.<br>• The project will define and distinguish mechanisms of alpha synuclein (asyn) related pathology between Parkinson's disease (PD) and LBD, which is an area that is currently not well understood.<br>• LBD and its relationships to other dementias remains an important area.<br>• The models used are standard, but the cells of origin (familial LBD) and their use to model LBD are novel<br>• The outcome will have a significant impact on LBD and diseases with asyn pathology like PD.<br>• The project aims to fill a critical knowledge gap in understanding how asyn contributes to neurodegeneration in LBD, using patient-derived iPSCs. This could lead to better understanding of LBD and PD pathogenesis, and the discovery of new diagnostic biomarkers and therapeutic targets<br>• If successful, the project could have a major impact by providing insights into cell-specific mechanisms of neurodegeneration in LBD and potentially informing the development of targeted therapies. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 14 **No:** 0 | • The project is very well motivated. One caveat is the use of cells from familial LBD, which is uncommon. It is not clear how the pathology in these patients relate to sporadic disease.<br>• Preliminary data, including differential gene expression analysis in iPSC-derived dopaminergic and cortical neurons from asyn gene (SNCA) triplication carriers, provide compelling evidence supporting the project's hypothesis and the feasibility of the proposed research.<br>• The project is highly relevant to human biology and disease, as it investigates the mechanisms underlying LBD, a condition affecting over a million Americans. The research could lead to significant advancements in understanding and potentially treating α-synucleinopathies<br>• The throughput of analysis possible via this application could help break this bottleneck. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 14 **No:** 0 | • The project is well designed and described and makes use of adequate methodology<br>• The project's design, which includes the use of patient-derived iPSCs and advanced imaging techniques, is poised to yield meaningful results that could advance our understanding of LBD and α-synuclein's role in neurodegeneration<br>• Given the significance of the research and the innovative approach, the project likely has a sense of urgency appropriate for CIRM's mission to accelerate stem cell treatments to patients with unmet medical needs<br>• Outstanding preliminary data indicate that the SCNA triplication ipsc lines overexpress synuclein and have degenerative disease relevant phenotypes in both dopaminergic (DA) and forebrain neurons |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 14 **No:** 0 | • The project is feasible given the applicants expertise, that the cell lines are already in place and also the gene corrected version.<br>• The project's aims and expected outcomes, which include elucidating the mechanisms of neurodegeneration in LBD using patient-derived iPSCs, appear logical and achievable. The preliminary data and the research plan suggest that the project is well-conceived and feasible within the proposed timeline.<br>• The team is good but limited to one PI and staff in that group.<br>• The PI and team are highly qualified, with a track record of relevant research and expertise in the field of neurodegeneration and the use of iPSC models.<br>• Might be overambitious within budget and timeline, but the PI lab has considerable resources so planning is probably reasonably realistic. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 14 **No:** 0 | • Partially so [re adequately addressing and accounting for the influence of race, ethnicity, sex, gender, and age diversity].<br>• Project outcomes extend or validate the applicability of regenerative medicine discoveries to underserved populations, including underserved racial/ethnic communities.<br>• Applicant described prior efforts or proposed plans for outreach, partnership, or educational activities to inform the development of DEI within the research project. |

| Application # | DISC0-16122 |
|---|---|
| **Title**<br>(as written by the applicant) | Mapping and modeling endothelial cell fate decisions for pulmonary arterial hypertension (PAH) |
| **Research Objective**<br>(as written by the applicant) | We will build a foundational model and experimental platform to catalog all genes that promote and protect against pulmonary arterial hypertension (PAH), with the potential to extend to many other developmental and disease processes. |
| **Impact**<br>(as written by the applicant) | The studies aim to identify genetic targets for pulmonary arterial hypertension (PAH) therapy and develop a predictive model to accelerate stem cell research and novel treatments. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Genomic datasets in healthy and diseased cell types<br>• Map of which genes affect cell fate decisions<br>• New predictive computational models<br>• Framework to take genetic information and better guide the diagnosis and treatment of pulmonary arterial hypertension (which can be extended to more diseases in the future)<br>• New technology that allows large-scale genetic perturbations in single cells with high temporal resolution<br>• Compiled large-scale single-cell data resources for predictive modeling |
| **Statement of Benefit to California**<br>(as written by the applicant) | The proposed research aims to harness the potential of foundational models and single-cell genomics to understand and predict cell fate transitions in the context of pulmonary arterial hypertension (PAH), allowing us to identify genes and pathways controlling cell fate decisions, leading to potential therapies for PAH. Therefore, it holds promise for benefiting the State of California and its citizens by advancing our understanding of disease mechanisms and potentially contributing to the development of regenerative therapies. |
| **Funds Requested** | $1,540,798 |
| **GWG Recommendation** | (85-100): Exceptional merit and warrants funding, if funds are available |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 90

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

| | |
|---|---|
| **Mean** | 90 |
| **Median** | 90 |
| **Standard Deviation** | 1 |
| **Highest** | 92 |
| **Lowest** | 90 |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 14 |
| **(1-84): Not recommended for funding** | 0 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 13 | • There are two important potential impacts: 1. Better understanding of pulmonary arterial hypertension (PAH), which is a rare condition (approximately 500-1000 new cases annually in |

| | |
|---|---|
| **No:** 0 | the US), with about 15-20% of cases heritable. It is poorly understood, and genetic components, e.g., a number of cases of heterozygous BMP receptor mutations, but with only 20% penetrance. 2. The project team's use of a global genetic approach with sophisticated bioinformatic analysis is highly ambitious and potentially paradigm-altering for disease analysis. If successful, it could be applied very broadly.<br>● PAH is driven by endothelial cell dysfunction and often results from heterozygous loss-of-function mutations in BMPR2. However, only 20% of PAH patients carry mutations in BMPR2, indicating that other genes yet to be identified may genetically interact with BMPR2 to cause the disease. Identifying these other genes will be essential to understanding PAH pathogenesis and designing targeted therapies.<br>● This proposal uses hiPSC-derived endothelial cells and CRISPRi screening and analysis platform for studying PAH.<br>● The proposal uses PAH as a test case. The applicant aims to develop a general-purpose technology that leverages advanced foundational models and genetic screens to create comprehensive predictive maps of the impacts of gene perturbations on cell state.<br>● If successful, the study will discover genes that could induce or reverse the effects of endothelial cell dysfunction in PAH. The study will develop and demonstrate a general-purpose technology that leverages advanced foundational models and genetic screens to create comprehensive predictive maps of the impacts of gene perturbations on cell state.<br>● This proposal is designed to understand the complexity of genetic and cellular factors that can drive pathogenic changes in endothelial cells in the context of pulmonary arterial hypertension. The study will use Perturb-seq and analysis strategy to define a Catalog of Gene Programs in hiPSC-derived endothelial cell differentiation with the aim of identifying genes and/or pathways important in the development of PAH.<br>● PAH currently has no cure, and therapeutic approaches manage symptoms only. Knowledge of the cellular and genetic changes that drive the disease could lead to opportunities for regenerative strategies.<br>● The study could have significant implications beyond its direct application to PAH. The technological advancement could be applied to drive regeneration in a number of different diseases through a greater understanding of the genetics that drive disease-dependent dysregulation of fate decisions. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 13<br>**No:** 0 | ● Use of global gene expression experiments and informatics to understand endothelial cell fate decisions is a unique approach. Strong peer-reviewed publications support the rationale.<br>● The study is based on the applicant team's previous studies.<br>  ● One co-investigator has pioneered the use of patient-derived human (h)iPSC-endothelial cells (EC) to study cellular mechanisms of PAH. They found that patient-derived hiPSC-ECs show characteristic defects that match PAECs in vivo, and used these systems to screen for small molecules that could reverse cellular pathology. The proposal is to use a genetic disturbance approach in iPSC-derived ECs to discover genes that could induce or reverse the effects of EC dysfunction in PAH.<br>  ● Another co-investigator has discovered phenotypes of pulmonary arterial endothelial cells (PAEC) from PAH patients. The data from PAH patient hiPSC-derived endothelial cells show transcriptional and phenotypic defects matching native patient PAEC.<br>  ● A third co-investigator has applied Perturb-seq in endothelial cells.<br>  ● The Principal Investigator (PI) recently published a predictive framework that learns vector field functions to represent the underlying gene regulatory network. The team has implemented a flexible, modular framework using a high-performance, scalable, and distributed interface.<br>● PAH is known to be a multifactorial disease with several disease-driving genetic mutations. However, targeting the related pathways has not led to a cure for the disease nor a therapeutic that can reverse the disease. Current models are not able to recapitulate the disease well, likely due to an incomplete understanding of the genetic factors driving the disease. This project has the potential to unravel the genetic complexity of PAH, which should significantly inform the therapeutic advancement for the disease.<br>● There are solid preliminary data supporting the proposed project, including a recent high-profile publication wherein a similar approach was applied to coronary artery disease.<br>● This project could provide precedent for many other projects based on the analysis of cell fate decisions, potentially benefiting the field.<br>● This was a difficult application to read and understand. The applicant should not assume knowledge of all key techniques by the reviewers and avoid reliance on jargon (e.g., esoteric names of techniques and analytic programs). Writing for a wider audience is advised, with more explanation of technology and accessible language. |

| GWG Votes | Is the project well planned and designed? |
|---|---|
| **Yes:** 13 **No:** 0 | • The project employs a comprehensive approach and cutting-edge technology to relate gene expression to cell fate and a specific disease phenotype.<br>• They have a strong project team with excellent, complementary expertise (clinical, lab experiments, bioinformatics). The applicant team also has the balance of a very experienced investigator and a newly-minted junior faculty member, plus other key personnel.<br>• Yes, the CRISPRi screening is based on previously discovered transcription and phenotype in hiPSC-derived endothelial cells from PAH. The study will directly measure the transcriptional effects of perturbing genes in hiPSC-derived endothelial cells from PAH and elucidate the consequences of BMPR2 mutations.<br>• However, one unaddressed issue is exactly which iPSC lines will be used for the disturbance screening, and the rationale for these choices.<br>• Overall, the project is well-planned to take into consideration controls, iPSC differentiation repeats, and differentiation variability. It would be nice to see some details of the functional implications of the genetic manipulations in the validation of the top 10 selected targets. As proposed, there is no detail beyond validation by bulk sequencing.<br>• The gene correction of the BMP mutations lacks some detail on exactly how this will be validated. This may have implications for the success of Aim 2.<br>• Pitfalls in the approach are discussed, and alternative/complementary approaches are discussed. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 13 **No:** 0 | • The approach is still somewhat theoretical. It will take time to see how the somewhat abstract conclusions about cell fate relate to disease phenotype and potentially treatment.<br>• The PI has extensive expertise in single-cell genomics technologies and data analysis, and developing analytic tools for large-scale scRNA-seq datasets.<br>• The planned collaboration is powerful. However, percent effort associated with the more experienced co-investigators should be increased. For example, a postdoc or PhD student from these labs could be involved in this project.<br>• Given the expertise and experience with similar approaches in other disease areas, the approach and timeline seem appropriate.<br>• This project should be feasible as proposed given the expertise of the research team, solid preliminary data, and recent publications showing the application of the computational approaches in other disease areas.<br>• The research environment in the specific laboratories and the institution has everything needed to support the project's success. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 13 **No:** 0 | • One of the stronger aspects is a co-investiagor's stated experience in global organizations focused on PAH and related conditions.<br>• The applicant will include hiPSC lines from underserved patient populations in the studies. The institutional biobank houses hiPSCs from diverse genetic backgrounds. To address the increased prevalence of PAH in females vs. males, the applicant will use control and PAH-derived hiPSCs from both sexes, matched for age.<br>• The tools, software, and data should be readily accessible to users all around the world. The applicant will continue to support and grow their community on GitHub, which currently has over 1,000 users worldwide according to the applicant.<br>• The fact that PAH impacts underserved populations that frequently present with more advanced disease has been taken into consideration, and iPSC lines will be selected to account for genetic diversity. Additionally, age-matched iPSCs from both sexes will be used, with males serving as controls for the female predominance of PAH.<br>• The outcomes would have applicability to underserved populations to better inform treatment options in the future.<br>• The research team has a longstanding history of supporting underrepresented groups. In addition, the institution has a number of DEI educational initiatives. |

| Application # | DISC0-15654 |
|---|---|
| **Title**<br>(as written by the applicant) | Modeling and understanding alveolar hypoplasia in Down syndrome (DS) using iPSCs-derived alveolar type II cells |
| **Research Objective**<br>(as written by the applicant) | Understanding alveolar progenitor cell defects in T21 and the genes/pathways associated with them will allow for developing therapeutic approaches for individuals with DS. |
| **Impact**<br>(as written by the applicant) | Although trisomy 21 affects multiple organ system, respiratory complications are the major cause of death in kids and adults with DS. The causes of lung disease in DS remain poorly understood. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Create an in vitro model to study alveolar defects in Trisomy 21 using T21 and euploid iPSCs lines<br>• Elucidate the defects in progenitor cell commitments to AT2 and AT1 cells in Trisomy 21<br>• Determine the role of the FGFR2 pathway in such defects and whether targeting this pathway in ex vivo organoid model can reverse the defects observed in T21<br>• Define the role of the mesenchymal niche in the alveolar hypoplasia phenotype observed in T21<br>• Generate unique resources (iPSCs) from diverse racial and ethnic background that will become available to the scienti<c community to study other co-morbidities of DS (intestine, pancreas, liver)<br>• Generate multi-omics data that will be made publicly available and serve as a tool for other scientist to develop other research questions |
| **Statement of Benefit to California**<br>(as written by the applicant) | In California, about 667 babies are born with Down Syndrome every year, with the highest DS rate for Hispanic infants. Respiratory complications are the most common cause for hospital admissions in DS. Healthcare cost is 12-13 times higher for children with Down Syndrome than those without. Understanding lung disease in Down Syndrome provides the opportunity to intervene early and adequately thus ameliorating outcomes and reducing healthcare burden for the state of California. |
| **Funds Requested** | $1,524,196 |
| **GWG Recommendation** | (85-100): Exceptional merit and warrants funding, if funds are available |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 90

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

| | |
|---|---|
| **Mean** | 88 |
| **Median** | 90 |
| **Standard Deviation** | 4 |
| **Highest** | 90 |
| **Lowest** | 75 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 14 |
| **(1-84): Not recommended for funding** | 1 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in

the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 14 **No:** 0 | • Down syndrome (DS), also commonly referred to as Trisomy 21 (T21) due to the partial or complete third copy of human chromosome 21, is the most prevalent chromosomal abnormality worldwide. T21 is a multisystem syndrome. Although more than half of hospitalizations of individuals with DS are due to lung disease, very little is known about the pathogenesis of lung disease in T21.<br>• The applicant proposes to generate iPSCs from people with T21 and age matched controls, and use iPSC-derived alveolar type II cells to model T21 lung disease in vitro. In this way they seek to understand the cellular and molecular defects governing alveolar defects in DS.<br>• This proposal aims to define the mechanisms inducing lung dysplasia in individuals with trisomy 21. This study is important since it could help to improve life expectancy in the T21 population. It could also have a broader impact since it could be used to model additional lung disorders.<br>• Modeling diseases related to T21 is a major challenge since animal models are not available. The lung is particularly complicated since the defect seems to appear before birth. The use of hiPSCs could address the current limitations and reveal key mechanisms.<br>• The applicant will generate multi-omic data to identify gene regulatory networks and epigenetic marks that represent risk factors for developing lung disease. They will also use de-identified human fetal and postnatal tissue for validation and comparison of findings.<br>• Animal models for T21 are limited due to lack of chromosome 21 in rodents. Additionally, the available animal models developed to study T21 do not recapitulate all hallmarks of human DS manifestations nor display the lung defects.<br>• The use of iPSCs-derived AT2 cell model allow one to replicate human lung development in a controlled and defined manner in a dish, and can be used to identify specific genes and epigenetic marks associated with alveolar hypoplasia in individuals with DS.<br>• The experiments in this proposal will advance our understanding of the biology of the human lung progenitor cells in health and in T21. This proposal will allow for the development of unique resources (T21 iPSCs lines, very few currently available), and single nucleus multi-ome sequencing data on primary alveolar cells and iPSC-derived alveolar cells.<br>• The project addresses a clear unmet clinical need and could have a broad impact. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 14 **No:** 0 | • The project will provide a new hiPSCs based platform to study lung development and to uncover mechanisms driving lung disease during fetal life. The rationale of the proposal is very strong and it will have a broad impact.<br>• The focus on the FGF2R pathway could be a weakness. Indeed, the preliminary data are not entirely convincing and FGF signaling is difficult to modulate in vitro. However, the rest of the grant is interesting and well prepared. So, there is no doubt that this proposal will generate exciting data.<br>• The project will take advantage of human fetal samples. This is a unique resource that will greatly help to validate results generated in vitro.<br>• Issues with differentiation medium and variability in differentiation efficiency between cell lines may introduce inconsistencies and affect the validity of the results. While the proposal intends to focus on reproducible changes across samples, the inherent variability in iPSC differentiation could lead to challenges in identifying consistent disease phenotypes.<br>• In recent years, with single cell technologies and the human cell atlas initiative, scientists are just starting to understand the processes of human alveolar development and regeneration. Nonetheless, understanding the process of alveologenesis in T21 lungs remains quite elusive.<br>• AT2 cells are essential for normal alveolar development, homeostasis, and regeneration after injury. The use of iPSCs-derived AT2 cell model allows the applicant to replicate human lung development in a controlled and defined manner in a dish. The model can be used to identify specific genes and epigenetic marks associated with alveolar hypoplasia in individuals with DS. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 13 **No:** 1 | • The project is divided into three aims subdivided into three subaims. The first aim is to define the molecular and cellular defects underlying alveolar hypoplasia in T21 developing lungs. The second aim will define the role of FGF2R signaling in these defects.<br>• The applicant has generated and characterized three T21 lines and three control lines using fibroblasts from fetal lung samples, demonstrating expertise and feasibility for this sub-aim. They will also generate iPSCs from human postnatal lung samples with and without alveolar anomalies. They have a diverse T21 and matched control tissue resource. |

| | |
|---|---|
| | • The proposal is well justified and the plan is well organized/articulated.<br>  • Differentiation and maturation of cells are well planned.<br>  • Bulk and single nucleus RNA- and ATAC-sequencing during differentiation will allow the applicant to identify gene regulatory networks and epigenetic marks in T21 derived AT2 cells.<br>  • Testing the hypotheses that decreased FGFR2 signaling contributes to AT2 defects in T21 is well planned.<br>• The proposal does not specify the total number of iPSC lines planned for generation and use, which could impact the robustness and reproducibility of the study.<br>• The project's hypothesis on the role of FGFR2 signaling in AT2 cell defects may be too narrow and could overlook other critical pathways involved in DS lung disease.<br>• Findings from iPSC-derived AT2 cells may not be fully generalizable to the in vivo context of lung disease in DS, limiting the translational potential of the research. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 13<br>**No:** 1 | • The preliminary data are impressive and convincing. They support the feasibility of the project.<br>• This is a high risk / high gain program. However, the applicant has included relevant back up plans and there is no doubt that large parts of the program are feasible.<br>• The team consists of human lung development and biology, T21 disease, iPSC modeling and AT2 cell differentiation expertise.<br>• The team is appropriately qualified and staffed, with collaborations established to address previous concerns about expertise in iPSC differentiation and bioinformatics.<br>• The team has access to necessary resources, including expertise in endothelial cell biology and single-cell transcriptomics, as well as a dedicated Data Project Manager.<br>• The Principal Investigator (PI) has extensive expertise in respiratory lung biology. S/he collaborated on and co-authored several publications addressing the role of epithelial-mesenchymal interactions as well as FGFR2b signaling on alveolar type 2 cells in mouse and human development and disease.<br>• A co-PI on this proposal has extensive expertise in lung developmental biology, human lung development and in particular T21. Another co-I on this proposal has a proven record in the differentiation of iAT2 cells. Another key person is a full-time computational biologist who will be in charge of the multi-omic data analysis.<br>• The project can be achieved within the proposed timeline. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 14<br>**No:** 0 | • The applicant underlines the importance of gender and genetic diversity in T21 development. They have a plan to derive diverse T21 hiPSCs to address the sex differences. Ancestral diversity is more challenging.<br>• While the project aims to generate iPSC lines from donors of diverse backgrounds, it is unclear how effectively the study will address the influence of race, ethnicity, sex, gender, and age diversity on disease outcomes<br>• Yes; the applicant will generate iPSCs lines from de-identified tissues from different sex, ethnic and racial backgrounds in order to compare the findings.<br>• DS rates are increasing over time among Black, Hispanic and American India/Alaska Native, but not white, populations. The project may help improve the lives of these underserved families and patients. |

| Application # | DISC0-15816 |
|---|---|
| **Title**<br>(as written by the applicant) | Investigating the SGF29/SAGA complex in regulation of normal and cancer stem cells |
| **Research Objective**<br>(as written by the applicant) | This study will address gaps in our understanding of how normal and cancer stem cells differ in their epigenetic states, helping develop new cancer-stem-cell-targeting therapis. |
| **Impact**<br>(as written by the applicant) | The long-term impact of our studies is the understanding of mechnistic differences between normal and cancer stem cells and the development of new therapies. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Evaluation of the role of SGF29 in normal hematopoiesis<br>• Evaluation of the role of SGF29 in LSC activity<br>• Preclinical evaluation of SGF29/KAT2A inhibitors in anti-LSC activity |
| **Statement of Benefit to California**<br>(as written by the applicant) | The population > 60 years of age is expected to show an overall increase of 166% in the next 4-5 decades in California, with an anticipated increase in the rise of several cancers. AML incidence rises with age and most elderly patients succumb to the disease. Our studies, aimed at evaluating novel treatments for AML may help benefit patients diagnosed with AML. Our studies on novel agents may also bring economic benefit if novel drug candidates proceed to clinical translation from our studies. |
| **Funds Requested** | $1,647,600 |
| **GWG Recommendation** | (85-100): Exceptional merit and warrants funding, if funds are available |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 87

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

| | |
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| **Mean** | 88 |
| **Median** | 87 |
| **Standard Deviation** | 2 |
| **Highest** | 90 |
| **Lowest** | 85 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 15 |
| **(1-84): Not recommended for funding** | 0 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 14<br>**No:** 0 | • Treatment of AML, especially in adults, has not progressed greatly in some years. There is a significant unmet need. American Cancer Society reports ca 21,000 cases/year of AML, mostly in adults, and > 11,000 deaths, almost all in adults.<br>• The proposal focuses on a novel approach for AML therapy based on on targeting chromatin readers, specifically one designated SGF29.<br>• Targeting of transcription factors (TFs) and defining distinguishing features of hematopoietic stem cells (HSCs) vs leukemic stem cells (LSCs) has been challenging. SBF29 is a co-factor |

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| | and given its potential potency, serves as a viable, and high priority candidate in an area that has had little impact beyond observational biology.<br>● SGF29 is a chromatin reader that could be selective and therapeutically targetable for AML LSC and potentially other common oncogenic pathways.<br>● The role of SBF29 in AML and, potentially, other cancers is unique and holds promise.<br>● There is potential for this approach if effective in primary AML cells, and second, if selective for AML and not normal HSCs.<br>● The connection to stem cell-based therapies is a little bit of a stretch. Yes, the investigators propose targeting LSCs, but they are trying to kill them. When I think of regenerative medicines, I think about using stem cells to replace damaged organs/tissues. With that said, in Aim 1, the investigators do propose to look at the role of SGF29 in regenerative hematopoiesis in normal cells, which could be beneficial to the regenerative disease. A bit of grantsmanship could turn this into a more "CIRMy" grant, but I really think this is a great and important proposal. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 14<br>**No:** 0 | ● Targeting TFs has been difficult due to their ubiquitous roles. The applicant uniquely aims at TF co-factors that have features of drug access, thus providing strong biological rationale and novelty to this proposal.<br>● Highly relevant given the direct use of human AML cells and projected use of primary AML and normal hematopoietic cells as secondary and tertiary validations proposed.<br>● There is already published support of highly promising clinical data on therapy targeting a distinct target in the same category, suggesting this has potential to be successful. Overall, targeting developmental/stem cell regulators seems a very attractive, novel approach.<br>● The recent identification of SGF29 by the PI makes this target ideal for study and likely to provide future clinical utility. Especially due to recent clinical use of an inhibitor of menin1 (MEN1) chromatin adapter (H3K79me2) that was able to induce remission in 1/3 of patients as a single therapeutic.<br>● Good preliminary evidence supporting approach of using lysine methyltransferase 2A (KAT2A) inhibitors to block overexpression of AML oncogenes mediated by chromatin modification (K9 acetylation) via recruitment by SGF29 of the SAGA complex.<br>● Strong data to reveal SGF29 in original CRISPR screen, and subsequent validation of SGF29 in cell lines.<br>● Strong preliminary data (CRISPR screen, validation in cell lines, CROP-seq, etc)<br>● The mouse studies and use of PDXs will give great insight about SGF29's role in LSCs.<br>● CROP-seq (ddPCR of sgRNAs) very clearly demonstrate the priority of SBF29 in AML. However, in Fig4a it is curious as to why MEIS-1 was not knocked out as a control for the droplet system. Raises concerns of the extent of KD in this system.<br>● The rationale and logic for patient derived LSC work, followed by CyTOF, is difficult to understand. The basis of using a CALM-AF10 driven leukemia and how this will be coupled with 26 primary AML samples is unclear. No secondary transplants are proposed, the only convincing measure of LSC self-renewal.<br>● There are concerns about using a prognostic signature for LSCs as evidence for effects on LSCs. This is a weighted score, and should not be compared to CD34 expression scores to determine specificity. This raises concerns on the role of SGF29 in LSCs vs. its clear role in AML given the data is generated in cell lines. Continued use of murine clonal induced leukemias and cell lines in Figs 6 and 7 are convincing for pan-AML, but no evidence for effects in stem populations is provided.<br>● The author states, “Taken together, our preliminary studies using CRISPR screens, single cell and bulk RNAseq, chromatin proteomics and leukemia assays strongly indicate that SGF29 plays an important role in leukemogenesis” This is consistent with published work, but lacks evidence for the effects in stem cells that is being inferred here. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 14<br>**No:** 0 | ● Two well-planned Specific Aims:<br>1) Basic study of SGF29 in context of function of normal HSCs and leukemic AML stem cells.<br>2) Test available inhibitors of the pathway as candidates to selectively target LSCs (based on long history that points to AML as a stem cell disease),<br>● Primary HSC and AML work is proposed, and conditional KO SGF29 mice is nearly in place. These are ideal tools for the foundational work proposed.<br>● Rationale for use of CyTOF is unclear, and complete absence progenitor readout or secondary transplants is disregarded.<br>● Aim 2 is a highly complex series of experiments using degron engineered cells lines and CHIP-seq. The requirement of this engineered sophistication in 2 cell lines, and expense, is unclear. |

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| | Simpler methods to support this interaction should be considered first. Eg. Simple CHIP-seq with SBF29 KO lines.<br>● Use of PF-4245 or PF-2315 provides an excellent and clinically relevant direction. However, this is standalone, and is poorly linked to force 2 Aims in this proposal. The connection to SAGA association to SGF29 and use of this drug is unclear.<br>● Studies with PF-4245 or PF-2315 lack detail. How was the dose and duration determined? Source of these drugs and expectations? The in vitro assays and multiplex of drugs is not feasible, and despite references provided poorly define EC50s. Unclear how and why RNA-seq is being done in the context of CFU data.<br>● No measure of endogenous mouse HSCs is being proposed, and effects on T-cells can not be determined in this readout, which is important for PF-4245 or PF-2315 treatment studies.<br>● The readout of colony forming units (CFUs) from AML has been characterized and requires distinguishing the effects from normal progenitors. The author should scan the recent literature on this topic to avoid false positive and negatives in this assay that does not simply readout pure AML progenitors unless carefully controlled.<br>● Rationale to use NRG recipient mice is unclear. This raises concerns if bonafide LSC are being measured vs. in vivo effects alone. Conclusions on LSCs require comparable standards of LSC measure.<br>● Not sure if whether the right mouse model is used for the PDX studies. Rationale was not explained.<br>● SBF29 could be compensated, and as per Aim 1 objectives, may have a deleterious role in normal HSC biology. This would create a challenge in terms of therapeutic directions. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 14<br>**No:** 0 | ● Have available appropriate model cell systems, including AML cell lines and patient-derived xenografts<br>● Good tool compounds to test, targeting the histone (lysine) acetyltransferase KAT2A, a key component of the SAGA complex (chromatin reader): 1) histone deacetylase inhibitor PF-4245; 2) a protein degrader<br>● Good opportunity to benchmark vs MLL/Menin inhibitor, in same category of blocking chromatin reader, which showed promising activity as single agent in trial in AML patients.<br>● The team is well assembled, including collaborators.<br>● Excellent and in position given the preliminary data generated.<br>● Research institute is dedicated to drug discovery for the future of this work and target validation in patients. All expertise and equipment is in place.<br>● Collaboration with a Cancer Epigenetics Team is great and could lead to drug candidates being identified.<br>● Use of commercially available inhibitors is great.<br>● The SGF29 CKO mouse model is nearly complete.<br>● Difficult to understand how LSC patient sample work in Aim 1 will possibly be done, as this is a stand alone project in itself. Highly unlikely.<br>● Unclear how this will be affordable, specifically the number of rounds and control of sequence work that is proposed in nearly every sub-aim. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 14<br>**No:** 0 | ● Medical need for better AML treatment is even more severe in underrepresented patient groups, e.g., non-Hispanic blacks and Hispanics than in white non-Hispanics.<br>● Well targeted to non-hispanic black patients, and diverse plan in place for future results in detail.<br>● AML outcomes are worse in African American, Hispanic, and lower socio-economic status individuals.<br>● The PI has a strong personal track record supporting DEI as Associate Director of a Cancer Center DEI program.<br>● Several symposia and outreach programs are in place annually that this proposal will be integrated.<br>● PI is Associate Director of DEI and has been heavily involved in DEI initiatives. Very strong in this area. Hosts a Cancer Center open house with the broader community.<br>● Excellent description and details provided. This is accounted for in xenografts, inhibitor use, and samples recruited.<br>● Study includes samples from diverse backgrounds and HLA types, and uses data from DepMap, which includes diverse cell line backgrounds. |

| Application # | DISC0-15774 |
|---|---|
| **Title**<br>(as written by the applicant) | Modeling of GATAD2B-associated neurodevelopmental disorder and NuRDopathies: Investigation of cellular & molecular anomalies altering neurodevelopment |
| **Research Objective**<br>(as written by the applicant) | Human and animal models of NuRD-deficiency will identify NuRD-subtype function in context of neurogenesis.<br>Multi-omic studies will identify/quantify molecular and cellular changes in NuRD-deficiency. |
| **Impact**<br>(as written by the applicant) | NuRD-deficiency causes several neurodevelopmental disorders (NDDs), our work will identify and quantify cellular and molecular changes in human and mouse models of corticogenesis with NuRD deficiency. |
| **Major Proposed Activities**<br>(as written by the applicant) | • We will generate GAND-IPSCs with inducible expression of HA-GATAD2B to correct these cells' phenotypes seen in NPC growth assays and in the spatial and temporal expression of cortical laminar markers.<br>• We will generate GAND-IPSCs with inducible expression of HA-GATAD2A to see if GATAD2B's paralog can function to correct the cellular phenotypes seen in NPC growth and cortical laminar marker assays.<br>• GAND-IPSCs with inducible knockdown of GATAD2A will determine if repression of GATAD2A can function to correct the cellular phenotypes seen in NPC growth and cortical laminar marker expression assays.<br>• GAND-IPSCs will be used to generate cerebral organoids and using immunohistochemistry will determine if NPC and cortical neuron subtypes are generated and coexpress cortical laminar markers.<br>• GAND-IPSCs will be differentiated into cerebral organoids (excitatory/inhibitory) and undergo snRNA-seq/snATAC-seq to identify dysregulated genetic pathways within NPCs/neurons with NuRD-deficiency.<br>• Gatad2b-deficient mouse cortices will undergo snRNA-seq/ATAC-seq to identify dysregulated genetic pathways and altered cellular subpopulations in NPCs/neurons to inform/confirm human IPSC data. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Neurodevelopmental disorders (NDDs) affect >3% of the world's population. Understanding the mechanisms of NDDs is imperative for developing potential therapies to assist families. The focus of our work is the use of patient-derived IPSCs and mouse models to study the epigenetic dysregulation found in NuRD-deficiency and other NDDs. We hope to identify abnormalities in NuRD-deficiency that can be applied to many NDDs, while also fulfilling CIRM'S goal of understanding brain disorders. |
| **Funds Requested** | $1,318,441 |
| **GWG Recommendation** | (85-100): Exceptional merit and warrants funding, if funds are available |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 87

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

| | |
|---|---|
| **Mean** | 87 |
| **Median** | 87 |
| **Standard Deviation** | 2 |
| **Highest** | 90 |
| **Lowest** | 85 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 15 |
| **(1-84): Not recommended for funding** | 0 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 14 **No:** 0 | • Understanding the mechanistic underpinnings in ultra rare diseases is not only important for patients affected, but is a core aspect of CIRMs mission.<br>• The insights from this study are likely to extend beyond the specific disease GATAD2B associated neurodevelopmental syndrome (GAND). Findings may be relevant for other NuRDopathies defined by chromatin remodeling dysfunction.<br>• The study, if successful, may provide insights relevant for other disorders associated with chromatin remodeling dysfunction.<br>• The project will provide understanding of the mechanisms behind an ultra rare disease which presents an unmet medical need, but the rarity of the disorder is "a problem" at the same time.<br>• The applicants plant to investigate the function of NuRD and GATADA and B paralogs, deficiency of which causes a neurological pan-NuRDopathy. The applicants suggest this may be critical for human brain development. |
| | *none* |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 14 **No:** 0 | • The applicants propose that characterizing the function of NuRD and GATAD2- paralogs will have a critical impact on understanding human brain development. This is likely to be true as any deficiency of either GATAD2 paralog causes a neurological "pan-NuRDopathy", a group of dominant genetic neurodevelopmental diseases.<br>• Supportive data have been published in a quite comprehensive and convincing study by the applicants showing in an animal model that GATAD2B is associated with the neurodevelopmental syndrome GAND and plays a critical role in neurodevelopment and cortical patterning. However, preliminary data to support whether human organoids display a phenotype is not yet available.<br>• The applicant generated five GAND-IPSC lines that all have confirmed GATAD2B variants. The data comparing differentially expressed genes between iPSC and NPC in these lines support the hypothesis that 2B-NuRD and 2A-NuRD play different roles during development.<br>• Differentiation of GAND- and control-NPCs into cortical neurons revealed cortical lamination abnormalities and overlapping defects in mice and human.<br>• The combination of human organoids and the mouse model is promising, although it is not yet known if the organoids show a phenotype. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 14 **No:** 0 | • The loss and gain of function experiments in Aim 1 are straightforward and appropriate. Aim 2 is an open-ended characterization of GATAD2A deficient cells from human organoids and mutant mouse samples and should be informative.<br>• The pitfall section has been extended and is informative, statistical analysis has been added However, reproducibility and variability of organoid cultures is still not addressed.<br>• The project is generally well planned and designed, but the question of organoid variability remains to be addressed.<br>• Human organoid data are very limited, and Figure 7 is not very informative. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 14 **No:** 0 | • The applicants have demonstrated in published and preliminary data that all tools are in place to conduct the study in the proposed timeline.<br>• There are no identifiable obstacles that would impact project feasibility.<br>• The PI's research has been dedicated to understanding gene regulation, potential treatment, and modeling of ultra-rare neurogenetic and neurodevelopmental disorders.<br>Other members of the team provide experience in "omics" analyses and animals model research.<br>• The host institution provides all the necessary resources.<br>• The project is well developed and planned. |

| GWG Votes | Does the project uphold the principles of diversity, equity and inclusion (DEI)? |
|---|---|
| **Yes:** 14<br>**No:** 0 | • NuRDopathies are caused almost exclusively by de novo mutations, and affected children are from all racial and ethnic groups.<br>• The proposal describes applicant fostered outreach to affected families with the disorders and other related gNDDs.<br>• Patient-derived cells come from racially and ethnically diverse backgrounds.<br>• The team plan to use cells derived from diverse ethnic backgrounds. |

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| Application # | DISC0-15972 |
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| **Title**<br>(as written by the applicant) | Immune cloaking of human stem cell-derived insulin-producing cells for curative cell therapy without immunosuppression |
| **Research Objective**<br>(as written by the applicant) | The goal of our project is to generate cells for replacement therapy in patients that have reduced ability to trigger the immune response in the recipient and therefore escape rejection. |
| **Impact**<br>(as written by the applicant) | We test a novel molecule that reduces immune activation upon transplantation of allogeneic stem cell products that can improve graft survival and as a result alleviate disease symptoms. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Remove a DNA modifying factor from human pluripotent stem cells and test if the edited cells can differentiate into functional insulin-producing cells<br>• Test the immune-resilience of edited human cells when combined with immune cells in culture as well as in mouse models. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Diabetes is largely a patient-managed disease, and health literacy has a strong correlation with glycemic control. Underserved communities suffer disproportionately from complications, further reducing their quality of life. Our intention is to reach all patients with insulin-dependent diabetes, many of whom are Spanish-speaking California residents. Making therapies accessible to patients who face daily challenges of living with diabetes addresses a clear need in California communities. |
| **Funds Requested** | $1,192,586 |
| **GWG Recommendation** | (85-100): Exceptional merit and warrants funding, if funds are available |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

**Final Score: 85**

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

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| **Mean** | 85 |
| **Median** | 85 |
| **Standard Deviation** | 4 |
| **Highest** | 90 |
| **Lowest** | 75 |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 8 |
| **(1-84): Not recommended for funding** | 6 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 12<br>**No:** 1 | • Development of immune cloaking hPSC lines is a pivotal hurdle in utilizing stem cells for effective cell replacement.<br>• The goal is to develop an effective strategy for obtaining human iPSC- and ESC -derived allogeneic hypoimmune or immune evasive insulin producing pancreatic beta-cells that would |

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| | not require a long-term immunosuppression for graft survival and function but would be surveilled and removed if they undergo undesirable transformation.<br>● Preventing rejection of grafted allogeneic pancreatic beta cells, derived from stem cells, would potentially provide an "off the shelf" treatment for a large number of individuals with Type I diabetes (T1D). This would have enormous medical and economic impact.<br>● Life-long immunosuppression is required for allogeneic beta-cell graft survival in T1D patients. This is a significant limitation for translation of beta-cell transplantation therapies to clinic. By focusing on this issue, this project addresses a major bottleneck in the use of stem cells for the treatment of T1D.<br>● If successful, this project may have a major impact on stem cell and regenerative medicine field as it applies to treatment of T1D and to strategies for inducing allogeneic graft tolerance in other tissues and organs.<br>● The problem of rejection of beta cells in patients with diabetes, along with problem of rejection of allogeneic cell grafts, is pivotal in regenerative medicine.<br>● The applicants will take advantage of a recent published finding and their own preliminary results showing that TET2, a member of the ten-eleven translocation (Tet) methylcytosine dioxygenase family, regulates interactions between beta- cells and immune cells that trigger immune rejection and elimination of grafted beta- cells in vivo.<br>● The applicants hypothesize that TET2 controls production of T-cell activating chemokines and cytokines by the beta-cells, and that deletion of TET2 in the beta-cells may lead to a resistance of the edited cells towards auto- and allo- host immunity. Therefore, TET2 deficiency may prevent immunologic rejection of transplanted allogeneic human beta cells by the host's immune system.<br>● The project may unravel molecular mechanisms of TET2-dependent transcriptomic, epigenomic, and metabolomic modification of the genome, and the role of these modifications in immune-mediated cell rejection.<br>● The efficiency of differentiating TET2 knockout hPSCs into beta cells remains a subject of inquiry, raising questions about whether it can match the efficiency of unmodified cells.<br>● Successful execution of this project promises a breakthrough in stem cell therapy. Establishment of immune cloaking stem cell lines has potential to revolutionize the field, potentially rendering the need for immune suppressive drugs obsolete, and significantly enhancing feasibility and success of allogeneic stem cell therapies.<br>● This project holds the promise of a transformative impact on stem cell therapy. Creation of authentic immune cloaking human stem cell lines opens possibilities for differentiation into diverse cell lineages. Clinical application of these cells presents a potential paradigm shift in disease treatment, offering a versatile and potent resource for addressing a wide range of medical conditions. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 11<br>**No:** 2 | ● The hypothesis that inactivation of the TET2 gene will protect allogeneic stem cell-derived beta islets from immune rejection is reasonable. It is worth advancing in DISC0 phase to see if the approach merits support in the TRAN and CLIN paths.<br>● Project team provides a strong data package responding to critiques.<br>● Not much attention is paid to the problem of immune attack by cells targeting beta-cell-specific antigen(s) in T1D. Perhaps implicitly, the applicants assume that mismatched HLA genes between graft and recipient will limit attack against the graft by the recipient's MHC-restricted anti-beta-cell T-cells?<br>● The project is based on sound scientific rationale. The first set of supportive evidence comes from the demonstration by multiple groups, including the applicant team, that it is possible to obtain large quantities of functional insulin producing beta-cell like clusters (IPCs) from hiPSCs and ESCs using a defined stepwise protocol. The second set of evidence comes from published results from a collaborator and from the applicant lab showing that knockout of Tet2 in the mouse model leads to immune evasion in the context of an autoimmune attack on pancreatic beta-cells.<br>● Preliminary results are compelling. They show:<br>  ● Correlation between increased TET2 levels and enhanced susceptibility to autoimmune attack in wild type vs. Tet2KO NOD mice.<br>  ● Increased protection of Tet2KO mice from developing diabetes upon challenge with diabetogenic splenocytes.<br>  ● Inhibition of transcription of chemokines and inflammatory mediators in co-cultures of Tet2 KO mouse islets with a cytokine cocktail (TNFα+IL-1β+IFNγ) when compared to WT islet co-cultures with the same cytokines.<br>● Collectively these results suggest that the Tet2-KO mouse islets have reduced response to inflammatory mediators and might be protected from the immune attack. |

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| | • The applicant appropriately addressed concerns of the previous application. In response to these concerns:<br>  ◦ They derived human ESCs lacking TET2 using CRISPR gene editing, differentiated cells into C-peptide-producing cells and showed beta-cell function in vitro in glucose stimulated insulin secretion (GSIS) assays.<br>  ◦ Demonstrated increased viability of TET2KO human IPCs in vitro upon exposure to activated T cells and improved survival of TET2KO IPCs in vivo upon challenge with reactive T cells suggesting a protective effect of TET2 deletion in human ESCs from an allogeneic-immune attack on islets derived from these cell.<br>• Tet2-KO beta cells exhibit diminished expression of IFN gamma-induced inflammatory genes crucial for activating T cells in mice. If this finding extends to human beta cells, TET2 KO beta cells could potentially evade rejection by T cell-mediated autoreactivity.<br>• Challenges persist for beta cell therapy, particularly in the context of rejection from other immune cells, such as NK cells, and macrophages. Effectively managing these immune cells becomes a critical consideration.<br>• The study demonstrated successful differentiation of normal hESCs into IPCs and transplantation into animals, certain critical aspects remain unaddressed. They did not establish whether gene-edited cells could differentiate into beta cells with similar efficiency to WT cells. It is essential not to assume that knockout (KO) of any gene will not impact hPSC differentiation, as exemplified by inability of beta-catenin KO hPSC lines to generate mesoderm and endoderm cells.<br>• TET2 deficiency has been shown to impede mesoderm and blood differentiation in human embryonic stem cells (see paper: TET2 Deficiency Inhibits Mesoderm and Hematopoietic Differentiation in Human Embryonic Stem Cells, 2014, doi.org/10.1002/stem.1718). It is imperative to avoid assumptions that TET2 deficiency will not affect endoderm and beta cell differentiation.<br>• The applicant focused on demonstrating their TET2 KO cells may be protected from T cells. There is no data showing NK cell, and macrophage-mediated cell lysis for human IPCs derived from TET2 KO clones. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 11<br>**No:** 2 | • The project is well-planned and designed.<br>• The project plan and timeline are commensurate with CIRM mission.<br>• Overall, this project will examine the role of TET2 in modifying cellular responses of the KO IPCs to an allogeneic immune assault. Further, this study may lead to the identification of additional novel candidates that mediate interactions between graft and host in other tissues and organs in the allogeneic settings.<br>• There is a clearly laid out developmental plan with well formulated milestones. Two straightforward aims:<br>1. Gene edit pluripotent stem cell lines (including at least one suitable for advancing to clinic) to knock out TET2 gene and show these can be differentiated to insulin-producing cell clusters<br>2. Test "immune evasion capabilities" of the KO cell clusters<br>• The potential pitfalls and alternative approaches are discussed.<br>• The application does not address potential toxic islet-specific and systemic effects of TET2 deletion. Given known pleotropic roles of TET2 in different organ systems; for example, a finding that TET2 deletion enables certain tumors to evade antitumor immunity, these issues could create difficulties in translation of this strategy to clinic.<br>• The PI showed that they will delete a big piece of genome DNA (11k) by using two sgRNAs to knock out TET2 in Fig 3. There is no direct evidence from genomic PCR to show that they obtained true KO clones. They showed an indirect evidence that some clones did not show TET2 expression after differentiation.<br>• Missing is clarification on the gene editing tools and techniques they plan to employ. Do they use plasmid-based approaches, modRNA, or RNP-based formats?<br>• Off-target concerns: The document lacks information on the steps the PIs will take to mitigate the risks of off-target editing in stem cells.<br>• TET2-deletion may not provide protection of IPC grafts from allogeneic immune attack. If this proves to be the case, the investigators will delete TET2 from the MHC class I and II deficient cells.<br>• This alternative approach may not be correct. MHC deletion will protect cells from T cell-mediated lysis, which may be the same effect as TET2 KO cells.<br>• There is no data showing that IPCs from TET2 KO hESCs are protected from NK cells and macrophages.<br>• The PI needs to show TET2 KO IPCs are protected from other immune cells, such as NK cells, and macrophages. Deletion of MHC will not help cells avoid NK cell or macrophage rejection. |

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| | ● The alternative of knocking out HLA genes seems less attractive. Multiple knockouts and elimination of HLA seem risky, even if a "suicide switch" were built into the cells.<br>● This is a two-year project with annual budgets above $400,000; no justification for this budget structure is provided. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 13 **No:** 0 | ● The project is technically feasible.<br>● While this is an early-stage discovery project, it carries a high reward, but also a high risk; the proposed aims might not be fully achievable within the project's timeline.<br>● The applicants acknowledge the high risk of the project in the potential pitfalls section by recognizing that TET2 deletion might not provide a full solution to the problem of allogeneic rejection, but believe that their study has potential to offer a promising strategy toward finding a clinically-relevant solution. This is a reasonable assumption.<br>● The team is appropriately staffed and qualified and letters of support from the collaborators are provided. One of the collaborators on the project supplied a seed set of data for this project.<br>● The team has access to all needed resources.<br>● Concerns about feasibility of this project are:<br>  ● Knockout of TET2 alone may not be adequate to prevent T cell rejection, rejection by NK cells, and rejection by macrophage. Additional strategies might be necessary to enhance immune compatibility and address these potential challenges.<br>  ● There is a possibility that TET2 knockout hPSCs may not efficiently differentiate into functional beta cells. There is a need to compare the differentiation efficiency between KO and WT cells. This raises the need for a thorough examination of the differentiation process and the consideration of alternative approaches to optimize generation of functional beta cells.<br>● The applicant claimed that they will hire a gene editing expert. But so far, they do not have such person on board.<br>● The budget appears to be appropriate. However, a justification for a two-year project with an annual budget above $400,000 is not provided. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 13 **No:** 0 | ● The applicant organization seems to have a strong track record with respect to DEi.<br>● To account for racial/ethnic and sex differences, the project will include iPSC lines generated from women, people of color, and people with diverse ethnic and genetic backgrounds. This will be accomplished in collaboration with an academic stem cell core that recruits patients with T1D to collect material for cellular reprogramming. The investigators will also prioritize lines with diverse HLA subtypes.<br>● Since T1D affects people of all races and ethnic groups at all socioeconomic levels, the outcomes of this project, which aims to develop approaches for immune protection of allogeneic IPCs, will validate the applicability of regenerative medicine to the underserved communities.<br>● Varying life experiences of the team members who stem from different socioeconomic backgrounds and with some members living with T1D, bring varied perspectives, opinions, and points of view into the project, and encourage an open dialog within the team as well as community outreach and forging new partnerships.<br>● At this stage of research, there is no discussion for recruitment of patients.<br>● Immune cloaking hPSC lines will be useful for underserved populations.<br>● The applicant did not describe prior efforts for outreach activities to inform the DEI development. |

| Application # | DISC0-15920 |
|---|---|
| **Title**<br>(as written by the applicant) | Harnessing the rejuvenating capacity of pregnancy-associated factors to restore aged stem cell function |
| **Research Objective**<br>(as written by the applicant) | Elucidation of pregnancy-related factors that mitigate cellular senescence and enhance regeneration has far-reaching implications for understanding the mechanisms of aging and rejuvenation. |
| **Impact**<br>(as written by the applicant) | The study will address the long-standing knowledge gaps related to the mechanisms of pro-regenerative impact of pregnant milieu and female muscle stem cell senescence |
| **Major Proposed Activities**<br>(as written by the applicant) | • Isolation of female pelvic muscle stem cells from diverse population of young and old donors<br>• Comparison of genetic, epigenetic and phenotypic signatures of young and old pelvic and non-pelvic female muscle stem cells<br>• Identifying key pro-regenerative factors associated with pregnancy<br>• Learning how pregnancy-associated factors impact aged female muscle stem cells' genotype, phenotype, and regenerative potential |
| **Statement of Benefit to California**<br>(as written by the applicant) | The proposed studies will bridge the long-standing knowledge gaps regarding aging of the female pelvic muscle stem cells and how pregnancy associated factors can be used to mitigate functional decline of these cells with age. The above is important because pelvic muscle dysfunction is a key risk factor for the development of pelvic floor disorders - a set of morbid and prevalent conditions that disproportionately affect older women and for which there are currently no preventative measures. |
| **Funds Requested** | $1,539,520 |
| **GWG Recommendation** | (85-100): Exceptional merit and warrants funding, if funds are available |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 85

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

| | |
|---|---|
| **Mean** | 84 |
| **Median** | 85 |
| **Standard Deviation** | 4 |
| **Highest** | 88 |
| **Lowest** | 75 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 10 |
| **(1-84): Not recommended for funding** | 5 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 14<br>**No:** | • The project addresses an important knowledge gap in our understanding of the biology and regenerative capacity of pelvic floor muscle (PFM) stem cells (hMuSC) in women. |

| | |
|---|---|
| 0 | Closing this gap will be important for developing regenerative medicine-based therapies for treatment of pelvic floor disorders (PFDs).<br>● PFDs represent a major public health problem, particularly in older women; these disorders are associated with significant morbidity and a diminished quality of life. Outside of surgical intervention, effective therapies are currently unavailable, in part, because of our poor knowledge of PFM biology. This project will attempt to overcome this bottleneck by elucidating the properties and regenerative capacity of the PFM MuSCs.<br>● This is a hypothesis -driven project. If successful, it will have a strong impact on the scientific knowledge of PFM biology and function and may lead to development of new therapies for treatment of the PFDs.<br>● PFDs are a major public health issue and incidence of the disease increases with age.<br>● Yes. Successful completion of the project may lead to an understanding of how pelvic muscles can be coaxed into regenerating the pelvic floor.<br>● Looking into pregnancy as a physiological state of parabiosis is extremely interesting and novel. Understanding how the hormonal alterations of pregnancy affect stem cells and regeneration will reveal basic mechanisms of muscle maintenance.<br>● This application is relevant to human biology and especially pelvic floor muscle disorders, which impact healthy aging, social life, and mental health.<br>● Understanding how aging affects pelvic human muscle stem cell (hMuSC) function will be important for women all over the world.<br>● The proposed study may provide data on intrinsic factors stimulating pelvic muscle division. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 13<br>**No:** 1 | ● The project is built on the idea that pelvic floor MuSCs can be activated and induced to proliferate, differentiate, and contribute to the pelvic floor muscle regeneration in response to various cues, including systemic factors elevated in pregnancy.<br>● The fact that activated MuSCs in the early half of pregnancy exhibit increased proliferation in vivo, and return to phenotypic quiescence by the end of gestation, is compelling and supportive of the fact that hormonal regulation during pregnancy affects MuSC behavior and function.<br>● The accelerated cell cycle entry of pelvic floor MuSCs, with higher EdU incorporation in pregnant compared to non-pregnant rats following mechanical stimulation, is intriguing.<br>● New preliminary data show that the "pseudo-quiescent state" of MuSCs in late pregnancy is similar to the "G Alert" phenotype that has been described previously.<br>● The new preliminary data shown in Fig. 7 showing pelvic floor muscle regeneration are very interesting.<br>● The rationale for Aim 1 where pelvic floor hMuSCs from younger and older pelvic skeletal muscles will be isolated, characterized, and compared to each other is strong, since little information is currently available about these clinically important MuSC type.<br>● The goal of Aim 2 is to discover systemic pregnancy-associated factors that regulate hMuSC ex vivo, and the goal of Aim 3 is to use these factors for regeneration of injured PFM in vivo in a murine model. A sound scientific rationale for these aims is missing.<br>● While there are many studies investigating the effect of stem cells in aged muscles from lower legs and trunk muscles, there is no information on the pelvic muscles.<br>● The application is based on sound scientific rationale, and the scientific topic is extremely interesting and understudied.<br>● The proposed assay based on activity of serum on muscle stem cells seems potentially limited. More preliminary data to justify the addition of serum in Aim 2 would have been important. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 12<br>**No:** 2 | ● The experimental design is well planned and will likely produce important and meaningful results.<br>● Aim 1 is properly designed. The design of Aims 2 and 3 is logical; however these aims would benefit from inclusion of data showing a positive effect of pregnant serum on PFM in vitro. Such data would validate the key assay of Aim 2 and will be important for achieving the goals of Aim 3.<br>● The new explanations on the transcriptomic data are greatly appreciated as well as bringing on the team experts with relevant knowledge.<br>● Potential pitfalls and alternative approaches are discussed. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 14<br>**No:** 0 | ● The team has all the necessary resources to conduct the proposed studies.<br>● The applicant has a collaboration agreement that will provide the necessary specimens from a variety of cadavers, allowing a wide range of ages to be investigated.<br>● A planned collaboration will provide excellent muscle stem cell resources. |

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|---|---|
| | ● The available biorepository resources are invaluable tools necessary to conduct the proposed studies.<br>● The team is ideal to perform the proposed studies.<br>● The budget is fine as proposed.<br>● Proposed experiments are on an ambitious timescale but can be accomplished.<br>● Adding a second surface marker could be helpful. Recent literature suggests that use of two biomarkers may be a better strategy for isolating hMuSCs. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 14<br>**No:** 0 | ● Pelvic floor disorders (PFDs) primarily affect the female population. Historically it has been studied in people of European origin. The applicant team is committed to extending their study to diverse populations.<br>● The applicant notes that the biospecimens to be used in the proposed studies are expected to represent the full diversity of people in the applicant institution's catchment area.<br>● Given that there are different kinetics in muscle regeneration between males and females, while the effect of hormonal regulation in females could also affect these processes, this proposal is uniquely positioned to address issues that have never been studied before.<br>● The Principal Investigator proposes to expand the educational resources available to the team via their clinical role as well as participation in women's organizations. |

| Application # | DISC0-15689 |
|---|---|
| **Title**<br>(as written by the applicant) | Utilizing Age-Specific Adipocyte Progenitor Cells for Cell Therapy in Older Patients |
| **Research Objective**<br>(as written by the applicant) | A new type of APC serves older patients as 1) better MSC in immunomodulation (reducing inflammation) for autologous transplantation; 2) better source of somatic cells for generating heathier hiPSCs. |
| **Impact**<br>(as written by the applicant) | Bottlenecks: 1) Older patients suffer from sarcopenic obesity, which has no safe and effective treatment. 2) Cell therapy in older patients is often not satisfactory due to stem cell aging. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Determination of the adipogenic capacity of human CP-As. Characterization of the ability of human CP-As to differentiate into other cell types, including osteoblasts, chondrocytes, and muscle cells.<br>• Determine the function of LIFR in human CP-As through LIFR agonist/antagonist treatments, virus-mediated gene manipulations, in a 3D culture system in vitro, and in a transplantation assay in vivo.<br>• Inducible eliminate new adipocytes generated during aging in a new mice model and determine the metabolic benefits. Comparison of the function of human adipocytes generated from CP-As vs. young APCs.<br>• Establish a high-throughput screening platform that is efficient, effective, and consistent for inhibiting human CP-A differentiation to prevent age-related fat expansion and select top hits.<br>• Evaluate the immunomodulating capacity of human CP-As, compared to classic bone marrow MSCs, in secreting anti-inflammatory cytokines and inhibiting immune cell proliferation.<br>• Evaluate the advantages of iPSCs derived from CP-As, compared to iPSCs derived from the blood cells of the same human donor, in DNA damage rate, iPSC marker expression, and differentiation efficiency. |
| **Statement of Benefit to California**<br>(as written by the applicant) | California has the largest number of older residents. Many older residents of underserved communities reside in so-called "food deserts", leading to sarcopenic obesity and many chronic disorders. We will explore the potential for using a newly identified adult fat stem cell to prevent and treat sarcopenic obesity, or be used for immunomodulation cell therapies, or serve as a source of somatic cell for generating better human induced pluripotent stem cells (hiPSCs) for autologous transplantation. |
| **Funds Requested** | $1,508,997 |
| **GWG Recommendation** | (85-100): Exceptional merit and warrants funding, if funds are available |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 85

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

| | |
|---|---|
| **Mean** | 83 |
| **Median** | 85 |
| **Standard Deviation** | 4 |
| **Highest** | 86 |
| **Lowest** | 72 |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 8 |
| **(1-84): Not recommended for funding** | 6 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 11 **No:** 2 | • This proposal describes, and potentially fills, knowledge gaps about the behavior of fat accumulation in older individuals as compared to younger individuals.<br>• The preliminary data suggest that a newly described cell type, committed pre-adipocytes age specific (C-PAs) becomes prevalent in older individuals, and that C-PAs contribute to age-related adipose tissue in aged individuals.<br>• The proposed work may lead to human stem cell therapeutics. C-PAs may be a problematic progenitor cell type in aged humans that contribute to medically harmful accumulations of white visceral fat in older individuals. Successful inhibition of this process, as proposed, is a potential therapeutic approach.<br>• C-PAs may also have therapeutic applications as immunomodulators or as a source for better iPSCs.<br>• The impact of this work has been somewhat diminished by the development of recent anti-obesity drugs (terzepatide and semaglutide). |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 12 **No:** 1 | • Over the course of about 10 years of research, this group and others have shown that white adipose tissue from older mice accumulate a new progenitor cell type (called C-PA: committed pre-adipocytes) that contribute to white adipose tissue (WAT) production. These older C-PAs outcompete similar cells from younger mice in competition engraftment assays, and exhibit different kinetics in vitro as well.<br>• The preliminary data support the applicants hypothesis that aging impacts the biology of C-PAs in vivo, resulting in a greater ability of "old" donor cells to engraft and contribute to WAT in recipients than those from "young" donors.<br>• Molecular analyses identify that C-PAs are a subpopulation of white adipose tissue (WAT) and identified that the leukemia inhibitory factor receptor (LIFR) is a marker of C-PAs.<br>• The applicant has demonstrated that LIFR plays a functional role in adipogenesis from transplanted C-PAs by up and down regulating LIFR expression.<br>• Since the last review, the applicants have now obtained WAT samples from 5 humans and identified cell populations with transcriptomes similar to C-PAs obtained from mice. This is an improvement over previous preliminary data, and the applicant will further explore the function of these cells during the award. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 12 **No:** 1 | • The project is designed based on the large primary data sets, specifically their newly identified CP-As cells. The design and plan follow logic and will give meaningful results.<br>• The work will build on the new preliminary data showing that human C-PAs probably exist based on RNAseq studies. The applicants will test these cells from several individual donor human samples for proliferation and differentiation in vivo, and in xenograft assays.<br>• The work proposed to compare the human C-PAs to another cell type and to explore the qualities of iPSC generated from C-PAs with those derived from different starting cell types seem less important and are not that attractive.<br>• A reviewer questioned the rationale for proposing to compare the iPSCs derived from C-PAs and iPSCs derived from skin fibroblasts and PBMCs and wondered how this would be conducted. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 13 **No:** 0 | • Overall, the aims are logical and not overly interdependent. Each of the 3 aims has good value in their own right.<br>• This is a large and qualified team with a good range of individuals with specialties that seem appropriate for the proposed research.<br>• The team has the necessary resources to complete the logically designed project.<br>• The PI has a long-standing interest in adipose tissue development, plasticity, and cellular heterogeneity. They had extensively studied the adipogenesis of adipocyte progenitor cells. |

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|---|---|
| | The co-investigator has extensive experience in stem cell biology and has expertise with developing human iPSC (hiPSC)-based disease models and cell therapies.<br>● Expertise in iPSC cells is not clearly demonstrated. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 13<br>**No:** 0 | ● This work might be very beneficial to overweight and obese older patients, who are over-represented in populations that experience economic disadvantages. Furthermore, since certain ethnicities experience higher rates of age-related obesity and insulin insensitivity (many of which reside in CA) this work may benefit them.<br>● Socioeconomically disadvantaged and ethnic minority groups have significantly increased risk for sarcopenic obesity, leading to various chronic diseases. Also sarcopenic obesity disproportionally impacts the older population.<br>This proposed project aims to identify new cell therapy for sarcopenic obesity that will benefit all population, including underserved racial/ethnic communities and old people. |

| Application # | DISC0-15755 |
|---|---|
| **Title**<br>(as written by the applicant) | Microglia replacement with non-myeloablative hematopoietic stem cell transplantation for Alzheimer's disease |
| **Research Objective**<br>(as written by the applicant) | Our objective is to resolve biological and pre-clinical bottlenecks to brain engraftment with microglia-like cells to hasten the application of stem cell therapy for AD and other CNS diseases. |
| **Impact**<br>(as written by the applicant) | Our project impacts the biological and pre-clinical bottlenecks in promising experimental studies that currently prevent clinical trials of HSCT for Alzheimer's disease. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Aim 1 will test new, safer approaches to translatability of HSCT as a potential route for regenerating brain microglia.<br>• Aim 2 will test the effectiveness of HSCT to regenerate microglia-like cells in an Alzheimer's disease model.<br>• Aim 3 will perform pre-clinical feasibility studies using human stem cells as a first step towards clinical trials. |
| **Statement of Benefit to California**<br>(as written by the applicant) | In 2020, the people of CA approved Prop. 14, authorizing $5.5 billion to CIRM, of which $1.5 billion is dedicated to research of CNS- and brain-specific disease. CA has more people living with Alzheimer's disease (AD) than any state, with 3 counties in the national top 10. Bone marrow transplantation (BMT) is widely available in CA, covered by Medi-Cal, and rapidly becoming safer. Our project will use CA taxpayer funds towards developing BMT as a viable AD therapy to benefit Californians. |
| **Funds Requested** | $1,540,801 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 84

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

| | |
|---|---|
| **Mean** | 84 |
| **Median** | 84 |
| **Standard Deviation** | 1 |
| **Highest** | 85 |
| **Lowest** | 80 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 4 |
| **(1-84): Not recommended for funding** | 11 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 14<br>**No:** 0 | • Alzheimer's disease (AD) is the most prevalent neurodegenerative disease, but current therapeutics for AD have limited effectiveness. This research aims to develop highly effective therapies for AD. The applicants propose pre-clinical studies in mice to move the proposed approach forward to future clinical trials of stem cell therapy for AD. |

| | |
|---|---|
| | • There is a large unmet need to develop new therapies for AD.<br>• The proposal is based on the observation that microglia are largely replaceable in adult mice, and engineered microglia, like stem cells, are associated with significant improvement in respect to neurodegeneration in preclinical models of AD.<br>• The project is in the domain of microglia-based approaches. Microglia are increasingly being recognized as key players (and therapeutic targets) in neurodegenerative diseases.<br>• Yes, targeting microglia in neurodegeneration can have implications for other diseases like Parkinson's disease.<br>• In addition, recent evidence suggests that stem cells can be differentiate into bone marrow derived microglia like cells in human brains. The objective of the proposal is to determine pre-clinical feasibility of hematopoietic stem cell transplant (HSCT) as the therapeutic approach for AD and to address important limitations: optimizing conditioning regimens, model optimization and using human bone marrow derived cells instead of mouse bone marrow derived cells as a graft population.<br>• If successful, the project will have a major impact on the allogeneic hematopoietic stem cell transplantation therapy for AD.<br>• Efficacy of engraftment and the toxic conditioning regimens that are required for replacement therapies represent significant bottlenecks.<br>• The path forward to a possible clinical application is not clear. For example, would microglia need to be ablated in human brains? How are endogenous microglia affecting a possible beneficial effect the transplanted cells might have? These issues are not addressed and impact enthusiasm. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 12<br>**No:** 2 | • The rationale for using this approach is supported by preclinical data that show very promising results in normalizing AD associated pathology using bone marrow transplantation.<br>• Microglia are key contributors to synaptic injury and neurodegeneration in AD and other neurodegenerative diseases. Microglial-based mechanisms of neuronal injury are significantly modulated by a specific genotype.<br>• The team have previously showed that microglia are largely replaceable in adult mice and that engineered donor cells engrafted in the microglial niche significantly improve molecular features of neurodegeneration in two murine models of neurodegeneration, including AD. They have also demonstrated regeneration of BM-derived microglia-like cells in human brain of older individuals.<br>• The applicant makes a valid case that BMT-derived cells bearing one genotype are superior to those that bear a different genotype in their ability to mitigate the behavioral and neuropathological changes in experimental Alzheimer disease.<br>• The experiments are based on the fact that microglia are implicated in neurodegeneration, but they do not take the potential protective role of microglia into account.<br>• Previous experiments use ablation of microglia but now they use a different method instead. This could potentially be developed to a clinical setting, but a reviewer was unsure about a therapy that is based on removing microglia globally in the brain. They deemed this too high risk.<br>• The data is supportive in terms of ability to conduct the project, but not in terms of translatability.<br>• The studies are based on allogeneic mouse to mouse grafting, and the translation to the human setting is not well described. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 11<br>**No:** 3 | • The project is well planned and designed in terms of experiments, models and methods, but not in terms of translation.<br>• In a second aim, the applicant will confirm behavioral deficits in the mouse model and test efficacy of the approach; this seems reasonable.<br>• Aim 3 will test efficiency of engraftment using human derived cells into NSG mice that will be challenged with an excitotoxin.<br>• Based on preliminary data, the applicant will evaluate the functionality of grafted stem cells by stimulating these cells through induction of neuronal injury using an excitotoxin. The relevance for AD is unclear.<br>• The potential pitfalls could be improved. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 14 | • The experimental plans are feasible given the expertise of applicants. |

| | |
|---|---|
| **No:** 0 | • The PI focuses on characterizing the molecular features of dementia-related neurodegenerative diseases, especially Alzheimer's disease. A co-investigator focuses on hematopoietic stem cell transplantation.<br>• The team is limited to scientists at one institution, but it does include experts in stem cells, neurobiology and translation.<br>• The applicant has the necessary resources, and the budget is appropriate.<br>• An allogenic transplantation using three month old recipients of mice and bone marrow from donor mice show the expected results.<br>• The role of an excitotoxin challenge as being relevant for AD is not clear. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 14<br>**No:** 0 | • A team member is pursuing allogeneic transplantation strategies for HLA-mismatching in cancer patients, specifically benefiting underrepresented or mixed genetic individuals, who often do not have a matched donor available and for whom matched donors are more difficult to identify.<br>• Addressing the bottlenecks would open up the therapy for more individuals especially for those where no fully HLA match donors can be identified.<br>• Diversity is acknowledged. Sex is considered as a variable.<br>• A general DEI statement is provided. |

| Application # | DISC0-15700 |
|---|---|
| **Title**<br>(as written by the applicant) | Gene-edited CD19 CAR-T cells with superior proliferation, persistence and serial-killing activity |
| **Research Objective**<br>(as written by the applicant) | Our studies are designed to modify the DNA inside of T-cells, so that they can function better in treating people who suffer from blood cancers such as leukemia or lymphoma. |
| **Impact**<br>(as written by the applicant) | Patients who receive a cancer therapy called CAR-T often see good initial results, but then the tumor come back. If successful, our study could allow patients to have longer remissions or cures. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Modify a gene in anti-cancer cells called CAR-Ts. Determine if this gene modification allows CAR-T cells to work better against tumor cells. Study how the gene modification improves CAR-Ts.<br>• Make CAR-T cells from different types of T cells that are present in people. Modify our gene of interest in the T-cell subtypes. Determine if our modification works best in a particular subset.<br>• Test our modified CAR-T cells against tumor cells for several different types of leukemia and lymphoma. If our CAR-Ts fail to work well against a specific type of tumor cell, try to understand why.<br>• Perform studies in mice which have been implanted with human cancer cells. Determine if the modified CAR-Ts work better in mice than the current FDA-approved CAR-Ts that are used in patients. |
| **Statement of Benefit to California**<br>(as written by the applicant) | According to statistics compiled by the Leukemia and Lymphoma Society, there were a predicted 10,860 new cases of blood cancers (leukemia, non-Hodgkin lymphoma, Hodgkin lymphoma and myeloma) in California in 2021, with 5,840 deaths from the same group of malignancies. These numbers are second highest in the country behind Florida. Improvements to CAR-T therapy, which is so far only approved for blood cancers, could potentially impact thousands of cancer patients, existing and new, in CA. |
| **Funds Requested** | $1,607,994 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 83

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

| | |
|---|---|
| **Mean** | 82 |
| **Median** | 83 |
| **Standard Deviation** | 4 |
| **Highest** | 85 |
| **Lowest** | 72 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 3 |
| **(1-84): Not recommended for funding** | 12 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 14 **No:** 0 | • CAR-T cells have revolutionized cancer therapies and are of major relevance. Relapse due to exhaustion of the CAR-Ts is a major issue that the applicant wants to address.<br>• CAR-Ts provide initial control of hematological tumors, but relapse is common. Various methods are being tried to improve persistence/overcome exhaustion. The applicants are proposing to investigate a relatively understudied pathway.<br>• Strength: Could provide an interesting new approach to substantially modulate CAR-Ts and prevent exhaustion.<br>• Weakness: Since the approach involves targeting an X-linked gene, more details on the effect and feasibility across the sexes are needed. While safety is not yet relevant, suggestions on how to move this forward would be needed.<br>• As proposed, the project is fundamentally translational. It addresses the problem that efficacy of CAR T-cells for cancer therapy is limited by their decreased proliferation and eventual death as they undergo multiple rounds of antigenic stimulation in cancer-bearing patients. The proposal aims to engineer CAR T-cells to mimic an X-linked genetic disease in which certain lymphocytes proliferate excessively. This may indeed enable greater expansion and longevity for CAR-T cells.<br>• It is not clear if the technology could help to reduce the very high cost of CAR T-cell therapy. Access to CAR T-cell products by members of disadvantaged groups is limited.<br>• While the work could provide insight into the underlying biology of CAR-T therapy (for example, the role of "stem cell memory" T-cells) in ways that might lead to improved products, the project could be better designed to achieve that goal.<br>• Improving CAR T cell persistence and function is an important goal and necessary for making CAR T therapy more potent. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 11 **No:** 3 | • The rationale is based on a specific hypothesis and selective disruption of a gene. The logic is very convincing. The preliminary data suggest the target as relevant and support the aims. However, there are little details on the mechanism proposed.<br>• Applicants propose to prevent CAR T-cells from ceasing proliferation and dying by removing a normal regulatory check. Preliminary data on CD19 CAR T-cells from three patients showed highly variable increases in population expansion, from 2.5-fold to approximately 75-fold, before cells become refractory to further antigenic stimulation and die.<br>• Whether this would significantly improve clinical outcomes is not yet clear. It also may increase risks associated with CAR T-cell therapy, such as the development of leukemias arising from the engineered CAR T-cells. This already occurs with sufficient frequency so that the FDA recently imposed a "black box" warning label on multiple approved CAR T-cell products. Potential risks should have been discussed in the application.<br>• The preliminary data are strong and derived from multiple subjects. The effects of this gene knockdown are dramatic in terms of expansion potential. Some of the observed effect sizes are a bit small (viability), but in other assays, the effects are dramatic.<br>• The project proposes to generate a new target for improving CAR T cell function, which has the potential to improve a wide range of CAR therapies, including solid tumor CARs which have been disappointing so far.<br>• The rationale is sound and based on extensive research in known pathways of T cell signaling. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 10 **No:** 4 | • It may be valuable for the applicant to explore alternatives to genetic editing that could achieve similar effects.<br>• Pitfalls are not adequately described and some may be missing; how milestones will be achieved is not clear.<br>• The "black box" labeling from the FDA appears to be the only discussion of safety.<br>• The project is organized under four milestones. However, these are presented as qualitative descriptions of activities, in rather general terms. Specific benchmarks that must be met to support translational development are lacking.<br>• Alternatively, the project could be framed around clear Specific Aims to test hypotheses about key factors for successful CAR T-cell therapy that might be augmented by the KO. An example would be a well-formulated test of the importance of the relevant T cells and whether these are preferentially expanded and maintained using the KO.<br>• Not taking into account the sex of T-cell donors as a likely source of heterogeneity in the degree of expansion seems a significant slip. Males have a single copy of the target gene, females two copies. If only one copy is KO'd in cells from a female, this would probably have much less effect on T-cell expansion than in cells of a male donor (consistent with X-linkage). |

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| | This effect could easily dwarf effects of racial/ethnic differences, etc. The project needs to address editing in males vs. females given the X-linked nature of the gene.<br>● The project is relatively straightforward and will give clear and interpretable results.<br>● Potential pitfalls are well-described. The "riskiest" part of the study is perhaps the CRISPR guides and the variation in phenotypes they are seeing with knockdown. Cleverly, they propose to exploit this to learn more about the relevant biology, but it might also be a source of significant "noise" that will force them to alter their strategy. It is well-considered though, and I think the applicants will be able to figure it out.<br>● The timeline is appropriate. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 14<br>**No:** 0 | ● Overall the proposal is clear and logical. The cell lines in Aim 3 are reasonable to at least capture some of the in vitro artifacts of all cancer cell line models. Limited alternatives are unfortunately available. In vitro and mouse model in vivo readouts are only capturing some of the performance.<br>● The CRISPR KO strategy seems reasonably straightforward, as are assays of CAR T-cell efficacy. The promise in the DEI section to systematically explore effects of the planned KO on cells from many individuals of diverse ethnicity, etc., unfortunately seems rather empty. The numbers required may have been impractical.<br>● The proposed aims are straightforward and logical and backed by clear examples in the preliminary data. It is very feasible in the timeline. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 13<br>**No:** 1 | ● The applicants acknowledge issues in the equitable delivery of CAR products.<br>● The DEI statement was well-considered and described several useful activities.<br>● The applicant includes a general statement on DEI but few actual efforts. The potential DEI-oriented impact depends on the applicant's collection of broadly representative samples; it is not clear that this will be actively pursued.<br>● Despite claims in the DEI section that CAR T-cells from many diverse individuals will be tested, the proposal is to test most steps on cells from three patients. Information on how patients will be selected is not included. As noted, male versus female may have biggest impact, unless CRISPR KO is carried out at high multiplicity so most cells would have KO of both copies of the targeted gene.<br>● Given the potential for variation in outcome based on biological sex, the authors should address this more directly.<br>● The targeted gene is on the X chromosome, but sex is not addressed. |

| Application # | DISC0-15764 |
|---|---|
| **Title**<br>(as written by the applicant) | Modeling Arealization to Understand Etiology of Neurodevelopment Disorders |
| **Research Objective**<br>(as written by the applicant) | This proposal will elucidate how neural stem cells create areas of the cortex, which will improve our understanding of neurological disorders and develop better organoid systems to model these areas. |
| **Impact**<br>(as written by the applicant) | This work will impact our understanding, detection, and eventual cell based treatment of neurodevelopmental and neuropsychiatric disorders. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Identify how cortical areas are generated by exploring the role of transcription factors in cortical organoids.<br>• Explore the role of external signals on refining cortical areas using assembloid systems.<br>• Share results of the experiments as a resource to the broader scientific community, including through our institutional cell browser. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Neurodevelopmental and neuropsychiatric disorders are on the rise amongst the general population. Our understanding of how these disorders emerge is limited by the models we can use to study potential treatment strategies. Organoids are stem cell derived models of the developing human brain, but do not accurately represent the areas impacted in these disorders. We will understand how these areas emerge normally in order to create better organoid models to study neurological disorders. |
| **Funds Requested** | $1,569,312 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 82

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

| | |
|---|---|
| **Mean** | 83 |
| **Median** | 82 |
| **Standard Deviation** | 3 |
| **Highest** | 90 |
| **Lowest** | 75 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 4 |
| **(1-84): Not recommended for funding** | 11 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 13<br>**No:** 1 | • The project seeks to understand key developmental factors (genetic and environmental) required for cortical area specialization, based on the observation that many neurological disorders, including autism and schizophrenia, have cortical-area specific phenotypes. |

| | |
|---|---|
| | • The project defines the key challenge as creating 3D human tissues models that differentiate neurons and other cells from a specific brain area (e.g. prefrontal cortex (PFC), temporal lobe, or primary visual cortex).<br>• This is more of a descriptive project, but this comment is not meant as a negative, Other funding agencies may not support this type of project since it is not hypothesis testing. Nonetheless it is invaluable data for the field of stem cell neurobiology.<br>• The project addresses a major bottleneck, since we have only a crude understanding of the identify of the vast majority of cells produced during neuronal differentiation. The project will open doors for multiple laboratories with different interests.<br>• The project defines and addresses a major bottleneck by attempting to understand how different cortical areas develop in the human brain in order to create area specific organoid models which may help shed light on neurodevelopmental disorders (e.g. autism, schizophrenia). The problem is they will use a model to make another model - making validity questionable. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 12<br>**No:** 2 | • The scientific rationale for developing methods to make neurons from specific cortical areas is sound.<br>• The preliminary data are supportive of the investigators ability to do a CRISPRa screen in human fetal tissue (an impressive model system). There is also a claim made that PFC-like tissue is derived from RORB activation but no quantification is given and, by eye, there does not seem to be marked difference from controls — all populations are highly overlapping. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 10<br>**No:** 4 | • Overall, the project is well designed. There is some circularity in that enriched TFs from PFC are used in the CRISPR library AND these same TFs are used on the readout to determine arealization. It would be helpful for the investigators to explain this, and/or discuss how they prevent "testing" on the exact gene they are perturbing to avoid uninformative results.<br>• Has the applicant claimed in the proposal that VP64 is superior to VPR ("slightly more effective")? Several labs have shown that second-generation CRISPR activators (such as VPR, SAM, etc.) are superior to VP64, both in vitro and in vivo. The applicant needs to check this and/or explain their meaning.<br>• Given the small scale of the library (<40 TFs), should the applicant test or try a cDNA overexpression approach? cDNA is easier to deliver and will drive higher levels of gene expression than a CRISPR activator. Please see work from Steve Elledge and others (human ORFeome) on this topic.<br>• Some discussion of MOI and multiple gene targeting is given. These are helpful. Given the long timelines (5 - 8 weeks), the applicant should undertake some pilot work to measure lentiviral silencing, which can be problematic for CRISPRa approaches.<br>• The project plan and timeline demonstrate an urgency that is commensurate with CIRM's mission. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 13<br>**No:** 1 | • Likely, but some CRISPR screen failure points are not well thought out. Also, the scope might be limited by the small library (>40 TFs vs. >1000 TFs in the human genome).<br>• The proposed team appropriately qualified and staffed.<br>• The team have access to all the necessary resources to conduct the proposed activities.<br>• The budget appropriate for the research proposed. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 14<br>**No:** 0 | • The project outcomes extend or validate the applicability of regenerative medicine discoveries to underserved populations, including underserved racial/ethnic communities.<br>• The applicant includes plan to use cell lines from various racial/ethnic backgrounds.<br>• The applicant describes prior efforts or proposed plans for outreach, partnership, or educational activities to inform the development of DEI within the research project, including serving on the molecular biology DEI committee, and several training programs. |

| Application # | DISC0-15693 |
|---|---|
| **Title**<br>(as written by the applicant) | Modeling Rett syndrome neurological disorder with human pluripotent stem cells to develop in cellulo screening platforms. |
| **Research Objective**<br>(as written by the applicant) | Study the neurological disorder Rett syndrome using neurons derived from genetically engineered human pluripotent stem cells and develop platforms to screen drugs for this disease in live cells. |
| **Impact**<br>(as written by the applicant) | Rett syndrome is the second most common cause of girls' intellectual disability, still lacking effective treatment. We hope to accelerate the discovery of more effective drugs for this orphan disease. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Model Rett syndrome neurological disease by genome-editing human pluripotent cells to carry a fluorescent MeCP2 protein and introduce a Rett-syndrome causing mutation.<br>• Convert the modified human pluripotent cells to neurons and determine how the MeCP2 mutation affects protein levels and DNA binding by measuring the dynamics of single MeCP2 molecules in live neurons.<br>• Miniaturize the conversion of human pluripotent cells to neurons to test hundreds of samples at once.<br>• Develop an automated microscopy platform to measure MeCP2 binding to DNA in real time in live neurons at high speed and throughput.<br>• Develop an automated microscopy platform to measure MeCP2 protein amounts and neurons' characteristics at high throughput.<br>• Perform an exploratory screening of drugs already approved by the Food and Drug Administration to test how they affect MeCP2 binding to DNA and/or protein amounts. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Our proposal enhances and combines California's preeminence in stem cell and single-molecule biology. Based on previous work, the applicant co-founded a Bay Area company using live cell single-molecule imaging for drug discovery. Our proposal will apply this imaging technology to more complex, disease-relevant systems based on human pluripotent cells, enabling the next generation of therapeutics that will benefit Californians and the world. |
| **Funds Requested** | $1,574,117 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 80

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

| | |
|---|---|
| **Mean** | 81 |
| **Median** | 80 |
| **Standard Deviation** | 2 |
| **Highest** | 85 |
| **Lowest** | 78 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 1 |
| **(1-84): Not recommended for funding** | 14 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 13 **No:** 1 | • Rett syndrome, which mainly affects females, is caused by MeCP2 mutations. No effective pharmacological approaches exist leaving Rett syndrome an unmet medical need. The applicants propose to develop a novel drug screening platform based on MeCP2 nuclear dynamics in hiPSCs differentiated to neurons to probe chromatin binding and protein levels in real time in live cells.<br>• Pharmacological approaches to directly restore MeCP2 levels or function are currently lacking.<br>• This proposal aims to accelerate drug discovery for Rett syndrome through modeling a MeCP2 mutation in a male patient hiPSC-derived neurons and developing in cellulo screening platforms by using single molecule tracking to quantify MeCP2 DNA binding ability and protein levels by advanced microscopy.<br>• If successful, the proposed research will develop in an imaging based drug screening platforms in hiPSC-derived live neurons and identify small molecules that modulate MeCP2 DNA binding and/or protein levels.<br>• The imaging system for tracking single MeCP2 RNA mobility/screening for agents that limit this mobility in a Rett-related mutation is high-risk high reward. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 13 **No:** 1 | • The applicants collaborators recently isolated a novel missense mutation in MeCP2 from a male diagnosed with classic Rett syndrome, where the mutation partially impairs MeCP2 function.<br>• This proposal aims to model the mutation in a male hiPSCs-derived neurons and develop in cellulo imaging screening platforms to quantify MeCP2 DNA binding ability and protein levels by advanced microscopy.<br>• The proposed project will advance molecular understanding of Rett syndrome by revealing how MeCP2 mutations disrupt DNA binding and protein levels in neurons derived from hiPSCs. Testing the proposed in cellulo platforms to screen a library of FDA-approved compounds might also reveal modulators of MeCP2 binding ability and protein levels, with impact on other Rett syndrome mutations as well as on MeCP2 duplication syndrome.<br>• The proposal has a wider impact potential because the proposed cellulo screening platforms could be adapted to other disease contexts with defined therapeutic targets.<br>• Despite potential benefits, it is concerning that all the study outcomes will be based on just one donor cell line - strongly limiting data generalizability and reproducibility. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 12 **No:** 2 | • The project is appropriately planned and designed.<br>• The project focuses on a single point mutation identified in a male patient diagnosed with Rett syndrome, starting with cell phenotyping analysis in mouse model brain samples, then transferring the mutation to a male iPSC line. Further, they will establish and optimize single molecule tracking and imaging methods to evaluate MeCP2 protein levels and function.<br>• They aim to scale up hiPSC differentiation to neurons in 96- and 384-well plates to develop a high-content platform to screen compounds that modulate MeCP2 DNA binding or protein levels.<br>• The study focuses on creating one point mutation in one established wild type male iPSC line. The phenotype can come from the off-target effects of the gene editing or the background of this iPSC line. The investigators should consider performing gene correction for the male patient iPSC line that carried the MeCP2 mutation to have additional isogenic pairs.<br>• To achieve highly homogeneous neuronal differentiation in 96 or 384 well plates at large scale starting from hiPSCs is technically challenging. More preliminary data for establishing robust neuron differentiation would be more supportive. For example, the applicant may consider differentiating large scale and splitting to the screening format to avoid the differentiation variation.<br>• The limitation of only proposing one line is not a "fatal flaw" - it would make sense to focus on one line then replicate key studies with a second, and that would likely be needed to publish. Given the very specific focus on MeCP2 mobility it would seem highly unlikely that background effects will be problematic, so this omission of standard practice of testing two lines is minor.<br>• While the reason for using only male donor cells is reasonably well justified, I am afraid that using only one donor line is not going to deliver reliable and generalizable results. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 14 | • Yes. The project is well planned and, provided appropriate staffing, achievable during the proposed timeline. |

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|---|---|
| **No:** 0 | • The project is designed logically and likely can be achieved within the proposed timeline.<br>• The team has extensive imaging and high-throughput drug discovery expertise, but lack of track record for neuron differentiation from iPSCs. Having an adviser or collaboration with expertise in neuron differentiation would be beneficial for the project.<br>• Great preliminary data from a great lab, highly likely to conduct the proposed experiments--high risk high reward so success is not assured. Rett syndrome is a major disease that is mostly untreatable. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 12<br>**No:** 2 | • Rett syndrome primarily affect females and those of all groups.<br>• The study focuses on one male iPSCs carrying a novel mutation isolated to avoid the cellular mosaicism in female cells when studying disease mechanisms and evaluating therapeutic strategies.<br>• Actually not, but the authors argue that the use of a male donor cells is justified due to female cellular mosaicism and that patients are not knockouts (as is the case in animal models which are knockouts to avoid female mosaicism).<br>• I suggest the proposal includes additional iPSC lines for validating mutation phenotype, and evaluating the potential compounds. |

| Application # | DISC0-15712 |
|---|---|
| **Title**<br>(as written by the applicant) | Interrogation of tandem repeat variants contributing to neurodevelopmental and psychiatric traits using stem cell models |
| **Research Objective**<br>(as written by the applicant) | Our project will identify molecular and cellular changes induced by specific genetic variants implicated in schizophrenia and autism spectrum disorder in stem cells, neuroprogenitor cells and neurons. |
| **Impact**<br>(as written by the applicant) | schizophrenia and autism spectrum disorder |
| **Major Proposed Activities**<br>(as written by the applicant) | • Perform multi-ancestry GWAS and fine-mapping to identify TRs associated with schizophrenia risk<br>• Identify high-impact de novo mutations at TRs associated with autism spectrum disorder<br>• Prioritize schizophrenia and autism associated TRs for genome editing experiments<br>• Perform genome-editing of target TRs in human iPSCs<br>• Differentiate edited iPSCs into neuroprogenitor cells and neurons<br>• Perform detailed molecular and cellular characterization of edited cells and their derivatives |
| **Statement of Benefit to California**<br>(as written by the applicant) | Our project has the potential to result in novel therapeutic targets for schizophrenia and autism spectrum disorder. We specifically leverage data from multiple ancestry groups, which can therefore benefit the State of California and its highly ethnically diverse citizens. Additionally, we focus on induced pluripotent stem cells (iPSCs), which has the potential to advance the understanding of physiology and disease by using samples obtained from individuals from various genetic backgrounds. |
| **Funds Requested** | $1,529,317 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 80

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

| | |
|---|---|
| **Mean** | 81 |
| **Median** | 80 |
| **Standard Deviation** | 2 |
| **Highest** | 85 |
| **Lowest** | 78 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 1 |
| **(1-84): Not recommended for funding** | 14 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 13 | • Schizophrenia and autism spectrum disorder impact a significant number of individuals and are highly complex diseases that have been studied for decades. Many unknowns remain. |

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| **No:** 1 | • The applicants propose to identify specific tandem repeats linked to schizophrenia and autism and use human induced pluripotent stem cells (hiPSCs) to study their effects on stem cell biology and neurodifferentiation.<br>• The project defines a very specific genetic alteration, expansion of tandem repeats (TRs) as key drivers.<br>The study might add to our understanding of root causes for these and possibly other neurological disorders.<br>• TRs are underexplored, and the group has developed approaches to study them.<br>• The problem with these two disorders is not that we don't know much about associated genetic mutations, but how those impact brain development and how come the same genetic mutations are seen in people with schizophrenia and autism.<br>• It is also not clear why the applicants are looking into these two disorders separately. The same genetic mutations are seen in both disorders, but the outcomes are different. Also, what about converging effects of of various genetic mutations? Looking into one of these disorders but more deeply, as opposed to the proposed approach of doing little bit in both, might be much more informative and productive. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 12<br>**No:** 2 | • There is a strong rationale for the focus on TRs.<br>• The design is very sound. Identify TRs associated with risk and then use iPSCs with genome-editing to assess the functional impact. Whether or not the TRs are really main drivers remains to be determined.<br>• Yes, rationale is strong when looking into those two disorders separately, but it is not clear why they are looking into both instead of going into more depth in one. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 11<br>**No:** 3 | • The aim of this proposal is to identify specific TRs with the strongest impact on schizophrenia and autism spectrum disorder risk, and experimentally study their effects on stem cell biology and neurodifferentiation. The project is well designed.<br>• The overall plan is clear and reasonable. Aim 1 will likely identify candidates that can be investigated in Aim 2. The major challenge will be to observe sufficient functional differences and accurately connect them to the TR perturbation.<br>• There is limited discussion that is largely focused on technical issues and less on the complexity of biological interpretation.<br>• Aim 1 is strong. The weakness is in Aim 2. How do the phenotypes to be measured relate to neuropathologies of schizophrenia and autism spectrum disorder? In the instance of schizophrenia, the main consistent neuropathology of this heterogenous disorder is in associational neocortex synapse formation/pruning/efficacy. Is this studied? |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 14<br>**No:** 0 | • Aim 1 and 2 are feasible from a technical point of view. Whether it is sufficient to establish new insights on causality is less clear.<br>• It is feasible to to identify TRs, but not to establish causality/biological effects. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 14<br>**No:** 0 | • The applicants are looking into ancestry-specific genetic variants associated with medically relevant phenotypes though all computationally. They are planning to take into consideration gender effects, but a detailed strategy is not provided.<br>• It's a largely computational effort, so there is limited influence on some aspects. |

| Application # | DISC0-15758 |
|---|---|
| **Title** (as written by the applicant) | Dissecting the cellular and molecular interactions established between human embryo and maternal endometrium at implantation |
| **Research Objective** (as written by the applicant) | Unlocking the Secrets of Human Embryo Implantation: Paving the Way for IVF Success via embryonic stem cell and advanced genetic technology. |
| **Impact** (as written by the applicant) | Infertility affects 1 in 6 people globally and leads to IVF, with less than 40% success, causing emotional and financial strain. Studying implantation can improve IVF and enhance reproductive care. |
| **Major Proposed Activities** (as written by the applicant) | • To film in real time the process of how the human embryo implants into the maternal uterus in a dish.<br>• To understand how the human embryo and maternal uterus communicates for successful implantation.<br>• To identify the genes responsible for the successful formation of the human placenta. |
| **Statement of Benefit to California** (as written by the applicant) | California nurtures a diverse population with various socio-economic backgrounds. The CDC 2020 report revealed that 54% of IVF procedures in CA didn't result in a live birth, with causes of failures remaining largely unknown. Leveraging the diversity within our research samples, we can address such problems to help couples in CA seeking IVF treatment to have high success rates and help reduce the financial burden on both patients and the CA healthcare system. |
| **Funds Requested** | $1,540,803 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 80

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

| | |
|---|---|
| **Mean** | 81 |
| **Median** | 80 |
| **Standard Deviation** | 2 |
| **Highest** | 84 |
| **Lowest** | 78 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 15 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 14<br>**No:** 0 | • Human embryo implantation is a huge black box and there are urgent needs for models that can help study the cellular and molecular details. The model could be extremely powerful, but the current aims are too vague to ensure any relevant impact.<br>• Our understanding of human embryo implantation remains limited due to challenges associated with direct observation and study of this phenomenon in vivo. Clinical applications of IVF |

| | |
|---|---|
| | demonstrate that majority of transplanted human blastocysts fail to establish clinical pregnancy, indicating that some these cases could be due to implantation defects.<br>● Gaining knowledge on human implantation is important and application of human PSCs for generation of embryoids may advance the field of regenerative medicine.<br>● The research could have a major impact on scientific knowledge in the stem cell/regenerative medicine field by providing insights into the cellular and molecular interactions during implantation.<br>● If successful, the project could improve the success rates of IVF procedures by identifying key therapeutic targets to overcome implantation barriers. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 12<br>**No:** 2 | ● The in vitro model presented could be the only way to gain some insights. Demonstration that in vitro endometrium model is or will be available supports the feasibility of planned aims.<br>● It is unclear if the proposed model can really mimic in vivo implantation.<br>● The proposal could be strengthened by providing more detailed information on the preliminary data, particularly regarding the blastocyst implantation in the 3D endometrial model.<br>● It is unclear if the proposed 3D endometrial model can truly mimic the in vivo conditions of human embryo implantation, which is critical for the validity of the study.<br>● Aim 1 is sound in general but too vague. Aims 2 and 3 are less convincing.<br>The applicant shows supporting data for the endometrial 3D model. Limited data for the blastocyst implantation are available. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 7<br>**No:** 7 | ● The experimental plan and design follows the study goals and should allow directly investigating the details of implantation dynamics.<br>● Aim 1 is likely to be very informative. The image-based acquisition plan is reasonable. The aim is however very vague though in terms of what we know already (Figure 3) and how this will be changed/improved with the model. No numbers or details are provided. Aim 2 and 3 have less clear focus. Aim 2 just describes some aspects of the omics approaches but nothing on what will be done with the large amount of data.<br>● Aim 3 will test known candidates which will be of limited value and it's unclear what will come of Aim 2 in terms of new candidates.<br>● The proposal lacks specific details, such as numbers of embryos or replicates and groups, which are necessary to evaluate if the expected outcomes will be statistically valid.<br>● Description of some experimental procedures and approaches is vague, making it difficult to evaluate if expected outcomes will be statistically valid as no numbers of embryos or replicates and groups are defined.<br>● Can in vitro culture and adaptation of endometrial cells change their function? Will this in vitro assembled endometrial model resemble and function as a uterus? What culture system and media will be used to maintain both the endometrial model and embryo to mimic natural implantation process? |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 12<br>**No:** 2 | ● Most experiments are feasible and can be achieved on time. Validation that expected results and conclusions are similar to the gold standard in vivo implantation is important but not discussed<br>● The aims are all feasible though details on what will be done are missing.<br>● The project team appears to be appropriately qualified and staffed for the proposed activities<br>● The team has access to necessary resources. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 12<br>**No:** 2 | ● Because of its general nature it could serve all individuals but also more specifically underserved populations if it increases efficiencies in IVF.<br>● The general nature of the approach to DEI could be seen as a lack of targeted effort in this area.<br>● The PI states that ability of a human embryo to implant, differentiate and give rise to a live birth is not influenced by race, ethnicity, sex, gender, and age diversity. Therefore, the research plan does not address and account for DEI criteria. |

| Application # | DISC0-15692 |
|---|---|
| **Title**<br>(as written by the applicant) | Generation of Functional Proximal Tubules in Organoids through Gradual Developmental Mimicry |
| **Research Objective**<br>(as written by the applicant) | We will develop protocols to generate clinically important kidney cell types that can be used for drug screening, disease modeling, and efforts to build an artificial kidney. |
| **Impact**<br>(as written by the applicant) | Clinical trials can perform efficient nephrotoxicity assays at low costs, cells can be produced to build an artificial kidney. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Genetically engineer stem cells to expressed genes important for kidney development and test their ability to make functional kidney cell types.<br>• Develop protocols using normal signaling cues to push human stem cells into kidney cell types.<br>• Test the physiological function of human stem cell derived kidney cells. |
| **Statement of Benefit to California**<br>(as written by the applicant) | In California, 15% of the population have Chronic Kidney Disease. This arises through several different paths but almost always, the proximal tubule cells in the nephron stop functioning as they should. We are developing ways to generate these cells from human stem cells. This will be an invaluable resource as they will allow us to develop safer drugs in clinical trials, allow us to understand the origins of kidney disease, and even pave the way to new kidney replacement therapies. |
| **Funds Requested** | $1,583,032 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 80

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

| | |
|---|---|
| **Mean** | 80 |
| **Median** | 80 |
| **Standard Deviation** | 1 |
| **Highest** | 80 |
| **Lowest** | 78 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 15 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 13<br>**No:** 1 | • Kidney diseases represent a major healthcare challenge and new therapies are urgently needed. For that, basic studies are necessary to further understand kidney development in vitro.<br>• Kidney disease is clearly a major health challenge, and end-stage renal disease (ESRD) a serious condition with limited options. Nephron proximal convoluted tubule cells (PCTs) are a key cell type with insufficient models available. This proposal aims to resolve this by |

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| | investigating molecular drivers of PCT differentiation and improving current protocols through signalling pathways.<br>● The proposal identifies a significant knowledge gap in understanding renal development, disease, and the generation of high-fidelity models for kidney diseases, which are responsible for a substantial portion of birth defects and chronic conditions.<br>● The proposal focuses upon nephron proximal convoluted tubule cells (PCTs) which are key functional cells in the kidney. These cells are also frequently damaged in diseases. Despite their importance, there is no human model functionally relevant for these cells. Thus, producing these cells in vitro is important. It will allow clinical development that's impossible so far.<br>● The overall objective of this proposal is to further understand the mechanisms controlling kidney development in human using hPSCs. This will address a major knowledge bottleneck. More precisely, the applicant proposes to study the transcriptional networks and signaling pathways driving the differentiation and maturation of PCTs. This will definitely reveal important information which could be useful to improve current protocols.<br>● The possibility to produce fully functional PCTs could have a broad impact. It will provide a new platform to model kidney diseases in vitro, and it could also give the opportunity to explore cells based therapies applications which are currently impossible.<br>● Overall, the project aims to improve the foundation (models) for future research and as such will have for now an indirect impact only. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 13<br>**No:** 1 | ● The project is based on a sound scientific rationale, leveraging insights into the developmental biology of proximal tubules and genetic underpinnings of kidney diseases.<br>● The rationale to stabilize presence or levels of two transcription factors as well as stimulating a signaling pathway is reasonable.<br>● The program is divided on two main hypotheses. The applicants suspect that key transcription factors are not expressed at the right level during kidney organoid differentiation to allow the generation of functional PCTs. This hypothesis is based on convincing single analyses on hPSCs kidney organoids.<br>● Their second hypothesis is that specific signaling pathways are necessary for PCTs functional maturation. This hypothesis is based on single cell analyses on human tissues. These preliminary data are also convincing. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 7<br>**No:** 7 | ● The program is divided in two parts which are complementary. The first part will first aim to overexpress transcription factors during differentiation using a dual inducible system, while the second aim will be to perform a detailed comparison between kidney organoid generated in vitro and primary tissue. The second aim will assess the state of a signaling pathway in organoids by comparing organoids generated in vitro with existing data on human tissues.<br>● Aim 1 is to establish the required inducible cell lines, followed by general and straight-forward molecular profiling. Technical details are provided, but how this is translated into conceptual advances is lacking.<br>● The project's first part, which involves overexpression of transcription factors, is technically challenging and may face issues with the inducible system being leaky or silenced during differentiation.<br>● The expression control of transcription factors could be problematic, with potential overexpression and variability in factor stability and duration.<br>● Concerns are the inducible expression systems, unclear levels and not enough details on how the induced signature leads to the next step.<br>● Functional tests for validating the maturation of proximal tubule cells (PCTs) are limited, questioning the functional validation of overexpressed target genes. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 12<br>**No:** 2 | ● The proposed aims and expected outcomes are logical and achievable within the proposed timeline, given the team's expertise and preliminary data.<br>● The team, led by the PI, appears highly qualified, with a track record in stem cell research and a commitment to diversity, equity, and inclusion.<br>● The team lead has a very strong technical expertise on kidney organoids. They have published key protocols, and thus, there is no doubt that they have all the expertise necessary to generate interesting data.<br>● The proposal contains a large quantity of preliminary data that strongly support the leading hypotheses of the project. However, some preliminary data on inducible systems could have been helpful.<br>● Most of the experiments are rather straightforward and could be accomplished faster. |

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| | Whether or not the induction has the desired effect is not clear.<br>● Part 1 relies heavily on inducible system to overexpress transcription factors after differentiation into kidney progenitors. This is not trivial and can be really challenging. The proposed system may be either leaky or strongly silenced during differentiation. The use of a safe harbor could help, but this might not be sufficient.<br>● Controlling the expression of the transcription factors might be very difficult, and these factors could be expressed at extremely high level. Duration and stability of each factor might also strongly vary. This is a very long shot.<br>● The inducible system is relatively new and has not yet been validated in hPSCs. This could be high risk.<br>● The functional test proposed to validated the maturation of the PCTs are limited. It is expected that overexpression of a transcription factor could induce the target gene but that does demonstrate functional validation.<br>● The second part of the project is more realistic and feasible. The combination of growth factors is more likely to increase the differentiation of PCTs. However, it might not work, and a more detailed back up plan could be useful. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 14<br>**No:** 0 | ● The proposal acknowledges the importance of genetic diversity and gender in kidney disease. However, this is not entirely clear how these aspects will be considered and addressed in the research plan.<br>● DEI is addressed but could be clarified more. The research plan lacks clarity on how aspects of genetic diversity and gender will be considered and addressed in the context of kidney disease. |

| Application # | DISC0-15902 |
|---|---|
| **Title** (as written by the applicant) | Regulatory map for guiding the generation of transplantable human hematopoietic stem cells |
| **Research Objective** (as written by the applicant) | Regulatory map for human blood stem cell development that will be used as a manual to guide pluripotent stem cell differentiation in culture to improve the treatment of blood disorders. |
| **Impact** (as written by the applicant) | This will improve the treatment of inherited and acquired blood and immune disorders by ultimately enabling new sources of blood stem cells for transplantation |
| **Major Proposed Activities** (as written by the applicant) | • Generation of epigenetic map of human developmental hematopoiesis<br>• Generate isoform level map of human developmental hematopoiesis<br>• Define the accuracy of blood stem cell specification in culture<br>• Define how to improve blood stem cell maturation in culture<br>• Define the unique features of transplantable blood stem cells<br>• Identify transplantable blood stem cells generated in culture |
| **Statement of Benefit to California** (as written by the applicant) | This work will benefit the citizens of California by ultimately enabling the development of new sources of blood stem cells and other blood and immune cells for therapies. These findings may help overcome the limitation of HLA matched blood stem cells for ethnic minorities and individuals of admixed backgrounds. These findings will help generate better culture models for blood diseases such as sickle cell anemia, and understanding blood diseases that originate before birth. |
| **Funds Requested** | $1,576,043 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 80

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

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| **Mean** | 80 |
| **Median** | 80 |
| **Standard Deviation** | 4 |
| **Highest** | 84 |
| **Lowest** | 70 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 15 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 14<br>**No:** 0 | • Yes. No studies in the epigenetic landscape of pluripotent stem cells (PSCs) to putative hematopoietic stem cell (HSC) transitions exist, and this proposal aims to complement pioneering studies of the applicant at the transcriptional level. To date, no somatic stem cells of any lineage have been derived that are similar to stem cells obtained from post-natal/adult tissue in humans. As such, the proposal is key to PSC-based regenerative medicine. Both hypotheses and resource data will be generated that will be unique worldwide. |

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| | • For decades, the research community has been trying to generate quality, fully-functional HSCs (and other cell types) from induced pluripotent stem cells (iPSCs). We lack the molecular determinants to define HSC origin and maturation stages.<br>• Progress has been made recently in differentiating transplantable HSCs, but the frequency is still quite low. A gene regulatory map of human hematopoietic ontogeny, including epigenomics and isoform level data, could yield new insights into how to further increase these initial successes.<br>• It is clear that full developmental knowledge about how HSCs are formed via human development is lacking, and it is probably connected to the difficulties of recapitulating such development via directed differentiation in vitro.<br>• The inability to generate functional HSCs from PSCs is a well-known bottleneck in the field of stem cell regenerative medicine. Progress on this would be welcome.<br>• A robust method to produce functional HSCs from PSCs would be transformative. This is somewhat a resource-type of grant in that detailed knowledge of the development of HSCs (a handbook) would constitute a knowledge resource of value. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 11<br>**No:** 3 | • The proposal utilizes a few protocols from collaborators for PSC differentiation. However, the applicant overlooks other established procedures for PSC differentiation to hematopoietic lineages in the proposal background sections and the list of references. The assumption is that the applicant either finds this work irrelevant or is unaware of these studies.<br>• The rationale for employing new single-cell RNA sequencing methods, without first comparing this type of data to previous data in Aim 1.1 and prior studies, is concerning. The applicant also does not investigate the depth loss of transcript for the two methods, missing an opportunity.<br>• The transcriptional phenotype and cell surface phenotype are not well connected, and the extent of heterogeneity is unknown. This is relevant to Activity 3-4, as fluorescence-activated cell sorting (FACS) purification of cells in the "best PSC to HSC" culture conditions is not used. The level of heterogeneity within a population is critical, especially given the massive number of cells required for minimal engraftment levels.<br>• There seems to be a disconnect between the use of single-cell omics and transplantation or clonal in vitro assays of low frequency, which doesn't compare to somatic hematopoietic tissues such as cord blood with progenitor and stem cell activity down to 1 in 3 and 1 in 8, respectively. Missing details in activities 5 and 6, such as cell lines, purified or not, stage or day of selection, add to concerns.<br>• Although the group has made major contributions to the field, a clearer rationale is needed for why conducting more of the same (mapping now at the epigenomic and isoform level) will yield better results. Specifically, how will they use the generated information to tweak their differentiation protocol?<br>• The rationale is reasonable enough, suggesting that knowledge of intermediate cell types obtained from in vivo human embryonic sources should provide information highly useful for developing successful in vitro directed differentiation methods.<br>• The preliminary data is detailed and complex but shows some progress, especially in developing early HSCs put to the aorta-gonad-mesonephros (AGM) phase, which can transplant into mice to yield HSCs (albeit at levels much lower than cord blood cells). |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 9<br>**No:** 5 | • The proposal is well-described, with a focus on technology and informatics. This group is well-positioned to troubleshoot and circumvent any issues that arise.<br>• Overall, the project is well-designed, although Aim 1 appears to focus mostly on refinements and additional data incorporation into a growing map of human HSC development. While somewhat incremental in nature, it is likely necessary, as key details are currently missing, as evidenced by the difficulty in achieving directed differentiation to HSCs.<br>• There is high potential for this work to have a significant impact, given the unique tissue and technology being used.<br>• Overall, the project is well-planned. However, more specifics are needed in Aim 3. Why is single-cell RNA sequencing needed when transplanting so many cells?<br>• Aim 3 is the weakest aim and could benefit from more details about the experimental plan.<br>• It is unclear how epigenetic information will be adapted for the improvement of directed differentiation procedures. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 12<br>**No:** | • The project is unlikely to be completed if the number of in vivo studies is not reduced. The details of experimental vs. biological replicates was difficult to follow or absent. E.g., for each |

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| 2 | study, the applicant should include both the number of lines and the number of replicates per line. This is important given the expensive nature of the technology.<br>● This project is probably feasible, but there is some concern that the transplantation model may be more problematic than the cells.<br>● The applicant team is very well positioned to do this work. They are world renowned experts and leaders in this area.<br>● The group has a pipeline to do this and access to the needed tissue.<br>● The budget seems low for the amount of animal and omics work. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 14<br>**No:** 0 | ● Sex balance will be incorporated, but no race/ethnicity information is provided or can be selected. Future intent to test on diverse PSCs using SNV analysis to capture broader background.<br>● It's unclear if there are genetic pre-dispositions associated with epigenetic regulation of PSCs differentiating into putative HSCs.<br>● HLA-matched donors are difficult to find for underrepresented groups; the proposed map includes tissues from both sexes.<br>● Yes - the need for diverse panels of PSCs matched to underrepresented individuals is inherent to the proposal.<br>● Assuming sample sizes in the proposed scRNA-seq are large enough, something might be learned about<br>the ability of diverse cell types to yield functional and transplantable HSCs.<br>● The Principal Investigator has created an online tool to teach the public about stem cells.<br>● Once protocols are optimized, they will test in sample of different ancestral origin. |

| Application # | DISC0-15763 |
|---|---|
| **Title**<br>(as written by the applicant) | Understanding the mechanisms and developing therapeutics for the neurodegenerative Parkinson's disease (PD) using human iPSCs derived models |
| **Research Objective**<br>(as written by the applicant) | This project aims to better understand the mechanisms of dopamine (DA) neuron degeneration in Parkinson's disease (PD) and develop new therapeutics using human induced pluripotent stem cells (hiPSCs). |
| **Impact**<br>(as written by the applicant) | If successful, this project will unravel new pro-survival pathways in human dopaminergic neurons and generate preclinical small molecule candidates for future disease-modifying therapeutics |
| **Major Proposed Activities**<br>(as written by the applicant) | • Develop a new scalable and live imaging-based high content screening platform to follow the survival of mDA neurons derived from hiPSCs using a CRISPR/Cas9 engineered TH-reporter hiPSC cell line.<br>• Investigate the neuroprotective potential of RAAS inhibitors and other candidate drugs using hiPSC derived human mDA neurons and mDA neuron-glia coculture models.<br>• Develop a 3D midbrain organoid and neuron-glia assembloid models from TH-reporter hiPSC cell lines that better mimic in vivo conditions to evaluate the neuroprotective potential of candidate drugs.<br>• Use CRISPR Knock-In technology to create the first CRISPRi and CRISPRa hiPSC lines with a TH-TdTom reporter that will pave the way for genome-wide studies in DA neurons in the context of PD.<br>• Develop the first hiPSC-based CRISPRi/a screening platform in mDA neurons to identify new genes, pathways, and small molecules that can synergize with RAAS inhibition for enhanced neuroprotection.<br>• Produce mDA neurons using hiPSC lines from different gender and background and test candidate neuroprotective compounds to discover therapeutics that will benefit all individuals. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Parkinson Disease (PD) is the most common movement disorder and the second most common neurodegenerative disorder. In 2020, California had the most deaths due to PD in the US (4147, NIH). The economic cost of PD is estimated to at least $51.9 billion a year in the US. There are no disease-modifying therapeutics for PD. This research promises to yield a collection of preclinical small molecule candidates that can pave the way for robust and effective disease-modifying therapeutics for PD. |
| **Funds Requested** | $1,593,011 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 80

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

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| **Mean** | 79 |
| **Median** | 80 |
| **Standard Deviation** | 3 |
| **Highest** | 84 |
| **Lowest** | 70 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 15 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 13 **No:** 1 | • Parkinson's Disease (PD) is a devastating and costly disease with no cure or effective disease-modifying therapeutics. The only symptomatic treatment, based on augmenting dopamine signaling, has only temporally effects and long term use is associated with severe side effects.<br>• The project aims to find new neuroprotective strategies in PD using human stem cell models.<br>• The project is grounded in a comprehensive understanding of PD and leverages innovative human induced pluripotent stem cell (iPSC) models to explore the disease mechanisms and therapeutic avenues.<br>• The research addresses a bottleneck in the development of stem cell-based therapies by focusing on the validation of renin-angiotensin-aldosterone system (RAAS) inhibitors and discovering new neuroprotective pathways.<br>• While the potential neuroprotective role of RAAS inhibitors are well documented and could provide a therapeutic avenue in PD, not all studies show beneficial effects, highlighting the need for a better understanding of the mechanism.<br>• Screens could yield new therapeutic candidates, but relevance for PD pathology is unclear.<br>• Establishment of a high-content screening platform using human midbrain dopaminergic (mDA) neurons derived from human iPSCs to identify additional targets as well as analyse the mechanisms that are underlying the neuroprotection will be important to advance this approach.<br>• Responsiveness to prior critiques is mixed. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 11 **No:** 3 | • The use of human iPSC-derived models and CRISPRi/a-based screens to identify neuroprotective pathways is cutting-edge and has the potential to uncover novel therapeutic targets for PD.<br>• Single-cell genomic profiling of human DA neurons in PD patients uncovered RAAS pathway receptor AGTR1 expressing dopaminergic (DA) neurons that are located in the substantial nigra pars compacta and are highly susceptible to degeneration in PD.<br>• AGTR1 inhibitor treatment showed significant neuroprotection in a zebrafish line where cells were challenged with CBE treatment that led to selective for DA neuron death.<br>• Inhibiting RAAS system associated proteins with drugs that are already in the clinic to treat hypertension have been shown to rescue DA neurons in PD. Re-purposing such drugs for PD provides an urgent and unique therapeutic opportunity.<br>• It is based on sound scientific rationale, but not very precise or based on PD-relevant degeneration. It is not clear whether PD disease features are sufficiently modeled.<br>• Preliminary data on the cell model exists. This data also show that tyrosine hydroxylase (TH) neurons die, but not the mechanism for this, and whether it is relevant for PD or specific to DA neurons.<br>• The applicant states (on page 11) that, in both the CBE- and Rot-induced neurodegeneration model in the neuron-glia co-culture, a RAAS inhibitor showed dose dependent neuroprotection when added a few hours before the neurotoxins. If pre-treatment is necessary to achieve protection, the approach is not translatable. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 7 **No:** 7 | • The proposal is supported by robust preliminary data, including the development of a chemogenetic model for dopaminergic neuron degeneration and the identification of RAAS inhibitors as potential neuroprotective agents.<br>• The project has a clear set of objectives and specific aims, which are supported by a robust chemogenetic model for inducing dopaminergic neuron degeneration suitable for high-content screening.<br>• The experimental plan is well described but the majority of projects are screens without sufficient plans for validation of hits or mechanistic insight.<br>• Aims are straightforward and consist of validation of already identified drugs and screening for new drugs<br>It is not clear whether the drugs have been or will be validated in an animal model.<br>• No patient derived neurons or organoids are used to test the drugs, which is a weakness. |

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| | • Candesartan showed no protective effect against Rotenone induced death (Fig 3I) which raises the question of why both models are used.<br>• Aim 2 seems premature. It might be necessary to first validate the efficacy of drugs analyzed in Aim 1 in a mouse model of PD. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 12<br>**No:** 2 | • The project's ambitious aims and the described timeline may be overly optimistic, raising concerns about feasibility within the proposed timeframe.<br>• The project team would benefit from more expertise on DA neuron differentiation and PD pathology specialists.<br>• To mitigate previous concerns of the "rejuvenation " of cells during reprogramming the applicant states that "Our new preliminary data have shown that neurotoxic chemicals (i.e., CBE or Rotenone) could mimic and possibly accelerate aging processes, resulting in mDA neuron degeneration in hiPSC-derived 2D and 3D models (Fig. 2,3)" . There are no data provided that would substantiate this claim..<br>• The PI has experience with hiPSC work and has successfully performed all preliminary experiments, demonstrating the capability to lead the project. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 14<br>**No:** 0 | • The proposal adequately addresses and account for the influence of race, ethnicity, sex, gender, and age diversity.<br>• Outcomes extend or validate the applicability of regenerative medicine discoveries to underserved populations, including underserved racial/ethnic communities.<br>• Prior efforts or proposed plans for outreach, partnership, or educational activities to inform the development of DEI within the research project are well described.<br>• The drug could be beneficial for a large number of patients, although route of delivery and timing are not well addressed as in vitro study use a pre- exposure.<br>• The initial focus on using an engineered hiPSC line derived from a white British male donor, seems reasonable considering the prevalance of PD in this population. An expansion to include other lines is discussed.<br>• The project's emphasis on using diverse hiPSC lines and its broader commitment to DEI principles in the research process are commendable. |

| Application # | DISC0-15916 |
|---|---|
| **Title** (as written by the applicant) | Exploring Potential Drugs to Enhance Neural Recovery in Combination with Neural Stem Cells after Spinal Cord Injury (SCI) |
| **Research Objective** (as written by the applicant) | Identify pharmacological compounds that can mimic rehab "pro-plasticity" state after SCI. Determine whether combination of this compound with transplanted NSCs will further enhance recovery after SCI. |
| **Impact** (as written by the applicant) | Identifying a pharmacological treatment that can be administered immediately following the injury could take advantage of this critical window of neuroplasticity. |
| **Major Proposed Activities** (as written by the applicant) | • Pharmacological compounds screened in vitro<br>• RNA-seq of cortical neurons in vitro<br>• Testing drug optimal dosage and method of delivery<br>• Spinal cord lesions<br>• Transplanted H9scNSCs<br>• Behavioral and histological Analysis |
| **Statement of Benefit to California** (as written by the applicant) | The proposed grant will find a therapeutic approach that can take a rapid path towards human intervention. If successful this compound can further improve anatomical and functional recovery in patients with spinal cord injury citizens in California State |
| **Funds Requested** | $1,583,998 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 80

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

| | |
|---|---|
| **Mean** | 77 |
| **Median** | 80 |
| **Standard Deviation** | 5 |
| **Highest** | 84 |
| **Lowest** | 65 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 15 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 12<br>**No:** 2 | • The project addresses key knowledge gaps in molecular mechanisms of training to promote brain/spinal cord plasticity. This is interesting from a basic science perspective and can also lead to better therapies in the future.<br>• The project seeks to enhance cell based repair of the spinal cord.<br>• The project has several interesting potential outputs - molecular understanding of how targeted training of neural grafts can promote graft survival and function - identify pharmacological compounds or small molecules that mimic neuroplasticity induced by task specific training - better treatments for spinal cord injury. |

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| | • The timing challenge during which a therapy would be most successful represents a significant bottleneck for optimizing therapies in humans who have access to rehabilitation and transplants shortly after injury. A new approach is needed to overcome this limitation.<br>• Drugs that could be used instead of rehab and that would extend and potentiate recovery after SCI would be highly valuable.<br>• No strong argument for use of stem cells. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 10 **No:** 4 | • The project is based on solid literature and findings by the applicant and follows a nice logic.<br>• The preliminary data supports the feasibility of the applicants ability to conduct the project, but offers limited data to support that successful candidates will be identified.<br>• The project is relevant for SCI and repair as well as in a broader context for cell based repair of the CNS.<br>• Rationale is based on the observation that rehabilitation alone (or in combination with other therapeutic interventions) can improve skilled forelimb functional recovery after SCI but only during a short window. The applicant suggests that rehabilitation may temporarily reinforce spared projections and stabilize newly sprouted axons within the spinal cord and brain.<br>• To identify mechanisms that are underlying this regenerative window, they mapped these transcriptional signature of rehabilitative training onto the Broad Connectivity Map (CMAP) and identified top compounds that potentially mimic this transcriptional pro-plasticity state on corticospinal neurons.<br>The next step is to screen these compounds in vivo and in vitro.<br>• Problem with the rationale: Published data in rodents show statistically significant recovery with rehabilitation + neural stem cells (NSC) but this recovery is compared to rehab alone. NSC alone have no effect. Thus the rationale of dedicating a major portion of the work to a cohort that receives both rehab and NSC is weak.<br>• A CIRM funded application has been dedicated to optimize the NSC transplant but, in this model, NSC are not effective.<br>• No information of top hit candidates is provided. As only five drugs were identified, a limited discussion would have been helpful (are these drugs already in the clinic? can they cross the blood brain barrier? etc). |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 8 **No:** 6 | • The project is well planned and designed following a logic progression and using state of the art methods.<br>• Potential pitfalls are identified and some alternative approaches suggested.<br>• Aim 1 is focused on screening the top compounds in the culture of adult cortical neurons, measure total outgrowth, number of branches formed, and maximum neurite length per cell through Tuj1-positive labeling. They will also assess the spontaneous electrical activity of cortical neurons and interrogate transcriptional signature of these neurons after treatment with the pharmacological compound.<br>• The rationale for screening the compounds based on neuronal outgrowth is not supported by data. Figure 3 only shows outgrowth in the presence of NSC, which by themselves have no functional benefit and the combination is not more effective than rehab alone. As no rehab only figures are shown, it is not apparent that outgrowth is a useful surrogate for the behavioral recovery seen in rehab.<br>• Screening in vitro is limited to neurons only and thus does not predict any effects on astrocytes, microglia activation, BBB effects etc.<br>• Aim 2 is highly translational and consists of determining whether pharmacological compound, when administered in combination with transplanted stem cells, would promote CST growth and functional recovery after SCI.<br>• Problem: How is drug load monitored? What outcomes in Aim 1 would define the used of what drug in Aim 2? Are all five drugs going to be tested? |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 13 **No:** 1 | • The project is ambitious but feasible given that the candidates are identified during the first year.<br>• For screening top hit drugs in vitro, they will use adult cortical rat neurons- such neurons are shown suggesting feasibility.<br>• The team is well composed but there are two concerns. One is that the co-PI has the relevant expertise and access to facilities needed for the in vivo work that is key to proposal. It would be better if this was with main applicant. Also bioinformatic support is remote.<br>• Many key resources are with co-applicants instead of main applicants. |

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| | ● Considering the very modest increase in recovery shown in the published work, it is questionable whether a n=10 per cohorts would be sufficient to see a difference. In fact, the published manuscript notes that " further expansion of group sizes would be required to establish whether there is a statistically significant benefit of rehabilitation alone in chronic SCI". There is no power analysis provided that would justify the numbers. Timing is not clear.<br>● Discussion about pitfalls and alternatives is inadequate. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 14<br>**No:** 0 | ● A pharmacological approach in a cervical bilateral contusion SCI model, would allow greater access to therapies.<br>● The use of female rats is well justified.<br>● Experiments will only be conducted in one sex. Considering that SCI is more severe in females, the general comment of having to reduce numbers is not reasonable. Running all experiments in both sexes a key principle of ensuring diversity.<br>● Very generic - not specific for SCI injury patient groups. |

| Application # | DISC0-15855 |
|---|---|
| **Title**<br>(as written by the applicant) | The functional impact of psychedelics on human brain organoids |
| **Research Objective**<br>(as written by the applicant) | This project aims to determine the mechanistic effects of psychedelics on the human brain using cortical organoids derived from stem cells, for the treatment of several neurological conditions. |
| **Impact**<br>(as written by the applicant) | Since psychedelics have been proposed for treating several psychiatric conditions and neurological disorders, the mechanisms underlying their actions will useful to ensure dosing, safety and efficacy. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Recording the electrical activity of brain organoids to evaluate the impact of psychedelics<br>• Evaluate alterations in the strength of neuronal communication triggered by psychedelics<br>• Determine alterations in neuronal communication using microscopic visualization caused by psychedelics<br>• Determine immediate, rapid changes in proteins caused by psychedelics<br>• Determine the long-lasting changes triggered by psychedelics on the expression of genes in individual cells. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Psychedelics are being proposed to treat several psychiatric and neurological conditions worldwide. Therefore, it is important to determine the mechanisms underlying their actions to ensure dosing, safety, and efficacy for millions of patients suffering from these conditions. Moreover, the psychedelics industry has a growing market that will stimulate new businesses and jobs in California. |
| **Funds Requested** | $1,584,000 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 78

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

| | |
|---|---|
| **Mean** | 78 |
| **Median** | 78 |
| **Standard Deviation** | 4 |
| **Highest** | 85 |
| **Lowest** | 70 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 1 |
| **(1-84): Not recommended for funding** | 14 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 9<br>**No:** | • The goal of this application is to define the impact of psychedelics on brain organoids as a model to understand their mode of action. Psychedelics are used to treat a broad number of |

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| 5 | neurological disorders. Thus, the impact of this proposal could be extremely broad and support the development of personalised therapies.<br>● The applicants propose to determine the immediate and long-term impact of psychedelics on the brain by using cortical organoids.<br>● The applicants propose to use brain organoids, electrophysiological recordings and proteomics to investigate impact of psychedelics on human brain. This is important due to the increased use of psychedelics as a potential therapy for various neuropsychiatric disorders.<br>● The project combines state of the art brain organoids with electrophysiology technology, single cell transcriptomics and proteomics. if successful, it could deliver exciting knowledge about the mode of action of psychedelics and general impact on the brain.<br>● The project could have an important impact. However, it will focus on a limited number of psychedelics and it would have been useful to better justify this selection in a clinical context.<br>● The applicants are using organoids, not mouse models, and therefore could investigate a larger number of substances. Furthermore, confirmation in an in vivo animal model (e.g. mouse) would strengthen the data (and translatability).<br>● The project uses state of the art methods to assess influences of psychedelics on organoid activity.<br>However, " this project will be able to reveal the impact of psychedelics using cutting-edge methods<br>and in-depth network analysis, offering valuable information about dosing, safety, and efficacy of the new therapies."<br>In this reviewer's opinion this statement, which seems to be a major point of grant impact, is inaccurate at all three levels of dosing, safety, and efficacy.<br>● 1) Dosing. Medication dosing is affected by route of administration, bioavailability, liver metabolism, liver/kidney excretion, blood brain barrier permeability, and other factors not present in your organoid system.<br>● 2) Safety of psychedelics might be partially addressable in your system, but only at the level of neuronal toxicity and that in relation to a very early and short term developmental exposure that does not model the adult usage. Most safety concerns with psychedelics are related to various toxicities in non-neural tissues, as well as long-term influences (2 weeks is not "long-term") on brain/psychiatric health that are not really addressable in your system.<br>● 3) Efficacy. Only a study design that somehow links clinical efficacy/lack of efficacy to a organoid-based metric that associates with this efficacy/lack of efficacy could really address efficacy. Your study design does not address efficacy.<br>● The organoids lack neurotransmitter innervation from midbrain catecholamine nuclei, such as dopamine, norepinephrine, and serotonin, and probably also acetylcholine from basal forebrain. These are known to play major roles in psychedelic actions, be they therapeutic or not.<br>In sum, changes on various measures will no doubt be found in response to various psychedelics, but the relevance of these changes to in human usage will be uninterpretable. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 9<br>**No:** 5 | ● The use of brain organoids to model the effect of psychedelics make sense and there is no doubt that the project could generate interesting data.<br>● Some validation using in vivo models or patient data would have been useful.<br>● Another challenge is the fact that the wiring within cerebral organoids has not been demonstrated to reasonably model the wiring of the cortex--this is due to the fact that arealization is not established, and also both inputs and outputs are not present (great though to try adding thalamic-like inputs here). The lack of output targets means that, if adequately specified and differentiated, most neurons in the organoid will not have their normal targets available.<br>● The lack of normal targets will highly likely result in aberrant connectivity and hence aberrant circuitry that is not modeling anything resembling human neocortex. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 12<br>**No:** 2 | ● The project is well organised and justified. However, few aspects could be improved. First, it is not clear at which stage of differentiation brain organoids will be used. Furthermore, phosphoproteomic studies look like a fishing expedition. The mult-omics part is interesting but might be challenging to achieve. Finally the selection of psychedelics could be better explained.<br>● Pitfalls have been identified and alternative approach have been proposed. However, these backup plans look like a totally new project and it would have been useful to integrate those in the main project.<br>● Well designed and achievable.<br>● State of the art multimodal analysis of cerebral organoids. |

| GWG Votes | Is the project feasible? |
|---|---|
| **Yes:** 12 **No:** 2 | • Yes, although ambitious.<br>• The preliminary data are convincing and there is no doubts that this team can generate important results.<br>• In vitro systems have limitations when compared to in vivo models, especially in therm of pharmacology. Human models systems are currently limited and brain organoids represent the best option. However, this model system presents severe limitations and can not be used in isolation.<br>• The protocol used to generate brain organoids seems to bypass variability between lines and also variation induced by genetic background, which is relatively rare. So, this protocol could also hide important effects on the psychedelics efficacy? Similarly, it would be useful to confirm that the right neurons are contained in the brain organoids. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 14 **No:** 0 | • This aspect is clearly explained in the proposal.<br>• The applicant indicate that there is no impact of genetic background on psychedelics effect. This could be better justified. The applicant does not see an impact of genetic background on brain organoids function or composition. Again, this is hard to believe. As a consequence, their experimental plan does not include diverse hIPSC lines which could be a limitation.<br>• Yes - takes into account different racial/ethnic backgrounds. |

| Application # | DISC0-15770 |
|---|---|
| **Title** (as written by the applicant) | Ultrasound Controllable CAR T Cell Therapy for Pediatric Glioblastoma |
| **Research Objective** (as written by the applicant) | Our study aims to bridge the ultrasound control with cell therapy, highlighting the integration of ultrasound technology with anti-GD2 CAR T cells to enhance GBM treatment efficiency and safety. |
| **Impact** (as written by the applicant) | Our approach should allow remote-controlled, noninvasive gene activation with high spatial and temporal precision for the delivery of immunotherapy to GBM in the depth of brain. |
| **Major Proposed Activities** (as written by the applicant) | • Selection of FUS-GD2CAR T cells exhibiting optimal killing efficacy against glioblastoma cells in vitro.<br>• Directed evolution-based screening of heat-sensitive promoters.<br>• Characterization of exhaustion in FUS-CAR T cells versus their constitutive counterparts.<br>• Characterize the GD2 FUS-CAR activation and killing efficacy against glioblastoma cells in vivo.<br>• Characterize the iGD2 FUS-CAR T cell profiles in the tumor. |
| **Statement of Benefit to California** (as written by the applicant) | By delving into and refining the genetic interface, our objective is to propel the development of CAR T cell therapy. The successful completion of this project is expected to yield a novel therapeutic strategy for the treatment of patients with lethal glioblastoma multiforme (GBM) in California. |
| **Funds Requested** | $1,200,000 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 77

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

| | |
|---|---|
| **Mean** | 77 |
| **Median** | 77 |
| **Standard Deviation** | 3 |
| **Highest** | 84 |
| **Lowest** | 70 |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 14 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 9<br>**No:** 4 | • CAR Ts are a powerful tool in the cancer therapeutics space. Some issues remain, and the proposal aims to engineer safer CAR T cells that are only activated locally via ultrasound.<br>• If successful, the project would provide another tool available for advanced CAR design.<br>• The data indicate that on-target off tumor effects could be an issue, but it's not clear that it's the primary issue in CAR T cell failures. |

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| | • The project's significance and possible target tumor-types for the technology beyond glioblastoma need to be clarified.<br>• The major issue with the project's significance is whether too much activity of solid tumor CARs is really the problem that needs focus. Further, there's concern that in a real tumor setting, the off tumor targets will be much closer to the tumor. Thus, the activated CAR T cells will be able to cause damage.<br>• The proposed technique is relevant only for very local tumors.<br>• While the technology is feasible, it has the major limitation that this would effectively restrict CAR T to a localized therapeutic modality. In general, the potential value of CAR T cells seems of far greater significance for systemic therapy in advanced disease.<br>• Even in glioblastoma, in which some tumors might be treatable by locally activated (via ultrasound) T cells that could not otherwise be treated surgically, the approach will only activate those CAR T cells in regions that already have tumor deposits large enough to be visible to a scan. This requirement obviates the major benefit of a systemic therapy that aims to be potentially curative by providing both short- and long-term protection against widely distributed, potentially scan silent, metastatic cells.<br>• Regulating CAR T cell activity is a key area of investigation, but a panelist was not convinced that local regulation of CAR induction addresses the primary success-limiting factors in current CAR T trials. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 8<br>**No:** 5 | • The project seems technically feasible based upon the preliminary data.<br>• The rationale to activate expression of a CAR through a use of heat-inducible promoter and locally focused ultrasound is supported by data from the applicants and others. However, as noted in comments on significance, restricting CAR T cells to a sophisticated mode of local therapy seems unsatisfactory. It undermines some of the greatest potential advantages of CAR T cells.<br>• The rationale that there are off tumor, on target effects is well established. Methods to mitigate those effects are important. The preliminary data are moderately supportive. I do not find the proximal/distal tumor study particularly compelling; the model presents a situation in which target antigen expression is very separated between tumor and normal tissues. In other settings, normal tissues might be intermixed with the tumor. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 12<br>**No:** 1 | • The project will test the specific hypotheses proposed and provide clear answers for them. Potential pitfalls are well described. The timeline is appropriate for the studies.<br>• If one ignores the limits of the concept and rationale, the proposed experiments are well planned and designed.<br>• Aim 1 is logical and well designed to iteratively optimize the heat-sensitive promoters needed for the focused ultrasound. It will also explore T cell exhaustion and how transient induction may overcome that.<br>• Aim 2 will test the CAR T activation and efficiency in vivo in both heterotopic and orthotopic glioblastoma models.<br>• Aim 3 will explore the distribution of the CAR T cells in the mouse models using cell sorting, spatially using tissue sections and molecularly using single cell sequencing. |
| | *none* |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 13 | • Based on the applicants expertise and proposed experiments, the project is feasible.<br>• It looks feasible to regulate expression of a CAR by T cells using ultrasound. However, the restriction to local expression seems a severe limitation, especially for such an expensive, individualized therapy with attendant high risks to go well beyond on-target, off-tumor side effects.<br>• The proposed aims are somewhat ambitious for the time period. The applicants are simultaneously proposing to address multiple aspects of CAR design that have otherwise remained intractable, and to do so in a three year period. The team has excellent qualification in the ultrasound method. The environment is suitable. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 13<br>**No:** 0 | • The host institution has a good track record upholding DEI principles.<br>• The applicants plan to recruit patients with attention to DEI.<br>• Diversity of donors will be considered. |

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| | • The DEI plan is well-described. There are limited opportunities for DEI involvement in the actual research, but it is addressed.<br>• The application provides a generic statement. |

| Application # | DISC0-15688 |
|---|---|
| **Title** (as written by the applicant) | Using Human Pluripotent Stem Cells for Biomedical Innovation Aimed at Improving the Health of Women and Girls. |
| **Research Objective** (as written by the applicant) | Engineering a model of the human ovary in order to understand ovarian disease and dysfunction. |
| **Impact** (as written by the applicant) | A human model of the ovary could be used to understand causes of primary ovarian insufficiency, polycystic ovarian syndrome or ovarian cancer. |
| **Major Proposed Activities** (as written by the applicant) | • To generate reporter lines for human ovarian somatic cell differentiation using stem cells.<br>• To assemble a model of the human ovary using stem cells for future biomedical innovation into ovarian disease and dysfunction. |
| **Statement of Benefit to California** (as written by the applicant) | Tens of thousands of Californian women, girls, and persons with ovaries under the age of 40 are living with ovarian disease or dysfunction. Solutions to overcome this will require biomedical innovation using human ovarian cells. This proposal aims to generate models of the human ovary using human pluripotent stem cells from people of diverse ancestry. |
| **Funds Requested** | $1,564,889 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 75

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

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|---|---|
| **Mean** | 76 |
| **Median** | 75 |
| **Standard Deviation** | 4 |
| **Highest** | 84 |
| **Lowest** | 70 |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 14 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 11<br>**No:** 3 | • The ability to differentiate iPSCs from a diverse population with known reproductive health histories into ovarian somatic and primordial germ cells would aid in our understanding of ovarian development. It's harder to determine if these models would be useful in understanding the pathophysiology of ovarian diseases.<br>• Somatic cells and primordial germ cells generated in vitro from human iPSCs could be useful to understand the basic mechanisms of early ovarian/germ cell development. However, generating in vitro models of complex human organs from pluripotent stem cells is very difficult, and in most cases, the models do not represent functional in vivo tissues or adult stage organs.<br>• The project aims to develop a stem cell-based model of the human ovary, addressing a significant gap in our understanding of ovarian biology and disease. This could lead to |

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| | advancements in the prevention and treatment of ovarian disorders such as primary ovarian insufficiency, ovarian cancer, and polycystic ovarian syndrome, which collectively affect millions of women and girls. However, it is unclear whether stem cell-generated in vitro ovarian models could address the complex pathophysiology underlying these conditions.<br>● It might be valuable if the proposal tied in in vitro gametogenesis. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 13 **No:** 1 | ● The rationale closely follows mouse protocols that require the induction of both ovarian somatic progenitors and primordial germ cells (PGCs) from pluripotent stem cells (PSCs), followed by their aggregation to form primordial follicles.<br>● Species-specific differences in early ovarian/germ cell development between mice and humans likely exist, but the proposal will follow mouse protocols. It's unclear how potential species-specific problems will be addressed to establish human models.<br>● Most preliminary concepts for proposed mouse protocols are developed by others, and human primordial germ cell-like cell (PGCLC) cell induction was also proposed by others. There's a lack of preliminary data demonstrating the feasibility of Aim 1 to use gene editing to make knock-in reporter human iPSC lines.<br>● The project heavily relies on hiPSCs for modeling, which, while innovative, may not fully recapitulate the complexity of ovarian tissue or disease states.<br>● The proposal aims to develop stem cell-based models of the ovary, but these models may not accurately mimic the intricate processes of ovarian function and disease.<br>● Most preliminary data are from studies using mouse cells; more preliminary data in human cells are needed to justify a funding recommendation. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 9 **No:** 5 | ● The project is well-planned, with clear objectives and specific aims that are likely to yield meaningful results. The use of hiPSCs from donors of diverse ancestry enhances the relevance and applicability of the research.<br>● The end goal of this proposal is vague. Once the ovarian somatic progenitors and human PGCLC are aggregated and primordial follicles are generated, what is expected? One would expect that these experiments advance the development of human PGCLCs to later stages of gametogenesis and female meiosis. The functionality of human ovaries generated in Aim 2 should be demonstrated by producing later stage human oocytes.<br>● It is critical for the success of this project for the applicant to provide step-by-step comparisons of experimental, in vitro generated cell and tissue types to the gold standard in vivo controls. Such controls are missing in this proposal.<br>● While it is well recognized that access to human fetal tissues at very early stages of development is difficult, the PI has unique collaborations and exclusive access to human samples. Therefore, inclusion of in vivo controls should be considered. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 9 **No:** 5 | ● The project's aims and expected outcomes are logical and achievable within the proposed timeline, given the team's expertise and the preliminary work already conducted.<br>● The team is well-qualified, with a diverse range of expertise relevant to the project's goals. Access to necessary resources and a supportive research environment further enhance the project's feasibility.<br>● The application does not include preliminary data establishing the lab's experience with genome editing.<br>● Aim 1 is feasible, but the proposed strategy for creating reporter iPSC lines is not a typical approach. Cas9 nickases have much lower rates of integration compared to WT Cas9.<br>● Most experiments are feasible and can be achieved on time. However, Aim 2 is dependent on availability of reporter cell lines. Verification of correct integration of reporter constructs into humans iPSCs by PCR and Sanger sequencing is not sufficient. It must be confirmed by more robust and up-to-date approaches, such as whole genome sequencing. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 13 **No:** 1 | ● Use of diverse iPSC lines with known reproductive health histories is proposed.<br>● The project is women's health focused.<br>● While the proposal aims to address diversity by including cell lines from different ancestries, these models may or may not represent the various genetic and environmental factors affecting ovarian health.<br>● Adult female iPSCs from diverse ethnic groups will be utilized. Inclusion of iPSCs from young girls or postmenopausal women would benefit these groups of infertility patients. It could also |

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| | be valuable to include XY iPSCs to address the rights of gay couples to have genetically related oocytes. |

| Application # | DISC0-15903 |
|---|---|
| **Title**<br>(as written by the applicant) | Instruction of Hematopoietic Stem Cell Fate from Human Pluripotent Precursors |
| **Research Objective**<br>(as written by the applicant) | Bone marrow transplantation is risky due to immune mismatch between donor and host. We seek to generate patient-specific blood stem cells to cure disease with a perfectly matched immune system. |
| **Impact**<br>(as written by the applicant) | Successful completion of the proposed research will result in robust and reliable production of blood stem cells directly from patients in need of a bone marrow transplant. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Optimize human blood stem cell production from pluripotent stem cells via modulation of the NOTCH signaling pathway.<br>• Optimize human blood stem cell production from pluripotent stem cells via modulation of the WNT signaling pathway.<br>• Optimize human blood stem cell production from pluripotent stem cells via comparison to checkpoints along the way of endogenous human stem cell generation. |
| **Statement of Benefit to California**<br>(as written by the applicant) | The proposed studies will inform methods to engineer blood stem cells from human pluripotent precursors. This will allow generation of patient-specific blood stem cells, enable transplantation in underserved populations where it is difficult due to lack of matched donors, and allow co-transplantation of blood stem cells to build tolerance for solid tissue grafts. In sum, our findings will help overcome obstacles to the effective treatment of diseases requiring hematopoietic cell transplantation. |
| **Funds Requested** | $1,529,743 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 75

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

| | |
|---|---|
| **Mean** | 74 |
| **Median** | 75 |
| **Standard Deviation** | 8 |
| **Highest** | 86 |
| **Lowest** | 50 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 2 |
| **(1-84): Not recommended for funding** | 13 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 13<br>**No:** 1 | • The project addresses the bottleneck of replicating certain key steps in the developmental pathway leading from pluripotent stem cells (PSC) to hematopoietic stem cells (HSC) that have not been adequately mimicked in cell culture to date. This, in turn, would resolve bottlenecks in |

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| | multiple experimental systems and in development of novel stem cell-based regenerative and genetic therapies.<br>● Successful production of human HSC from PSC would be transformative for research in stem cell biology relative to all blood cell types, both normal and in disease. This has been a holy grail of stem cell biology and regenerative medicine for decades.<br>● Accomplishment of the goals of the proposal would have tremendous impact on scientific knowledge and also huge medical implications. It would facilitate a myriad of cell and gene therapy programs. Providing donor HSC enabling hematopoietic stem cell transplantation for any recipient would be just the tip of the translational iceberg. Important applications would transform hematology, immunology, oncology (at least leukemias) and potentially other fields.<br>● The ability to make HSCs from patient-derived iPSCs would open up many opportunities for cell and gene therapy. HLA-matched donors are often difficult to find especially in underrepresented populations and individuals from admixed ancestry.<br>● After decades of work, we still can't make HSCs from iPSCs.<br>● The applicant does not sufficiently acknowledge the current state of the field to allow a detailed evaluation of the novelty of their approach |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 10<br>**No:** 4 | ● There is no doubt that, in principle, PSCs should be able to serve as a source of HSC. The applicant has assembled a robust, international team of collaborators who bring specialize d knowledge, skills, and tools to understanding the developmental pathway from PSC to HSC. Leveraging this knowledge and skills has real potential to crack the practical problem of replicating that pathway from pluripotent SC in the laboratory.<br>● Strong step-by-step approach built on "key checkpoints" in developmental biology of HSCs from early mesoderm forward in organisms from zebra fish to mice and humans.<br>● The specific focus on more sophisticated building in of Notch and WNT signaling to differentiation protocols that already have generated some HSC from PSC seems well justified. The proposal addresses finding the optimal sequence, timing, and presentation (e.g., juxtacrine) for certain developmental signals, and sourcing key factors (e.g., WNT 9A) that have not previously been available for HSC differentiation protocols.<br>● Evidence is presented that key developmental factors, specific NOTCH and WNT family members, may have been limiting in previous studies, in part because of appropriate forms of the factors were not available. WNT 9a is one specific example - acting on complex receptor system of Fzd9 plus EGF receptor as a cofactor. Project aims to overcome these barriers.<br>● The data show some initial success in generating HSC from PSC. The rationale and preliminary data for the application of NOTCH and WNT family members are compelling and supportive of the project.<br>● The differentiation scheme and possible manipulation including functional assessment (Aim 1), specific manipulation of WNT with FZD9-EGFR signaling (Aim 2) and comparison of derived versus actual HSPCs (Aim 3) is reasonable.<br>● The data support the specific questions.<br>● Concern was expressed during review that application does not reference previous work in the field in sufficient detail, including efforts that have used NOTCH and WNT family members. This leaves some uncertainty about which elements of the proposal are fundamentally different from prior work in a field in which a number of strong groups have failed previously to generate HSCs efficiently from pluripotent stem cells.<br>● Major literature is not referenced, making it very much unclear how these defined changes compared to years of prior work on Notch and WNT.<br>● It is disappointing that the authors do not reference or discuss any of the other differentiation methods that have been tried by other groups that include Notch and Wnt signaling.<br>● It's very unclear whether these rather simple adjustments are sufficient for the proposed breakthrough.<br>● It is not clear that manipulations in Notch and Wnt Signaling will be enough to create a breakthrough that the field has been trying to achieve for decades.<br>● Relevance to human biology and disease cannot be overstated. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 9<br>**No:** 5 | ● Within its design, the specific experiments are reasonable and logic and will provide meaningful though more incrementally appearing results.<br>● Careful systematic approach based on developmental biology is a major strength. The project is laid out in exquisite step-by-step detail with clear milestones and metrics for success.<br>● Final comparison of HSC derived from PSCs vs authentic HSC from early in human development seems an important strength.<br>● "Deep dive" into WNT protein production and WNT mimetics seems wise, as this is a difficult family of factors to produce consistently in active form as lipid-modified recombinant proteins. |

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| | • Similarly, intense focus on details of NOTCH signaling seems highly apt for this problem.<br>• The project is laid out clearly for the expected 3 year donation. After decades of frustration, the timeline demonstrates both understanding of the level of detail that must be accomplished and the urgency of achieving the goal.<br>• Pitfalls are identified, especially with respect to provision of the WNT9A protein, as WNT proteins are notoriously difficult to produce in active form in part because of their lipid modification. Alternatives are presented with supporting evidence of feasibility.<br>• Budget is justified.<br>• Question remains of which key steps are fundamentally distinct from what already has been incorporated into previous attempts to generate HSCs carried out by other strong teams.<br>• Alternatives focus mostly on technical aspects.<br>• It should be made clearer what this group is specifically doing in terms of novelty in using Notch and Wnt Signaling to guide PSC-HSC differentiation. Others have tried.<br>• Aim #3 can't be done if Aim #1 and #2 aren't successful. This seems like a big problem given that the field has been trying to do this for decades and have yet to be successful.<br>• More data in human iPSCs should have been presented. The figure showing zebrafish development/signaling is not needed. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 13<br>**No:** 1 | • The project appears feasible. While there is no guarantee of success, it strikes this reviewer as an extremely well thought-out "shot on goal" to achieve what would be an extraordinarily valuable outcome.<br>• The PI is deeply experienced and has a strong track record. They have assembled a stellar international team that already collaborates well and brings a high level of expertise in each of the key areas that present potential roadblocks to success.<br>• The applicants make a good case that that they have identified some important developmental steps that have not been adequately mimicked in prior protocols. They also have paid close attention to sourcing some key reagents that may not have been available to other groups that tried previously to generate HSCs from pluripotent stem cells.<br>• Resources look excellent, and are well matched in the international labs represented on the project team.<br>• Proposed experiments can be conducted in the timeline.<br>• The experiments are feasible, the overall outcome on deriving true HSCs is less clear<br>• There is no guarantee that a complex, multi-step protocol can be developed, especially in light of many previous failures.<br>• While many iPS lines are available, ostensibly to be sure a successful protocol can be replicated across ethnic groups (something of which I personally have little doubt - as key developmental pathways have tended to be consistent for the broad human population), it is not obvious that focusing initially rather narrowly on "tried and true" H1 and H9 lines is the optimal approach. Is there possibly a way to identify upfront iPS lines with greatest propensity for successful HSC differentiation? |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 12<br>**No:** 2 | • Applicants note explicitly that there is a significant disparity in availability of matched HSC donors for transplantation in white (high) vs Hispanic (low) vs non-Hispanic Black (very low) populations. Effort to test the protocol across ethnic groups is crucial, even if it seems very likely that a successful protocol will work for all groups. An appropriate iPS cell collection is available.<br>• Successful production of HSC from PSC potentially would overcome current major racial/ethnicity disparities in the availability of HSC for transplantation in a range of clinical applications.<br>• HLA-matched donors harder to find in underrepresented populations and admixed populations.<br>• It is extremely likely that the method would enable production of HSC representing the full range of the human population. Banked PSC are available to test that thesis and enable production of a diverse range of HSC.<br>• HLA matching is harder for underrepresented groups. Being able to make PSC-derived HSCs would be helpful to these groups.<br>• If successful in H1 and H9 cells, the investigators will use iPSCORE lines from various ethnicities to validate the universality of the approach.<br>• The PI presents a good case showing their own interest and commitment to DEI during their career.<br>• The PI was the Divisional Diversity Officer for the Minority Outreach and Recruitment Program at their institution for many years and has helped shaped several initiatives/programs to provide opportunities to underrepresented individuals. |

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| | ● The applicant has been deeply involved in development of DEI within their home institution and in the context of the project.<br>● While the lab efforts are clear, details for the actual project are very limited.<br>● It would be nice to include other lines than H1 and H9 in the primary work to have a broader representation of MHC haplotypes. |

| Application # | DISC0-15793 |
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| **Title**<br>(as written by the applicant) | Development of a humanized swine model for translational studies of CNS-targeting cell therapies |
| **Research Objective**<br>(as written by the applicant) | We aim to develop a humanized, immunodeficient swine model for the study of our iPSC derived microglia replacement therapy and CNS therapeutics in general. |
| **Impact**<br>(as written by the applicant) | The proposed work will allow advanced studies of our lead product for the treatment of ALSP and support future work for therapies designed to treat neurological disease and damage. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Select xenopermissive pig model.<br>• Select strategy of hCSF1 delivery to humanize pig.<br>• Demonstrate proof-of-concept translational utility of xenocompatible, humanized pig model. |
| **Statement of Benefit to California**<br>(as written by the applicant) | [The applicant organization] is a California-based company, as are two of the primary contractors. Funding of this work will provide direct economic benefits to these companies and their employees, in addition to providing needed therapies to California citizens suffering from neurological damage and disease. |
| **Funds Requested** | $999,124 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 75

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

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| **Mean** | 74 |
| **Median** | 75 |
| **Standard Deviation** | 2 |
| **Highest** | 75 |
| **Lowest** | 70 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 15 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
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| **Yes:** 8<br>**No:** 6 | • This application will address the safety of transplantation of human microglia derived from iPSCs for future treatment of adult-onset leukoencephalopathy with axonal spheroids and pigmented glia (ALSP) into a pig model.<br>• The aim is to develop a humanized, immunodeficient swine model and demonstrate its utility for translational studies with ALSP as a model.<br>• The goal is to establish a humanized, immunodeficient pig model that could be used for translational studies with a focus on ALSP, which is a severe disease with no cure.<br>• There is a great need for large animal models in stem cell research and this is addressed here.<br>• The project has relevance for ALSP but can be much more broadly applied and have broad impact on CNS-targeting therapies. |

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| | • In addition, applicants argue that the work would provide a good model to analyze migration patterns of engrafted cells in a bigger brain. It is generally true that limited scaling in the context of cell transplantation in mice, particularly in the CNS, could be a significant bottleneck to advance therapies, however whether such a model would be actually required by the FDA to move a transplantation approach forward is not discussed.<br>• Pigs are a good and economical large animal model for surgical engraftment of human cells into a brain and to optimize cell dosage and cell delivery strategies of a candidate regenerative cell therapy. Immune-deficient, homozygous knockout pigs are available from the National Swine Resource and Research Center (NSRRC). |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 10<br>**No:** 4 | • ALSP is caused by mutations in the colony stimulating factor-1 receptor (CSF1R) microglial gene. This leads to dysfunction of microglia, thus, replacement of these cells should provide benefit.<br>• The critical preclinical data showing that humanized microglia impact the pathology of ALSP are not discussed or provided. It would have been necessary to show that the cellular component that is developed under a new CIRM grant (beginning in 2024) has clinically relevant efficacy in an existing mouse model.<br>• The vision is large animal studies with which to evaluate safety, toxicity, and preliminary efficacy. But is this necessary? Porcine models may be more cost effective than other large animal models but not cheap. Some more consideration of when pigs vs. rodents are most suitable would be good.<br>• Most preliminary concepts were developed in the mouse. Human iPSC derived microglia induction was done in the past, but is insufficiently described here. There is a lack of preliminary data demonstrating the feasibility of producing pigs expressing human CSF1. Since the survival of human microglia implanted into pig brains is critical, the proposed studies are not feasible without transgenic pigs.<br>• Even if one believes that the approach would work, the pig model that will be developed will require immune suppression or will use a genetic immune compromised animal. Therefore it seems difficult to quantify and understand the role of humanized transplanted microglia to any pathology that also involves endogenous inflammation. This is especially important in light of data showing that CSF1R is also expressed on other myeloid cells.<br>• A suitable strategy in ALSP might involve removal of dysfunctional microglia followed by replacement. This is not discussed.<br>• The project is based on sound rationale with some minor deviations.<br>• Limited preliminary data. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 5<br>**No:** 9 | • Since preliminary results have already demonstrated that endogenous pig CSF1 does not support the growth of human iPS derived microglia, Aim 1 will unlikely produce any results on engraftment and other outcomes.<br>• Aim 1: The applicant discusses that in the absence of additional human CSF1 they expect little microglia establishment in the pig brain. Based on this, it is thus not clear how they will measure the inflammatory response. Aim 1 thus seems to be reduced to performing surgical procedures using existing protocols.<br>• The expected outcome of Aim 2 is not clear. The applicant does not provide any data that show efficacy of microglia on pathology in ALSP. Therefore it is not discussed how much microglia engraftment and migration is actually needed and the applicant also does not comment on whether too much microglia or too extended migration could even be detrimental.<br>• Transduction of pig brains with human CSF1 is unlikely to produce desirable high level of transgene expression required for cell survival. This notion is supported by the preliminary results where heterozygous expression of hCSF1 in mice supported microglia engraftment and survival at a lesser extent than homozygous expression.<br>• The purpose and rationale of transplanting glioblastoma cells into the pig model is not clear. Glioblastoma models in pigs have already been established and while some studies in mice suggest that microglia cells could lead to tumor progression, this is not tested here. The analysis of the role of human microglia in a context of a pig cancer model seems to be an independent project that would need to be supported by robust preliminary data.<br>• Unclear what is the purpose of the neuroinflammatory response tests to toxic glioblastoma cells in Aim 1? As the applicant points, glioblastoma cells may cause animal death and these experiments may not produce any expected results. I don't see any point to conduct Aim 1.<br>• The rationale for the Aim 2 is weak. If human CSF1 is critical for the survival of human microglia in pig brains, it will be more effective and feasible to produce such pigs by gene editing. |

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| | • The applicant proposes to use transplantation into the neonatal brains. As microglia plays different roles during development and during inflammation, it is not clear what endpoint analysis will be used to determine that the transplanted microglia acts "normally".<br>• The engraftment of microglia into newborn pig brains is a limitation since ALSP is an adult-onset disease and clinical applications of future stem cell replacement therapy will be on adult patients. I understand the choice of engraftment of iMGL into newborn pigs is driven by the fact that these immunodeficient pigs do not survive long in conventional housing.<br>• The use of piglets is not well justified. Their immune system is not same as adults so the translational relevance is unclear.<br>• It would be critical to provide clean animal containment facilities so that cell transplantation studies can be performed in adult pigs with brains sufficiently developed in terms of anatomical structure, physiological function, and immune response characteristics.<br>• Aim 3 combines Aims 1 and 2 and assesses engraftment. The goal of Aim 3 remains unclear as the applicant has not discussed to what extent microglia will be needed to modulate ALSP pathology.<br>• Aim 3 is highly depended on Aims 1 and 2 and since the success of these aims is uncertain the Aim 3 may never proceed.<br>• There is no information on iPSCs and their differentiation to microglia. How they will be quality controlled? How many cells injected? And in what area of the brain? |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 9<br>**No:** 5 | • The project is feasible but has a tight timeline.<br>• The research grant funded in 2021 to develop microglia cell therapy for ALSP has not yielded any publications and it is not clear whether the approach yielded positive results.<br>• Engraftment, survival and migration of human microglia in Aim 1 is uncertain since endogenous pig CSF1 does not support the growth of human cells.<br>• Contract research organizations will provide equipment, veterinary support and holding rooms for large animals, such as pigs. What the applicant organization provides is unclear. Unclear if and what IACUC oversight will be provided to support the project. No justification of humane use of pigs is provided. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 12<br>**No:** 2 | • The applicant describes the effort to generate iPSC derived microglia from diverse patient pools, and cells will be transplanted in pigs of both sexes. It is reasonable to suggest that not only the sex of the graft but also the sex of the host might influence the outcome.<br>• The applicants describe efforts to engage with the ALSP community and have made progress in enrolling patients from different backgrounds to generate cellular components.<br>• They have also implemented other therapies with DEI in mind.<br>• iPSCs from 4 ALSP patients including one African-American is mentioned. However, it is unclear if and how many of these cell lines will used in this study. Other aspects of DEI including race, gender and age are considered not applicable to the proposed work. |

| Application # | DISC0-15943 |
|---|---|
| **Title**<br>(as written by the applicant) | Investigating epigenetic reprogramming and cell extrinsic signaling events in the specification and maturation of human primordial germ cells |
| **Research Objective**<br>(as written by the applicant) | This project will identify the progenitors that get specified to primordial germ cells in humans and map the molecular reprogramming events that are key to specify and mature this cell state. |
| **Impact**<br>(as written by the applicant) | Due to a lack of access to early human embryos, the project will employ 3D gastruloids/extended PGCLC cultures to impact our understanding of the specification and maturation of primordial germ cells. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Identify the progenitors that specify human primordial germ cell-like cells (hPGCLCs)<br>• Elucidate epigenetic and spatial determinants of hPGCLC specification<br>• Identify factors that drive hPGCLC demethylation and maturation |
| **Statement of Benefit to California**<br>(as written by the applicant) | Primordial germ cells (PGC) are the founder cells that differentiate to sperm or egg, and dysregulation in this germline development results in infertility, a disorder that affects 10% of adults. Despite its significance, our understanding of PGC specification and maturation is obscured by a lack of access to human embryos. This project will gain insights into these processes, thereby enabling improved strategies for in vitro gametogenesis, disease modeling and identification of drug targets. |
| **Funds Requested** | $1,492,031 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 75

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

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| **Mean** | 74 |
| **Median** | 75 |
| **Standard Deviation** | 3 |
| **Highest** | 80 |
| **Lowest** | 70 |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 14 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
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| **Yes:** 11<br>**No:** 3 | • The project aims to fill a significant knowledge gap in understanding the biology of human primordial germ cells (hPGCLCs), which is crucial for advancing human biology and disease treatment, particularly in the context of infertility.<br>• The project addresses the origins of germ cells in the human embryo and the epigenetic processes that need to occur to set aside the germ line. This is not understood due to the inaccessibility of the human embryo at this stage. This work will inform infertility-male and |

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| | female- and effects of aging on fertility. The project has the potential to enhance the formation of functional gametes from human pluripotent stem cells for reproductive purposes.<br>● Germ cells are from a biological and practical point of view tremendously important. Little is currently known about their specification and emergence in human embryos.<br>● The gastruloid model may be too simple, and resubmission comments have not sufficiently addressed this. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 8<br>**No:** 6 | ● While the proposal argues for the biological relevance of the 3D gastruloid model to early post-implantation human embryos, concerns may arise regarding the direct applicability and comparability of these models to actual human embryonic development due to inherent limitations in mimicking complex in vivo conditions.<br>● The project is based on a stem cell derived gastruloid model developed in the lab of co-investigators and modified here to show that primordial germ cell precursors can arise in the structures. There are some concerns that this model is not well-justified in terms of similarity to intact embryo.<br>● A lentivirus tagging system to follow lineages with barcodes will be used; the applicant has a collaborator for this. This looks feasible. If not, they may move to a different genetic tagging method. This is an important aim as relying on pseudotime trajectories to identify lineage precursors is not sufficient. The PI recognizes this and will focus on direct lineage analysis; this is very important.<br>● The technical tour de force is too much for the simple model.<br>Using the model with a effective genetic (static or evolving) barcode plus validation would be already complicated enough.<br>● The suggested experiments are way too many and complex, with little convincing data or examples of how trees will be built and what will be done with the large amount of data. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 8<br>**No:** 6 | ● Aims are clear, technologies in place and interpretation of likely results are well described. The timeline is appropriate to complete experiments proposed and publish them.<br>● The application is much improved since previous submission. The applicant has a paper under review at a high impact journal on the spatially resolved single cell transcriptomics and epigenomics used here and posted a preprint paper on the organoid model.<br>● The applicant has the long-term culture system of PGCLCs working and has data suggesting two groups of cells. At least two candidate genes have been found, are upregulated and are known to be important in mouse PGC maturation. This suggests the screen will work.<br>● The development of spatial single-cell multiomics technology was initially considered outside the proposal's scope, indicating a potential gap in integrating cutting-edge spatial transcriptomics methods to enhance the understanding of cell extrinsic signaling events. Although the proposal outlines plans for epigenomic analysis, including DNA accessibility, methylation, and histone marks, the complexity and technical challenges associated with these analyses could pose significant hurdles.<br>● All aims lack sufficient analysis plans. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 12<br>**No:** 2 | ● The PI and the research team are highly qualified, with a track record of relevant publications and experience in the field of developmental biology and genomics.<br>● The PI has a strong background in multiomics. The PI has developed methods to quantify epigenetic inheritance at single cell level and to perform endogenous label-free lineage tracing. Collaborators are appropriate and enhance the team.<br>● Cell systems are in place, the technologies are all in hand and core facilities are available to undertake genomic analysis. Data analysis and microscopy tools are within the expertise of the team.<br>● The proposal acknowledges the necessity of careful experiments for lineage tracing, which involves technical challenges such as infecting gastruloids with lentiviral libraries and computational reconstruction of lineage trees, potentially complicating the identification of hPGCLC progenitors.<br>● Yes, the data data collection is feasible. Identification of true progenitors may be feasible.<br>● Robustness of data is a concern and overambitious to the point of not being feasible. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 14<br>**No:** | ● The proposal outlines efforts to involve underrepresented minority (URM) students in research and educational activities, but the effectiveness and impact of these initiatives on promoting DEI in STEM fields require further elaboration and evidence of outcomes. |

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| 0 | • Age and sex will be covered by use of iPS cells from different ages. Only white and Asian cell lines are available. At this early stage, the issue of any ethnicity differences is not very relevant and it will not be feasible to study so many different cell lines.<br>• DEI principles are at least partially addressed, though the cell lines only capture some diversity. |

| Application # | DISC0-15890 |
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| **Title** (as written by the applicant) | Immune exhaustion as a mediator of hepatic stem cell expansion in pediatric acute liver failure |
| **Research Objective** (as written by the applicant) | Defining the role of liver progenitor/stem cells in mediating liver regeneration during pediatric acute liver failure (PALF) |
| **Impact** (as written by the applicant) | This project will advance our understanding stem cells by studying liver progenitor cells in PALF, including how different immune subsets mediate liver injury and capacity for regeneration. |
| **Major Proposed Activities** (as written by the applicant) | • Define the phenotype, trajectory, and spatial relationships between exhaustion phenotype immune subpopulations and proliferating hepatic progenitor cells during PALF.<br>• Establish key intrahepatic liver progenitor cell and immune cell types associated with liver regeneration and clinical recovery during PALF using predictive models.<br>• Define the phenotype and functional status of peripheral immune cells and plasma proteomic profile in PALF to establish features associated with liver regeneration and clinical recovery.<br>• Develop an ex vivo, patient derived liver organoid model of PALF to enable investigation of therapeutic targets with the potential to promote liver progenitor cell expansion and differentiation. |
| **Statement of Benefit to California** (as written by the applicant) | This project will benefit California and its citizens by advancing our understanding of PALF, a condition that affects previously healthy children. PALF is a very serious acute illness that carries a significant chance of needing an emergency liver transplant or dying. Our project will help us understand how the liver can repair itself, enable discovery of biomarkers of liver regeneration during PALF, and result in a new model of PALF so we can test new drugs to promote liver recovery. |
| **Funds Requested** | $1,509,999 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 75

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

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| **Mean** | 73 |
| **Median** | 75 |
| **Standard Deviation** | 2 |
| **Highest** | 75 |
| **Lowest** | 70 |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 14 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
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| **Yes:** 11 **No:** 2 | • Pediatric acute liver failure (PALF) is a complex, devastating illness that affects healthy children and is associated with high mortality. PALF causes hepatic necrosis with liver dysfunction. There is no test that can establish clinical trajectory or inform the decision to proceed with transplantation or survival with native liver (SNL).<br>• Evolution of PALF is difficult to predict and alternative treatments are needed since only a limited number of patients can benefit from liver transplant.<br>• There is no test to predict the course of disease. There are no suitable animal models or in vitro models available to adequately mimic the conditions surrounding the human immune response in PALF.<br>• The aim is to examine how the immune system interacts with and influences hepatic progenitor cell expansion and regeneration during PALF. The long-term goal is to develop predictive biomarkers of liver progenitor cell expansion to identify patients with capacity for SNL following PALF.<br>• The main objective is to understand interplay between liver progenitor cells (or stem cells) and immune cells in the context of PALF. PALF is certainly an important health care challenge and the clinical need is real. However, little information is provided about incidence, available therapy, etc.<br>• The project addresses a gap in our knowledge about PALF, but is largely about finding immune and non-immune cell phenotypic correlates of disease progression. The planned program does not address the etiology of PALF. The major outcomes of the project would largely have application to diagnostics.<br>• In theory, correlates found could suggest mechanisms of disease and therapy, but there is no understanding of the initiating etiology, Finding that T cells are implicated does not really address cause without information on the specificity of the cells.<br>• The investigators should decide whether they want to pursue largely biomarker discovery--and what the implications of that would be--OR whether they want to go after etiology, in which case Aim 3 needs to be improved and the immunology significantly more in depth.<br>• The impact part could be be better articulated and presented. The application seems rushed. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 5 **No:** 8 | • There is no test to predict course of disease. There are no suitable animal models or in vitro models available to mimic the conditions surrounding the human immune response in PALF. Thus, there is a critical need to understand the underlying disease process, ability of hepatic progenitor cells to promote regeneration, identify biomarkers of clinical trajectory, and create a pre-clinical model to test strategies to enable liver regeneration and survival with native liver.<br>• It's important to address a major bottleneck, that the lack of any prior data evaluating the regenerative capacity of liver progenitor cells during PALF. However, the focus of this biology grant is not clear, and whether there is any mechanism related to liver stem cell dysfunction in the disease would be studied.<br>• The role of the immune system in PLAF is important but the key is to define if it drives the disease or is simply a consequence of the massive inflammation provoked by cell death of hepatocytes.<br>• Understanding cross talk between immune system and regenerative process is definitely interesting. However, there are already studies showing that TNFa and other pro-inflammatory cytokines can induce proliferation of hepatocytes after injury.<br>• The definition of liver stem cells used by the applicants does not exist in human. Their knowledge is based on studies performed on mouse models with different type of injuries which are not related to human disease. The existence of a stem cell population is simply unproven in human. Furthermore, it is well known that proliferation of hepatocytes is the main mechanisms of regeneration in acute liver failure.<br>• This proposal is not a stem cell grant. The goal of the applicant is to identify new immune biomarkers to predict disease progression.<br>• The rationale is strong in one sense and weak in another. Strong in the sense that it seems likely that biomarkers could be found for PALF. Weak in the sense that the search by phenotyping is unlikely to reveal much about mechanism. Will how the organoids be validated as a model of disease? |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 6 **No:** 7 | • Patient cohorts are well detailed. Based on preliminary data, they hypothesize that immune exhaustion mediates liver injury and the regenerative capacity of hepatic progenitor cells in PALF.<br>• The project is divided into 3 Aims. Aim 1 will define the interplay between immune cells and proliferation of hepatic stem cells during PLAF. Multiplex immunofluorescence (IF) is definitely interesting. However, the selection of the hepatic stem cell marker is problematic since these proteins will be expressed in cholangiocytes. Why not perform single nuclei RNA-Seq and then |

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| | multiplex IF? This will allow to go beyond the simple histopathology which have been so confusing.<br>● Aim 2 will study the phenotype and functional status of peripheral immune cells and plasma proteomic profile in PALF using a proteomic approach. The main goal is to identify additional predictive markers. This could generate interesting biomarkers but those are always very challenging to validate. Also, there is no link with Aim 1.<br>● Aim 3 will develop a platform to study interplays between immune cells and liver cells in vitro using co-culture organoids. This part is very problematic. First, there is no evidence that liver organoids contain any stem cells. They are only cholangiocytes. Furthermore, they will obtain these organoids from extremely damaged liver which have failed to regenerate. This part will need much more work.<br>● There is little link between each part. Ideally, Each part should be complementary and inform each other. This is not clearly explained.<br>● Aim 1 is straightforward. Aim 2 could easily fail if such correlates are not detectable in the blood and Aim 3 is poorly defined in terms of how the model itself will be validated as a proper recapitulation of the PALF phenotype.<br>● Pitfalls do not address the concerns raised in 3a.<br>● The time line is appropriate. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 9 **No:** 4 | ● The analysis could be completed in the proposed timeline. For the proposed work, the team is qualified. The environment is ideal for the studies as the applicant organization is a major center for these surgeries.<br>● The PI is a transplant-surgeon scientist. The team consists of multiple clinician-scientists related the liver diseases and expert in pediatric liver pathology.<br>The teams have expertise for immunology,' omic' data analysis liver physiology and clinical hepatology.<br>● Aims 1 and 2 look feasible.<br>● The lead applicant has a very strong background in immunology and the identification of markers to predict disease progression. However, their experience with mechanistic studies or in vitro models is more limited. The co-applicant could bring this expertise, but they don't seem to have any experience with liver organoids hence problems in Aim 3. Otherwise, this is a very strong group with a lot of clinical expertise. Thus, there is no doubt that they will deliver important data.<br>● The preliminary data are interesting. However, it is difficult to understand how they support the leading hypothesis of the program. For example, figure 4 shows a decrease in hepatocyte markers (which make sense since these cells are massively dying). However, these results don't support the activation of a stem cell population. Figure 7 looks random, there is no information about marker. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 12 **No:** 1 | ● This aspect is well described and well addressed by the applicants.<br>● The plan accommodates these factors in a reasonable manner. The applicant described an excellent history of these activities.<br>● Yes, they'll recruit highly diverse, and often underserved patient population from the local catchment area to study how biological sex and social determinants of health impact liver-related outcomes in children with PALF. |

| Application # | DISC0-15713 |
|---|---|
| **Title** (as written by the applicant) | An iPSC-derived approach to studying how NOD2 affects Paneth cells in Crohn's disease |
| **Research Objective** (as written by the applicant) | This proposal aims to develop a human iPSC-derived approach to determine how genetic variants in NOD2 affects the functioning of Paneth cells in Crohn's disease |
| **Impact** (as written by the applicant) | If successful, this would allow a study to determine how NOD2 genetic variants intrinsically affect Paneth cells, or affect their response to inflammation or microbes. |
| **Major Proposed Activities** (as written by the applicant) | • Selection of Crohn's disease patients with the L1007fs mutation and subsequent generation of their iPSCs<br>• Genetic editing of each patients iPSCs to generate corresponding isogenic iPSC lines that correct the mutation<br>• Directing all iPSC lines to intestinal organoids and directing towards a Paneth cell fate. Then examining how the L1007fs mutation intrinsically affects Paneth cells or their response to inflammation<br>• Examining how the L1007fs mutation affects Paneth cell responses to microbial ligands, either under control or inflamed conditions<br>• Examining how the L1007fs mutation affects Paneth cell responses to live microbes under control or inflamed conditions |
| **Statement of Benefit to California** (as written by the applicant) | Crohn's disease affects 1,000,000 individuals in the US, and as the largest state in the country, will undoubtedly have a large population of such patients. We will be investigating how one mutation, which is associated with a more severe form of the disease, affects a cell type heavily implicated in the disease and thus may one day lead to new avenues of treatments for such patients. Economically, services from Californian businesses will be required to successfully complete this proposal. |
| **Funds Requested** | $1,503,866 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 75

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

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| **Mean** | 72 |
| **Median** | 75 |
| **Standard Deviation** | 5 |
| **Highest** | 80 |
| **Lowest** | 65 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 15 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
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| **Yes:** 10 **No:** 4 | • The goal of this proposal is understand mechanisms behind Crohn's disease (CD) which affects up to 1 million individuals. It has major consequences on quality of life and there is currently no treatment.<br>• CD is supposed to be induced by abnormal cross talk between the immune system and the microbiome and variants in the bacterial sensor NOD2 have a strong association with the disease. NOD2 is highly expressed in Paneth cells. However, its function in these cells is unknown.<br>• The applicant proposes to use a new model of human intestinal organoids derived from hPSCs to study the function of NOD2 and the impact of genetic variants on NOD2 function in Paneth cells. This will provide important information necessary to develop new treatment blocking CD progression.<br>• It has not been possibleto define the functional impact of genetic variants in NOD2 on Paneth cell function so far since protocols available only generate limited number of Paneth cells.<br>• Resulting information could help to personalize treatment based on NOD2 genetic variants.<br>• This study will assess if NOD2/L1007fs variant is implicated in CD using iPSC and human intestinal organdies (HIO) technology to model in vitro CD conditions. Currently, the causative link between NOD2 genetic variants and CD is uncertain, this application will determine direct mechanisms by which NOD2/L1007fs variant is implicated in CD.<br>• In vivo, mechanisms of CD in adult patients are very complex. The application does provide any assurance that this simple HIO model correctly mimics the complex adult gastrointestinal system and can be used to study the CD.<br>• The proposal is not novel- purely descriptive and limited in scope. Does not go beyond cell characterization<br>• The proposal will only look at the most severe NOD2 mutation, L1007fs, and how it affects human Paneth cell function. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 12 **No:** 2 | • The scientific rationale is very strong as genetic variant in NOD2 are strongly associated with CD.<br>• The applicant will use their iPSC Core to generate four lines (2M and 2F).<br>• NOD2 variants have been linked to CD and this proposal will examine if the L1007fs mutation affects Paneth cell function and thus may cause CD.<br>• The rationale is that stem cell-based in vitro organoid approach will eliminate the need to obtain invasive biopsies from CD patients<br>• NOD2 is also expressed in immune cells and thus the role of NOD2 in Paneth cells is not established. While this is the goal of this application, the importance of immune cells is lacking.<br>• It remains unclear if iPSC derived organoids accurate represent complex in vivo gastrointestinal system with all mature cell types, tissue complexity and environment |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 10 **No:** 4 | • This plan seems rational and well organised.<br>• The project is divided into 3 Aims. Aim 1 will define the impact of the L1007fs mutation on Paneth cell development, number and function under basal or inflammatory conditions. Aims 2 and Aim 3 will assess how this mutation affects the capacity of Paneth cells to respond to microbial ligand and microbial community respectively.<br>• The experimental plan is well designed. Aims are focused on different aspects of Paneth cell function affected by the homozygous L1007fs variant vs. isogenic CRISPS corrected controls<br>• The focus on L1007fs could be better justified since this variant is extremely high in specific populations. It could have been interesting to include additional variants in a limited number of experiments. This would have derisked the project.<br>• The proposal will generate Paneth cells and characterize these cells. While creating a population of Paneth cells will be of use to the field, the proposal is VERY limited in scope.<br>• The cells will not be characterized at the molecular level.<br>• Use of lymphoblastoid cell lines (LCLs) as a surrogate for iPSC induction is a potential concern. LCLs are established by in vitro infection of resting B cells from peripheral blood with Epstein Barr Virus (EBV) bearing genetic, cytogenetic and phenotypic abnormalities of transformed cell lines. It would be more desirable to produce iPSCs from traditional fibroblasts or PBMC. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 12 **No:** 2 | • Proposed experiments can be accomplished in the proposed timeframe.<br>• The proposed experiments should not take the proposed three years.<br>• Proposed studies can be accomplished within 3 years. However, iPSCs lines are not available, likely causing delays. |

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| | • Preliminary data are convincing. They clearly demonstrate that the applicant can generate Paneth cells which are functional. Thus the platform necessary for this proposal is already available.<br>• hiPSC derived from lymphoblastoid cell lines can be difficult to differentiate. The method used to transform the original T or B cells can interfere with pluripotency and capacity of differentiation. It would have been useful to demonstrate that hiPSCs derived from LCL can indeed generate Paneth cells.<br>• The main risk is that NOD2 or NOAD2 variant has simply no function in Paneth cells. The applicants do mention this risk, but their back up plan is not really clear. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 4 **No:** 10 | • The proposal does describe the importance of genetic background and gender on disease. Thus, they plan to use female/male hiPSCs. However, there is little information about the genetic background of their hiPSC line or if they will consider this aspect in their analyses.<br>• No outreach is planned. There is no evidence that the group is working with partners in the community.<br>• It is not sufficient to note that there are issues in population biases and not address them.<br>• Need to try more to get African American samples. Justification that they don't have access to the samples isn't acceptable.<br>• Prevalence of CD in mostly European and Ashkenazi Jewish populations is noted, thus no information how patients will be selected is provided besides their male and female origin. |

| Application # | DISC0-15808 |
|---|---|
| **Title**<br>(as written by the applicant) | The influence of human neural stem cells on autoimmune and regenerative function in mouse models of multiple sclerosis (MS) |
| **Research Objective**<br>(as written by the applicant) | We will investigate the immunoregulatory influence of neural precursor cells (NPCs) on inflammation vs. remyelination in viral and autoimmune models of multiple sclerosis (MS). |
| **Impact**<br>(as written by the applicant) | There are currently no clinically approved treatments for progressive MS. We will determine if NPCs induce repair or instead influence autoimmune cells as a first step toward their use to treat MS. |
| **Major Proposed Activities**<br>(as written by the applicant) | • We will clarify the role of regulatory T cells (Tregs) in hNPC-induced remyelination and clinical recovery using mouse MS models.<br>• We will characterize a novel molecule expressed in Tregs that may be involved in maturation of oligodendrocyte precursor cells (OPCs) into functional oligodendrocytes.<br>• We will determine if Tregs that accumulate in the central nervous system following hNPC transplant promote neurological repair in viral and autoimmune mouse MS models affect microglial function.<br>• We will assess the impact of hNPC-induced Tregs on gene expression within the damaged CNS to determine how these immune cells affect repair and remyelination via microglia and oligodendrocytes.<br>• We will establish the influence of Tregs on microglial pro-inflammatory vs. remyelination gene expression in response to hNPC administration to mouse MS models.<br>• We will employ imaging mass cytometry to determine how hNPC-induced Tregs influence the pro-inflammatory vs. regenerative cellular topology within the CNS of MS mouse models. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Multiple sclerosis afflicts many Californians, a disease that typically presents in early adulthood. It is a highly debilitating disease for which there is currently no cure. While there are therapies that limit autoimmune damage, these therapies are ineffective for progressive forms of MS. We will characterize a population of anti-inflammatory T cells called Tregs induced by NPC transplantation that may facilitate the neurological repair and clinical recovery in progressive MS patients. |
| **Funds Requested** | $1,506,309 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 70

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

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| **Mean** | 71 |
| **Median** | 70 |
| **Standard Deviation** | 2 |
| **Highest** | 75 |
| **Lowest** | 70 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 15 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to

indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 7 **No:** 7 | • Multiple sclerosis (MS) is a severe disease that would benefit from newer treatments, in particular already progressed disease.<br>• New therapies are needed and a better understanding of how regulatory T cells and other immune cells in the central nervous system (CNS) influence remyelination and recovery induced by neural precursor cells (NPCs) in distinct animal models of multiple sclerosis (MS) will contribute to this.<br>• This project will explore the mechanisms whereby introducing hNPCs into mouse MS models cause activity of Treg cells (T suppressor cells) that result in remyelination. At present, these mechanisms are not well known, and this work might help fill in this knowledge gap.<br>• The project aims to develop a therapy for MS based on promoting remyelination via transplantation of NPCs.<br>• Past studies show that adding NSCs derived from iPSCs into mouse MS models can induce remyelination and suppress demyleination. If this can be advanced to a clinical use, then this might be helpful for MS.<br>• Could have implications also for other diseases like autoimmune encephalitis.<br>• Overall, this is a grant that focuses on NSC-induced local repair of myelin in the spinal cord, but the lack of a convincing and robust clinical-grade response is concerning. It is not clear if the effects observed by this group could be robust enough (or pervasive enough throughout the CNS) to yield clinically relevant responses.<br>• The effects of the injections are very limited and don't provide a strong translation case beyond slightly and locally improving aspects in the mouse model.<br>• Very localized response - not clear on the relevance. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 5 **No:** 9 | • The project is based on previous literature and follows a good rationale.<br>• An amplitude of preliminary data is given and this supports many aspects of the project.<br>• Detailed study of an observation (hNPC presented antigen -> Tregs) that has local and modest effects.<br>• Depletion of Tregs using a specific antibody negated the action of NSCs, thus showing that the effect is mediated by Tregs.<br>• Overall, the preliminary data documents a very localized remyelination response to nNSC injection. However, Fig. 1A shows clinical responses, but no corresponding histology more broadly throughout the CNS is presented in the preliminary data. This is a major concern, since if responses to NSCs are only very localized, then an effective treatment from this work may not be forthcoming. At present, the clinical impact of this work rests entirely upon Fig. 1A, which is data from ten years ago.<br>• hNSCs are introduced into the CNS by spinal injection, and are rejected within a few days (the recipient mice are not immunocompromised). However, this apparently induces a Treg response leading to remyelination mediated by oligodendrocytes. This seems to work best in the viral model as compared to the second model, but in the second model some remyelination is detected, though this is not sufficient to alleviate motor symptoms.<br>• Figure 2 shows that NSC demyelination is reduced in the spinal cord near the NSC injection site. There is some concern that this benefit is very localized, and it is not clear to what extent remyelination in the brain (sites distant from the injection) or even at more distant sites in the spinal cord has occurred. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 9 **No:** 5 | • Aim 1 will expand upon in vitro studies to study the action of hNPCs in vivo. In this aim, Tregs after NPC injection will be checked to see if they recognize CNS antigens present in the hNPCs. In addition, Tregs conditioned by NPCs (adoptive transfer) will be tested for their ability to act in their own right after transplantation.<br>• The project is well designed and appropriate methodology is used.<br>• The plan and timeline are well designed and the budget appropriately justified. Pitfalls are partially identified. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 13 | • Yes, the proposed aims are entirely logical and can be easily achieved in 3 years. |

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| **No:** 1 | • Two mouse MS models will be used. These are proven models and consist of one which results in demyelination and MS like presentations, and the second causes an auto-immune attack on myelin sheaths, which exhibit MS-like features.<br>• All resources are in place and the budget is appropriate.<br>• The team is well-composed but PIs have little time committed to project.<br>• The project is ambitious but feasible.<br>• Yes, but impact likely very limited. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 13<br>**No:** 1 | • The applicants note that MS is distributed across all ethnicities. However, there is a higher incidence of relapsing remitting MS (RRMS) in females, and this is taken into account. Both male and female mice will be used for the proposed work.<br>• Project is focused on mice and hence limited on its true DEI impact. |

| Application # | DISC0-15886 |
|---|---|
| **Title**<br>(as written by the applicant) | Evolutionarily Conserved Mechanisms that Control Stem Cell Aging and Rejuvenation |
| **Research Objective**<br>(as written by the applicant) | Elucidate the stepwise mechanisms underlying HSC and SSC aging and devise methods to reverse aging based on the successful rejuvenation model we discovered in a colonial chordate |
| **Impact**<br>(as written by the applicant) | Identifying the causal factors of HSC and SSC aging, uncovering additional molecular mechanisms of SC loss of function and evaluating a potential method for rejuvenating human SCs. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Elucidate the molecular underpinnings of Botryllus HSC and niche aging by comprehensively analyzing genetic, epigenetic and transcriptomic changes across the age continuum<br>• Multi-omic analysis of Botryllus blood stem cells and niche samples across ages after pulsatile electric current (PEC)<br>• Evaluate multi-omics of mouse and human HSC and SSC after in vitro PEC treatment Milestone: Identify post-PEC genetic, epigenetic and transcriptomic footprints of human HSC and SSC<br>• Study multi-omics and reconstitution profiles of PEC-treated mouse and human HSC after transplantation<br>Milestone: Identify regimens resulting in mouse and human in vivo HSC revitalization post PEC<br>• Study multi-omics and reconstitution profiles of PEC-treated mouse and human SSC after transplantation<br>Milestone: Identify regimens resulting in mouse and human in vivo SSC revitalization post PEC<br>• Employing the integrative Boolean Network Machine learning model to identify multi-omic drivers of stem cell aging and rejuvenation |
| **Statement of Benefit to California**<br>(as written by the applicant) | Age-related changes in HSCs are linked to increased susceptibility to infections, reduced vaccine efficacy, and leukemia. Our project aims to develop innovative strategies and treatments for these age-related diseases that benefit all ethnic groups that live in California. By targeting human SCs, we can advance the understanding of physiology and disease across various genetic backgrounds and ages, leading to new and more effective treatments. |
| **Funds Requested** | $1,141,490 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 70

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

| | |
|---|---|
| **Mean** | 71 |
| **Median** | 70 |
| **Standard Deviation** | 5 |
| **Highest** | 75 |
| **Lowest** | 60 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 15 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 12 **No:** 2 | • The aging of stem cells themselves is an interesting and relevant fundamental knowledge gap in eukaryotic biology in general. This group studies Botryllus (a tunicate) which comprises a novel model organism that has several features that make it a promising model in which to study stem compartment cell aging.<br>• This is a very new idea that may have unknown but potentially significant impacts on a variety of age-related diseases and perhaps aging itself.<br>• This is a very risky, but highly innovative and possibly high reward, proposal. Impacts from this work in the clinic are likely quite far down the road, but the idea and use of a non-standard model (Botryllus) could yield unanticipated but significant knowledge that would be hard to obtain with standard models such as mice only.<br>• The project would have been much stronger if it focused on tunicates. Only Aim 1 uses this very interesting model system. Humans age, and most people would rather not, so investigating aging is relevant.<br>• The team argues that the bottleneck to reversing aging is the lack of a model organism in which this area can be studied, but only Aim 1 uses Botryllus. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 7 **No:** 7 | • The applicants discovered that pulsed electrical current (PEC) reverses aging in Botryllus, but it's not clear the method similarly affects humans and mice (Aim 2 and 3).<br>• The hypothesis to be tested is that PEC causes a change in stem cells, resulting in a long-lasting, rejuvenating outcome. The hope is that PEC changes are preserved evolutionarily but there is no strong evidence that this is the case.<br>• The proposal rests on the notion that PEC treatment has a beneficial impact on stem cell compartments in Botryllus colonies. However, there are some concerns that the behavior of stem cells due to this treatment is not yet firmly established.<br>• The key data to suggest that PEC has impacts on stem cell compartments is presented in Figs. 3 and 4. However, these data are not completely compelling. Fig. 3 shows that PEC induces increased stem cell-derived Botryllus structures and overall survival. However, stem cells are not directly studied, so the interpretation that these observations are due to stem cell effects is somewhat dubious.<br>• Overall, the PEC data does not convincingly demonstrate that stem cell populations are responsible for the rejuvenation seen upon PEC treatment. This preliminary data would be much more convincing if stem cells were directly assessed.<br>• Fig. 4 is composed of transcriptional changes that occur upon PEC treatment. It is not clear from this data what is really going on with stem cells, even if many of the transcripts shown have some stem cell function. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 4 **No:** 10 | • Organisms show some signs of aging as they get older (Botryllus). Additional characterization is needed.<br>• Overall, it appears that more work is needed to identify, define, and work with Botryllus stem cells.<br>• Aims 1, 2, and 3 are all the same experiment but in different species. What is the rationale for performing these similar experiments instead of testing pathways identified in Aim 1?<br>• The significance for PEC upon Botryllus cells consists only of effects upon their transcription and proliferation, but the stem cell phenotype in said cells is rather speculative.<br>• It is not clear that machine learning will yield interpretable data in this system as the basic biology of cell types and their behaviors are not clearly defined. Also, the use of bulk RNAseq approaches is somewhat concerning, since there is likely to be RNA extracted from mixtures of cell types. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 12 | • This project may be moving too fast. The results of pulsed electrical current (PEC) in the Botryllus model system are not yet known. Hence the need for Aim 1, which will analyze this. |

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| | • What is the goal of Aim 1? Aim 1 will identify genes/pathways required for "anti-aging" using pulsed electrical current. None of this data will be used in Aims 2 and 3.<br>• Aim 2 hopes to apply PEC to mouse HSCs, to see if this might affect their competitive survival and differentiation upon transplantation, but this is risky, as the effects of PEC on mouse HSCs has not yet been explored.<br>• Aim 3 will look at effects of PEC on mouse and human skeletal stem cells. It is unclear if these are satellite cells.<br>• Effort does not appear to line up with the amount of work needed to complete the project (only Aim 1 is in the PI's area of expertise).<br>• Experiments can be performed, but justification of why they should be conducted is weak. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 12 | • The project will use stem cells from diverse races, genders and ages.<br>• Human HSCs sampled for the studies will include diverse races, genders and ages. |

| Application # | DISC0-15904 |
|---|---|
| **Title** (as written by the applicant) | Neural Stem Cell Aging and Neurodegeneration |
| **Research Objective** (as written by the applicant) | Dissecting the mechanism of neural stem cell (NSC) aging will advance our understanding of the biology of stem cells with implications in treating neurodegenerative diseases. |
| **Impact** (as written by the applicant) | Successful completion of the proposed studies could lead to the identification of a novel pathway regulating neural stem cell aging and a druggable target for treating neurodegeneration. |
| **Major Proposed Activities** (as written by the applicant) | • Determine the role of CD38 in NSC maintenance and cognition at young age.<br>• Determine the role of CD38 in NSC maintenance and cognition at old age and in Alzheimer's disease.<br>• Determine the mechanism by which NAD boosting improves aging- and Alzheimer's disease-associated cognitive decline. |
| **Statement of Benefit to California** (as written by the applicant) | The proposed research will advance our understanding of the biology of stem cells that is relevant to human biology and disease and provide Californian students a training opportunity for stem cell research. |
| **Funds Requested** | $1,610,930 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 70

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

| | |
|---|---|
| **Mean** | 71 |
| **Median** | 70 |
| **Standard Deviation** | 4 |
| **Highest** | 80 |
| **Lowest** | 65 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 15 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 8<br>**No:** 6 | • Aging results in a decline in numbers of neural stem cells (NSCs), neurogenesis, and cognitive function. NAD levels also decrease as a consequence of aging. NAD is a potent neuroprotective and anti-inflammatory molecule. The aim of the proposal is to understand if and how NAD metabolism regulates NSCs, neurogenesis, and cognition throughout the lifespan and during the development of Alzheimer's disease. The applicant also proposes to explore effective strategies for boosting NAD to improve NSC maintenance and cognition. |

| | |
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| | • The applicant will determine the roles of CD38, an NAD degrading enzyme that increases in expression during aging, and NAD boosting on NSC maintenance, neurogenesis, neural activity, and cognition.<br>• If successful, the study will provide mechanistic understanding of how mitochondria and NAD metabolism regulate stem cell maintenance and tissue homeostasis.<br>• The proposal is focused on how NAD metabolism affects aging- and Alzheimer's disease-associated changes in NSC maintenance and cognition. This could be of relevance for human disease, but there is no support for this in the application.<br>• This project does not address a bottleneck. Despite excellent evidence for CD38 involvement in aging and Alzheimer's, analogous or stronger data in is available for many other molecules that have failed to address cognitive decline in humans.<br>• The applicant has not used human omics data to prioritize genes or pathways to study. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 5 **No:** 9 | • NAD levels decrease as a consequence of aging, including in the human brain and cerebrospinal fluid (CSF), and NAD is a potent neuroprotective and anti-inflammatory molecule. How NAD metabolism regulates NSCs, neurogenesis, and cognition throughout the lifespan is largely unknown.<br>• The applicant has recently shown that SIRT7, a NAD-dependent enzyme, suppresses mitochondrial stress and prevents NSC aging and aging-associated neural hyperactivation and cognitive decline.<br>• The relative efficacy of NAD boosting via the NAD precursor nicotinamide riboside or inhibition of NAD degradation by CD38 has never been tested.<br>• Data supporting the hypothesis have recently been published and are provided in the project plan.<br>• The immunohistochemistry results shown are compelling for a role for CD38 in models of Alzheimer's disease.<br>• The proposal is based on the assumption that adult hippocampal neurogenesis a disease-relevant process in the human brain. This is highly debated in the field. While hippocampal neurogenesis occurs in humans, it is not yet clear whether the levels of adult neurogenesis at this site correlate with cognitive abilities in human.<br>• The narrow focus ignores major scientific developments of the last 20 years in understanding the complexity of cellular responses and the insufficiency of the one-gene-at-time approach.<br>• The limitations of C. elegans and mouse models of Alzheimer's disease are well known - effects in animals have repeatedly failed to translate to humans. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 6 **No:** 8 | • The experiments in mice are well planned.<br>• The project plan does not sufficiently validate findings from nonhuman models in human cells or tissues.<br>• The project plan is appropriate as a follow-up study to clarify the role of CD38 in NAD metabolism during aging.<br>• Technical plans for imaging and studies in cellular and animal models are adequate.<br>• There is a claim near the end of the proposal that the applicant will validate CD38 findings in NSCs of the dentate gyrus in older people and people with Alzheimer's disease. This should be the first activity, to determine whether CD38 is more relevant to Alzheimer's disease than many other molecules with strong supporting evidence.<br>• No. This same proposal could be written for any number of targets. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 12 **No:** 2 | • Most studies will be carried out in mouse models that are available, but depend on breeding a knock-out line with AD mice, which can be complicated.<br>• Conducting studies in aged mice is challenging, but the applicant's use of pharmacological knock down instead of transgenic mice may be suitable.<br>• The preliminary data showing that SIRT7 regulates NSC maintenance, neurogenesis, neural activity, and cognition have been recently published. The preliminary data for determining CD38's role in NSC maintenance and cognition in old mice were also shown.<br>• The preliminary data are a very strong demonstration of the two-photon microscopy the applicant plans to employ, and results on related disease models.<br>• The Principal Investigator is very well published, externally well-recognized within this topic, and (normalizing for career length) possibly one of the best people to carry out this project.<br>• The Principal Investigator is an expert in sirtuin biology, biology of aging, and stem cell biology.<br>• Some of the investigators on the applicant team have very low time commitment to the project. Most strikingly, 1.25% effort is not sufficient. |

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| | ● With the notable exception of claims for accurately representing the effects of CD3 on Alzheimer's disease, yes. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 13<br>**No:** 1 | ● The project outcomes extend or validate the applicability of regenerative medicine discoveries to underserved populations, including underserved racial/ethnic communities.<br>● The applicant has described prior efforts or proposed plans for outreach, partnership, or educational activities to inform the development of DEI within the research project.<br>● Both male and female mice will be examined in the proposed studies. The applicant collects human samples based on the available demographic information, such as gender and ethnicity/race. Samples from underserved racial/ethnic groups will be prioritized.<br>● For animal studies, the key is to test in both sexes. Exploring any influence/effect of race/ethnicity in animal studies would require a tremendous budget for development of humanized rodents, and would be premature at this stage.<br>● The Principal Investigator has a definitely above average and possibly excellent track record of educating a diverse group of scientists. The activities listed in this regard require time and energy; her personal commitment is evidently quite strong. Based on this, the PI's efforts to incorporate DEI into the proposed work will likely come to pass (even without specific numeric targets).<br>● The focus is on the team, not on research participants or patients. |

| Application # | DISC0-16005 |
|---|---|
| **Title** (as written by the applicant) | Regenerative medicine meets oncology: hiPSCs crafting the future of immune competent human skin cancer models |
| **Research Objective** (as written by the applicant) | Our research aims to create an innovative mouse model using human stem cells with a functional immune system to better understand and treat skin cancer. |
| **Impact** (as written by the applicant) | Our project aims to greatly improve how skin cancers are studied and treated, potentially leading to more effective therapies and benefiting public health. |
| **Major Proposed Activities** (as written by the applicant) | • Ensuring our lab-created skin cells develop properly to pave the way for the next steps in our research.<br>• Testing whether skin cancer cells from patients can provide us with the key insights we need for our study.<br>• Carefully introducing these lab-grown skin cells into specially designed mouse models to build a more accurate skin cancer research tool.<br>• Checking if the human-like skin we've created on mice truly resembles what we have, to confirm the reliability of our model.<br>• Finally, evaluating if our new mouse model can effectively mirror the human body's reaction to skin cancer, which could be a game-changer for future research and treatments. |
| **Statement of Benefit to California** (as written by the applicant) | With over 200K estimated new cancer cases in 2023, California faces a substantial health burden. Our research aims to revolutionize skin cancer models, improving patient outcomes and providing a foundation for more effective treatments. This could significantly alleviate the healthcare system's economic strain and serve as a model for combating other cancer types across the globe. |
| **Funds Requested** | $1,540,193 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 70

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

| | |
|---|---|
| **Mean** | 70 |
| **Median** | 70 |
| **Standard Deviation** | 5 |
| **Highest** | 85 |
| **Lowest** | 65 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 1 |
| **(1-84): Not recommended for funding** | 14 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 11 | • The project proposes to generate an interesting mouse model of human skin engrafted in a fully immune competent mouse model for examining skin cancer development and treatment. Basic biological studies of tumorigenesis and skin biology could be facilitated by such a model. |

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| **No:** 3 | • In principle, this model could improve the fidelity of human models of skin cancer and disease.<br>• It's unclear what the impact will be, but there is some potential for new discovery in this model. There are some concerns with the relevance of the immune phenotypes.<br>• The project team proposes creation of a new approach to generating models to study human cancers in the laboratory in an immune-competent animal (mouse) model, without the high cost and other limitations of "humanized" mice (i.e., extremely immune-deficient mouse strains reconstituted with human immune cells). The applicant proposes to establish proof of concept with a common human skin cancer.<br>• The proposed skin cancer to be studied for proof of concept is very common, and its incidence is increasing, so improved treatment would have real impact. Even though the mortality rate for the skin cancer studied is low, the prevalence of the disease results in a high mortality burden.<br>• Despite an outstanding team and some truly impressive preliminary data on generation of mice with a human epidermis, the application contains some intrinsic weaknesses and at least one major flaw in the conception of the project. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 4<br>**No:** 10 | • The applicants remarkable demonstration of their method to generate mice with good-sized patches of human epidermis derived from a human keratinocyte cell line represents a major strength of the proposal. This is a biological tour de force, even while raising the question of how difficult it may be to transition to normal primary human keratinocyte lineage cells.<br>• The applicant proposes to use cancer cell lines established decades ago in the model. Researchers generally concur such lines serve as questionable representatives of patient cancers. This weakens the ability of the model to mimic the complexity and pathogenesis of the human disease.<br>• With appreciation that developing a model system may take years of effort, the need for an aim to optimize the generation and injection of hiPSC-derived keratinocyte progenitor cells indicates that the system is still far from robust. It is quite possible that two full years into the project there will be very little, if any, significant new information about human skin cancer.<br>• How do the investigators propose to generate synthetic skin cancer models representative of primary human cancers? Will they be able to generate patient derived xenograft models in the context of the proposed immune-competent mice?<br>• The criteria for selecting the final tumor driver and tumor suppressors used to make the model among multiple options are not clear. Notably, the applicants criticize mouse carcinogen-induced skin cancer models for having a disproportionately high fraction of a specific proto oncogene mutants, yet will use activated and mutated forms of the same oncogene in this model.<br>• The preliminary data supports that the model will be successful, but it's not clear what the major improvements are over the NSG model. The NSG model is of course flawed, but I'm not sure a chimeric mouse is a major advance.<br>• Presumably aspects of the non-specific innate and lymphocyte response will be improved in this setting.<br>• A most important concern was that the host mouse has murine MHC genes, while the grafted human keratinocytes and tumors have human HLA genes. Adaptive cellular immune responses of T-cells depend upon matching of the MHC haplotypes between the immune and target cells. While the mice in the model proposed in the experiment are "immune competent," in the absence of matched MHC, the model misses out a crucial element of cellular immunity against cancer.<br>• The major concern is that while technically immune competent, the skin will have a distinct MHC from from the rest of the mouse. This will lead to a mismatch between priming (from dendritic cells) and effector function. Given the importance of T cell specificity in immune control and immunotherapy of skin tumors, it doesn't seem that the immune competence here will be relevant for most human disease and treatment. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 9<br>**No:** 5 | • The project is well-designed to test the feasibility of creating the model. Potential pitfalls are well-described. The time line is appropriate.<br>• As noted, the design of the project misses a fundamental aspect of cellular immunity against cancer - for which Peter Doherty and Rolf Zinkernagel were awarded a Nobel Prize in 1996.<br>• After three years of funding, the work proposed by an excellent team unfortunately will not yet yield a model of skin cancer that we can be sure will accurately represent the typical genetic profile of the human cancer. This remains a big challenge. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** | • It's feasible to generate mice with human skin cancer-like tumors. However, this will not provide a system that fully reflects growth of skin cancers in a fully immune competent patient. |

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| 12<br>**No:**<br>2 | • The aims are feasible and likely to be achieved. The staff is well qualified. The environment is outstanding. The budget is appropriate. The applicant describes various outreach activities. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:**<br>12<br>**No:**<br>2 | • The plan adequately addresses principles of diversity equity and inclusion and will include diverse demographics in the modeling. The outcomes of the research should be equally applicable across populations.<br>• Applicants cite the host institution's commitment to progress and a Presidential Initiative on inclusion, diversity, equity, and access in learning. However laudable these institutional efforts may be, the applicants offer nothing specific about how their specific project advances principles of DEI. This is non-responsive to CIRM's DEI criteria. |

| Application # | DISC0-16038 |
|---|---|
| **Title**<br>(as written by the applicant) | Making of Geometry: Mapping the mechanics that shapes the human neural tube. |
| **Research Objective**<br>(as written by the applicant) | Many human conditions associate with misshaped organs. Our study will identify the forces required to shape the earliest precursor of the human brain. |
| **Impact**<br>(as written by the applicant) | Neural tube defects remain a major birth defect despite folate treatment. We will use stem cells to identify molecular mechanisms that coordinate forces to shape this structure. |
| **Major Proposed Activities**<br>(as written by the applicant) | • We will map out the forces involved in neural tube closure<br>• We will systematically test how the neural tube responds to physical variations in their environment, such as substrate stiffness<br>• We will uncover the molecular mechanisms involved in coordinating forces in response to variations in physical features |
| **Statement of Benefit to California**<br>(as written by the applicant) | Neural tube defects have devastating consequences for those affected. While hundreds of candidate genes have been discovered in animal model systems, solving this problem in a human genetic context remained out of reach. Our new platform, together with a physical understanding of the closure mechanisms at the molecular level will enable new treatment strategies, and thus contribute to the development of novel prevention strategies. |
| **Funds Requested** | $1,478,078 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 70

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

| | |
|---|---|
| **Mean** | 70 |
| **Median** | 70 |
| **Standard Deviation** | 4 |
| **Highest** | 80 |
| **Lowest** | 65 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 15 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 6 | • Modeling in vitro neural tube closure using hPCSs could be useful for our understanding the in vivo processes and potentially the neural tube closure defects. |

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| **No:** 8 | • The knowledge gap is defined as understanding the mechanics underlying human neural tube defects. We cannot access human embryos easily at the requisite time and stem cell models allow potential access.<br>• The project as defined is based on trying to study the mechanical aspects of neural tube closure (NTC) in their stem cell model system. It is biophysically based, and they do not actually propose to explore further any specific models of neural tube defects (NTD) in humans.<br>• The biophysics of neural tube folding and closure has been studied in model organisms, and while the shape and details may vary, the fundamental principles of epithelial folding and closure are likely conserved. It is not clear that new concepts will be discovered in the human system.<br>• Generating in vitro models for development of complex human organs from pluripotent stem cells is very difficult, and in most cases, they do not represent actual in vivo processes, tissues or organs.<br>• It is unclear how this in vitro model of neural tube closure that is completely or partially dependent on artificial scaffolds can represent the natural processes that occur during early embryonic development.<br>• The relevance of this biophysics application to human disease is questionable.<br>• Insights that would be gained into new concepts from the work are not clear; folding is well conserved in other models. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 10<br>**No:** 4 | • The systems to measure mechanics of morphogenesis have been worked out well in prior studies by the PI in other systems and can be applied here.<br>• The rationale is to measure the impact of mechanical properties of substrates (spatial and temporal gradients in substrate stiffness) on NTC. However, it is unclear how these results could "lay the foundations for specific models of human neural tube defects, derived from patients".<br>• If this system is to be a powerful way to actually model human NTDs, then it is critical to assess whether genetic and environmental factors proposed to be involved in human NTDs can produce measurable changes in this system. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 6<br>**No:** 8 | • The end goal of this proposal is vague. Once the NTC model is generated, what is expected? One would expect that these experiments advance the knowledge of human biology or promote development of therapies. But how would this artificial system based on mechanical closure of neural tube with synthetic scaffolds be informative for in vivo NTC?<br>• The PI has developed a cell line mutant for a gene known to be associated with neural defects in mutant mice and humans. A very brief description of the behavior of this cell line in the model system is provided but there is no specific aim focused on extending the analysis of this or any other NTD-related mutant line.<br>• The project is designed to provide information on the mechanical properties and involvement of integrin signaling in neural tube folding and closure. It is not designed well to demonstrate usefulness as a model of specific human NTDs. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 11<br>**No:** 3 | • For most experiments, pitfalls and alternatives are considered. However, it would be critical to develop better NTC model using solely stem cells rather than silicone or alginate substrates.<br>• The measures of the physical parameters of neural tube folding can be achieved in the timeline. The studies on the role of integrin signaling can also be completed. Examination of how to actually provide human patient models of NTDs is not in the timeline provided.<br>• The team is well equipped to address the physics and hydrogel work. They could benefit from a biologist interested in neural development. The PI has a lot of experience in quantitative assessment of cell behavior in response to mechanical shifts. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 14<br>**No:** 0 | • Women of Hispanic origin are disproportionately at risk of a neural tube defects. Two hiPSC cell lines originating from Hispanic populations we will obtained from the CIRM Human Induced Pluripotent Stem Cell Repository.<br>• NTDs are more prevalent in Hispanic populations, and they will use cell lines from these populations. It's not really clear how they will quantify any differences among cell lines. This will be a longer term issue. |

| Application # | DISC0-15944 |
|---|---|
| **Title** (as written by the applicant) | Using an iPSC Derived Human Lung Airway Model to Determine the Antiviral Effects of Airway Surfactants |
| **Research Objective** (as written by the applicant) | A novel model system of the human airway and progenitor cells to study the pathogenesis of viral infections. |
| **Impact** (as written by the applicant) | Primary lung tissue humans is difficult to obtain, thus our iPSC derived airway cultures overcome the bottleneck of studying human airway injury |
| **Major Proposed Activities** (as written by the applicant) | • Making human iPSC derived progenitor airways<br>• Analyzing the human iPSC derived airways at different oxygen levels<br>• Analyzing extent of injury of the airway cultures at different oxygen levels<br>• Infection of iAirways with live respiratory syncytial virus (RSV)<br>• Determining the therapeutic role of recombinant BPIFA1 and surfactant against RSV |
| **Statement of Benefit to California** (as written by the applicant) | This novel human airway model system will simplify and hasten the development of anti-viral therapeutics and reduce the burden on the medical system in California. |
| **Funds Requested** | $1,392,506 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 70

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

| | |
|---|---|
| **Mean** | 69 |
| **Median** | 70 |
| **Standard Deviation** | 2 |
| **Highest** | 70 |
| **Lowest** | 65 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 15 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 9<br>**No:** 5 | • The proposal focuses on respiratory syncytial virus (RSV) but, if the proposed experiments are successful, the approach could be applicable to many other respiratory diseases and even non-genetic respiratory issues.<br>• Using an iPSC-derived lung airway model the study proposes to discover how airway progenitor cells are influenced by prematurity and subsequently, how this impacts their ability to respond to infection with RSV, from which pre-term infants are at high risk of developing severe lung injury.<br>• The study also hopes to evaluate the antiviral properties of airway surfactants in the iPSC-derived airway model, while also determining how RSV infection impacts stem cells in the immature airway. |

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| | • Studying the pre-term airway in a relevant model, i.e. human model, is extremely challenging and limited to accessibility to tissues. This study will apply an iPSC approach which has the potential to be able to evaluate developmental aspects of the human airway.<br>• If successful, the project has the potential to inform on physiologically relevant changes in the airway composition and function that may inform future therapeutic advances to prevent lung injury due to viral infections.<br>• The project explores the role of oxygen and surfactant in regulating lung pathology, with the primary innovation coming from the proposed advanced organoid model. The proposed airway model is posited as a breakthrough platform for understanding lung responses to infection. The proposal explores the role of a particular surfactant. The applicant will explore responses during deficiency or with prophylaxis, but the project will not really address the mechanism of action. |

| **GWG Votes** | **Is the rationale sound?** |
|---|---|
| **Yes:** 6<br>**No:** 8 | • The rationale for the choice of genes to knock out is not clear. It's not clear what the applicant is trying to model.<br>• Is the study trying to model pre-natal or post-natal airway development? Note that many of the references are incorrectly placed throughout the proposal and critical references on which the model is based are not included.<br>• Yes, the model has the potential to generate data that could impact our ability to prevent chronic lung damage induced by viral infections in pre-term infants. Further validation of the model may have implications for other airway diseases.<br>• There are exciting preliminary data, but they do not sufficiently support the rationale for proceeding with this project.<br>• The model needs to be further studied before the applicant proposes these experiments.<br>• The proposed airway model would allow for the future investigation of many other types of treatments. This is only if the model works, which is not clear.<br>• The airway model appears well established, which allows for the proposed work to be done rigorously. The justification for the focus on a single surfacant is limited - this is an interesting discovery project, but the analysis proposed is relatively superficial.<br>• The project addresses RSV, which is an important human disease. |

| **GWG Votes** | **Is the project well planned and designed?** |
|---|---|
| **Yes:** 4<br>**No:** 10 | • The results are likely to be interpretable, but we already know that surfactant, hypoxia, and hyperoxia all regulate lung epithelial and stromal cell biology. The studies here don't seem to advance mechanistic insight. Pitfalls and alternatives are presented and generally well-considered. The timeline is appropriate. The applicants discuss selecting diverse samples but then propose only three replicates, which may create complications.<br>• While preliminary data is presented showing differentiation of human iPSC toward lung lineage cells it is not convincing that the cells generated represent a specific time in human lung/airway development.<br>• There is no preliminary data showing differentiated cell type markers of specific lung lineage markers over other secretory epithelium. Transcriptomic analysis shows that the profiles align with lung progenitors and lung centric cell types, however it is not clear how well these overlaps with specific time points in the developing, pre-term or postnatal lung, specific to this study.<br>• Based on the cultures shown, the airway has both epithelium and supporting mesenchyme. This usually makes the epithelial barrier challenging to evaluate by transepithelial electrical resistance (TEER). It was not clear how TEER would otherwise be recorded.<br>• Reproducibility of the organoids, including the epithelium and distribution of cells in the mesenchyme is not clear, these could be significant factors that impact the experimental outcomes.<br>• Preliminary data supporting the impact of hyperoxia on the cultures is not significant. But, more importantly, it is not clear how many experimental repeats from how many individual donor iPSC lines this data represents. Inter and Intra experiment variability are thus difficult to evaluate.<br>• It is not entirely clear what level of airways the model is poised to recapitulate. The presence of submucosal glands suggests the proximal cartilaginous airways but the simplified epithelium in the images would suggest a more distal airway. The applicant describes [*gene name redacted]*3A2 as their secretory cell marker, but this gene is typically more distally expressed in place of *[gene name redacted]*1A1.<br>• The distribution of stem cells (basal) and ciliated cells is not clear in the model. Ciliated cells seem absent or lacking which could significantly impact the data on surfactants given the importance of cilia in airway clearance and immune responses. This could also substantially impact infectivity so knowing the reproducibility of the ciliated airway is critical. |

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| | • The gene editing in description is vague and it is not clear how lines will be selected for use. For example,<br>  ◦ Will single cell cloning be used to purify edited clones?<br>  ◦ How will KO be validated?<br>  ◦ If the applicant is proposing to generate KO in multiple lines, will there be a selection process for the lines to be used in the study?<br>  ◦ If the KO impacts progenitors, then that will also impact other surfactant proteins - how will this be adapted to ensure specific focus on the impact of *[gene name redacted]*FA1?<br>• There may not be an advantage of the current model over other models currently in the field. |

| **GWG Votes** | **Is the project feasible?** |
|---|---|
| **Yes:** 11<br>**No:** 3 | • The proposed aims are achievable, the team is well-qualified, and the environment is outstanding.<br>• With (i) no preliminary data on the model reproducibility and (ii) no information on the number of independent iPSC lines and repeats used in the preliminary studies or proposed for the new study, it is challenging to determine whether variability in iPSC differentiation and organoid development will supersede the impact of the biological parameters being evaluated.<br>• The aims are vague. For example, in Aim 1 a knock-out will be generated, but there is limited description of what it will be used for. Sequencing will be employed, but what specifically will be studied in the datasets? Diversity is discussed, how many replicates or lines will be used, what statistical analyses are planned?<br>• The timeline seems ambitious. However, if all the lung progenitors are pre-differentiated and ready for use in the experiments it is feasible.<br>• The proposed airway model may not represent what happens in humans.<br>• The project is feasible, but may not work due to the lack of characterization of the proposed airway model.<br>• The team seems to have most of the expertise needed to carry out the experiments.<br>• Expertise in CRISPR editing is not clear. |

| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
|---|---|
| **Yes:** 12<br>**No:** 2 | • The hiPSCs used for making the model come from African American, Hispanic, and white men and women.<br>• The DEI section is not well developed.<br>• The proposal includes a robust plan for diverse demographic representation in the experimental systems - but the feasibility of this is a concern.<br>• The results of the study will be applicable across populations.<br>• The applicant has described outreach efforts to increase representation in research.<br>• Throughout the proposal, race, ethnicity, sex, gender and age are mentioned and it is stated that these will be specifically evaluated. However, this is not evident from the sample sizes and planned replicates in the project plan.<br>• It is not clear that sufficient iPSC-lines are available to provide meaningful and significant data related to diversity. While there is some evidence for sociodemographic mechanisms underlying disparate health outcomes for Black infants with severe disease, the applicant did not specifically discuss this or design the study to address this issue. |

| Application # | DISC0-15925 |
|---|---|
| **Title** (as written by the applicant) | Mechanisms of synaptic neurotransmitter dysregulation in human Alzheimer's disease (AD) neurons |
| **Research Objective** (as written by the applicant) | New knowledge of mechanisms of synaptic neurotransmitter dysregulation in human Alzheimer's disease (AD) neurons will be gained. |
| **Impact** (as written by the applicant) | The bottleneck of defining neurotransmitter signatures applied to human iPSC neurons and induced neurons has been solved with our development of neuropeptidomics and metabolomics technology. |
| **Major Proposed Activities** (as written by the applicant) | • Analyze neuropeptide signatures combined with classical neurotransmitters secreted from human neurons derived from MCI and Alzheimer's disease (AD), with comparison to human AD brain.<br>• Evaluate dysregulated neuropeptides, as well as classical transmitters, for neurotoxicities of cell death, inflammation, diminished neural network activity, and compromised mitochondrial metabolism.<br>• Analyze protease mechanisms utilized to generate dysregulated AD and MCI neuropeptides. |
| **Statement of Benefit to California** (as written by the applicant) | The benefit of this research to the State of California is that findings will advance development of mechanistic biomarkers and new drug targets for Alzheimer's disease (AD) which is suffered by numerous citizens of the State of California. AD is a devastating disease of the aged that results in severe loss of memory that compromises daily living. Elucidation of brain mechanisms responsible for AD brain neurodegeneration is critical to advance new therapeutic approaches for Californians. |
| **Funds Requested** | $1,435,016 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 70

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

| | |
|---|---|
| **Mean** | 68 |
| **Median** | 70 |
| **Standard Deviation** | 3 |
| **Highest** | 70 |
| **Lowest** | 60 |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 14 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 8 **No:** | • The applicants propose to investigate synaptic neurotransmitter dysregulation in Alzheimer's disease (AD) by using human iPSC neurons and age-equivalent 'induced neurons' (iN) representing early and late stages of AD. |

| | |
|---|---|
| 5 | • This project proposes to fill the gap in understanding synaptic dysregulation of various neurotransmitters in AD, both early and late stages, This is commendable, but it is unclear exactly what they study will provide that is not already known.<br>• The project appears to too small scale to make sufficient impact.<br>• It's likely that neuropeptides have some disease relevance but their relative importance or causality is not explored in this application. This type of evidence is expected to initiate any AD experiments.<br>• There are thousands of genes and proteins that significantly change between AD and control cells or tissues. The authors haven't described how their targets are the king pin in all of that, or at least how they are particularly significant in terms of the etiology of the disease.<br>• This targeted experiment in a small number of cell lines will be useful to very few, as the field has moved on to more robust collections. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 4<br>**No:** 9 | • The rationale may be sound, but it is not well developed and not well supported by the preliminary data in the proposal. One of the major issues with this type of work is variability in IPSC lines. No power calculations are provided and the applicants propose to use a small number - limiting the generalizability and potential for success.<br>• The preliminary data are too weak to support funding the project.<br>• Given the high variability in IPSC lines, the push in the field for ever larger collections to adequately capture the variability in AD molecular phenotypes, genetic diversity, and intrinsic noise, a single-digit sample size is not adequate. The proposal did not include a detailed the power calculation, and the cited reference also did not appear to have a power calculation.<br>• A project like this is not poised for success until donor-line similarity scores are established. These are typically in the range of n=40+. Perhaps this particular system is highly conserved, but there's no evidence of that in the proposal.<br>• The proposal would need evidence of synaptic maturity or culture conditions designed to promote synaptic maturity.<br>• With hundreds of proteomics samples available, the proposal should include some evidence this synaptic neurotransmitter dysregulation is critically implicated in AD. The only relevant reference looks at only a handful of samples.<br>• The evidence they cite from a tetrodotoxin model (which isn't a mainstream AD model) actually utilizes closer to 20 lines, so again, I don't see evidence that a single digit number of lines will work. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 4<br>**No:** 9 | • This project is not well developed nor well designed - too small scale.<br>• No - no screening in large-scale omics data has been or will be undertaken to establish the relevance of synaptic neurotransmitter dysregulation in AD.<br>• In terms of urgency, this system has not been proven to be a priority. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 7<br>**No:** 6 | • Feasible, but not very impactful.<br>• The team seems very well versed in the details of this particular system.<br>• The throughput of the proteomics isn't described in detail and doesn't sound high, though maybe it doesn't have to be for a small project.<br>• No - there's a certain scale on which IPSC work in AD needs to be done, and work significantly below that scale is not justified. For instance, for isogenic comparison a small number of lines might suffice, but here there's no evidence the experiment will succeed with such a small number of lines (among other limitations mentioned above). |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 11<br>**No:** 2 | • With a single digit number of lines, it's not possible to capture significant diversity-related data.<br>• In theory, yes, but in practice no, due to sample size.<br>• The lab has an above average and substantive track record of DEI-related educational efforts. |

| Application # | DISC0-15847 |
|---|---|
| **Title**<br>(as written by the applicant) | Transplantation of hiPSC-neurons to treat inflammation-based cognitive deficits in central nervous system (CNS) injury |
| **Research Objective**<br>(as written by the applicant) | These studies will help inform a larger body of work designed to understand how stem cell-based spinal transplantation therapies impact distal regions along the continuum of the neuroaxis. |
| **Impact**<br>(as written by the applicant) | If successful, the proposed studies will help mechanistically shed light on how stem cell-based spinal transplantation therapies impact distal regions along the continuum of the neuroaxis. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Differentiation of hiPSCs and synthesis of proprietary hydrogel<br>• Mid-cervical contusion SCI of animals<br>• Transplantation surgery of animals<br>• Retrograde tracing surgery of animals<br>• Euthanasia, processing of brain and spinal cord tissue, and immunohistochemistry<br>• Sensorimotor and cognitive behavioral testing |
| **Statement of Benefit to California**<br>(as written by the applicant) | Spinal cord injury patients often experience cognitive dysfunction suggesting that injury impacts areas of the brain responsible for learning and memory. Stem cell-derived neuron transplantation therapies have the benefit of improving sensorimotor function but studying how long-term engraftment impacts other parts of the nervous system is of both basic and translational importance. |
| **Funds Requested** | $1,540,499 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 70

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

| | |
|---|---|
| **Mean** | 57 |
| **Median** | 70 |
| **Standard Deviation** | 21 |
| **Highest** | 70 |
| **Lowest** | 1 |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 14 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 7<br>**No:** 6 | • Impact on cognitive acuity and memory is a significant co-morbidity of spinal cord injury (SCI) that affects quality of life beyond the already devastating burden of paralysis.<br>• Understanding the impact of how long-term engraftment of stem cell-derived therapies impact on inflammatory and cognitive consequences represents a current bottleneck for devising a holistic therapeutic approach. |

| | |
|---|---|
| | • Investigating how hiPSC-based spinal transplantation alters neuroaxis systems distal from the site of engraftment is significant and potentially impactful.<br>• The applicants propose to study how long-term engraftment of stem cell-derived therapies impact distal sequelae along the neuroaxis. They suggest that transplantation of stem cell-derived therapies can not only improve sensorimotor function, but also improve cognitive outcomes through attenuation of inflammation along the neuroaxis.<br>• The proposal incorporated a figure from another group's publication, modified in a way that made the statistics (error bars) misleading. Regardless of whether an applicant includes a statement such as 'adapted from' or 'borrowed from', and regardless of whether the original source is cited, this constitutes a form of scientific misconduct. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 5<br>**No:** 8 | • It has already been shown that 28% of rats with T12 contusion SCI suffered from depression (Brakel et al., 2019), which was associated with increased serum levels of interleukin (IL)-6 and IL-1α before and IL-6 and tumor necrosis factor (TNF)-α after injury (Brakel et al., 2021). While these reports are not cited, they provide a rationale for testing whether cell transplantation can prevent this decline.<br>• Associations between hippocampal neurogenesis and neurological diseases - as well as neurogenesis after SCI - have been recognized, but it is not established that these occur in humans and are causally related.<br>• While the use of athymic rats rests on the assumption that T cell mediated effects are not involved in the immune response and inflammatory environments, robust neuroinflammation has been shown in this model and would at least address responses of macrophages and B cells.<br>• The applicants have published that their hydrogel technology prevents transplanted Schwann cell loss after SCI years ago, but they remain to publish the same is true for iPSCs in a peer reviewed journal.<br>• In their prior paper, the applicant states their cell transplants reduce neuroinflammation after SCI and improve connectivity and regeneration of cortical neurons. The novel aspect of this proposal is testing learning/memory; why was this not conducted in prior work?<br>• The applicants prior work supports the project rationale. However, the athymic rat model poses a major issue to studying inflammation, since T cells will be absent. Xenogeneic differences between human and rat will also present immunological challenges that raise uncertainty about the experimental design.<br>• Fig. 2 shows a newly created figure that incorporated a figure from a published manuscript. In the applicant's version, data bars had been re-colored and/or spliced together. It is not disclosed whether the applicant had permission from the original authors to alter the figure. Using and altering a figure from another group's published paper without explicit permission is not acceptable and affected the score. |
| | *none* |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 3<br>**No:** 10 | • SCI have been shown to be a risk factors for age-associated disorders, like AD, suggesting that older individuals with SCI would be more susceptible to aberrant immune function and cognitive decline. Age of the animals is not considered; this is a limitation.<br>• The Y maze is not testing memory but, rather, anxiety. Pain might affect the outcomes in the test and is not discussed.<br>• The applicants state they will use both male and female rats and that their power calculations show n=10/group. However, they don't state how many rats of each sex will be used per group or what their power calculations say is a minimal number per sex. This should be clearly stated as using both sexes just to "tick boxes" and may not bring meaningful results.<br>• The topic of adequate animal numbers is also important because the effects of biological sex mismatch between host rodents and donor cells have been reported previously. Due to all that, this study does not seem to be powered to bring meaningful conclusions.<br>• With the proposed cohort sizes, a panelist wondered why the applicants need three years for this study.<br>• This project could be improved by addressing the immunological limitations of the model and performing more sophisticated measurements of inflammation beyond IHC and Luminex. Adding an immunologist to the team could help with this.<br>• While the aims are straightforward, they are highly descriptive.<br>• The applicant proposes in Aim 2 to use systemic pharmacological depletion of immune cells to test the impact on memory. However, immuno-depletion experiments are not described. The proposed pharmacologic treatment blunts systemic inflammation, but does not deplete specific immune cells. |

| | |
|---|---|
| | • Models of cognitive and memory impairments in rodents are highly associated with impaired hippocampal neurogenesis. In fact, large decreases in hippocampal neurogenesis have been reported in studies using the compression and contusion model. Hippocampal neurogenesis, which might not occur in humans after SCI, could impact the interpretations of result and lead to wrong conclusions. A project co investigator is an expert in rodent and human neurogenesis; thus, this point should have been addressed. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 10 **No:** 3 | • Relevant papers cited by the group are only posted on bioRxiv since 2021 and have not since been published, but preliminary data show efficacy of the hydrogel approach.<br>• Applicants do not show that their animals have a memory defect post injury; thus, it is not clear whether they have a phenotype they can study.<br>• It is not discussed where Aim 2 will lead should the animals show no significant effects in the novel object recognition or elevated maze tests.<br>• The applicant reports good progress on previous biomaterial grants but produced limited data on a CIRM transplantation grant that ends soon. Overall publication productivity is limited; most papers are on bioRxiv.<br>• This is an all or none type of project. If it fails to confirm the applicants' hypothesis, all we will know is that transplantation of stem cell-derived therapies cannot improve cognitive. While such a finding would be important, it is just not enough value for the investment.<br>• Feasibility would be fine if rationale and design concerns are addressed. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 10 **No:** 3 | • Both sexes will be used in the animal model studies, and patient lines will be tested from both sexes and various ethnicities.<br>• Cervical SCI research is underrepresented, and preservation of cognitive function is highly relevant for all patients.<br>• The applicants' effort to investigate cervical SCI, which is underrepresented in SCI research, is commendable.<br>• African Americans represent the largest growing minority demographic of SCI patients, and a portion of the applicants patient lines are derived from African American donors. Also, the applicant states that via CIRM funding, they are testing therapeutic efficacy across multiple patient lines of male and female origin. The applicant's decision to use a cell line from a single white female individual is questionable from more than the DEI perspective. |

| Application # | DISC0-15827 |
|---|---|
| **Title**<br>(as written by the applicant) | Addressing Fundamental Neurobiology of Autism with Brain Organoid Models |
| **Research Objective**<br>(as written by the applicant) | Our study describes a transformative approach to autism. Combining several risk loci to find deeper points of convergence could reveal dysregulation of shared neuron classes among autism subtypes. |
| **Impact**<br>(as written by the applicant) | Our study could reveal new biological targets for autism mutations, and potentially identify convergent neurobiological pathways amenable to therapeutic intervention in autism. |
| **Major Proposed Activities**<br>(as written by the applicant) | • Investigate whether Cul3 mutations and 16p11.2 deletions converge on shared neuron classes by multiome profiling<br>• Evaluate posttranscriptional convergence of Cul3 mutations and 16p11.2 deletions by global proteomic profiling and by single cell proteomics.<br>• "Functionalize" obtained results by electrophysiological recordings of calcium dynamics, neuronal excitability, and network activity in intact organoids<br>• Develop chimeroids as clinical trial in a dish models<br>• Rescue observed deficits using brain chimeroid models by exposing them to potential drug candidates |
| **Statement of Benefit to California**<br>(as written by the applicant) | California's diagnosed autism incidence rate rose from 0.49 per 1,000 of 3–6y olds in 1998 to 3.49 per 1,000 of 3–6 year olds in 2018, a 612% increase. The latest March 23, 2023 report examined 4-year-old children in the same 11 communities and found similarly high rates of autism (2.2%) in the network overall, with an increase to 4.6% in California in particular. Investigating molecular mechanisms of autism and developing therapies based on these mechanisms would greatly benefit California. |
| **Funds Requested** | $1,196,603 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 65

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

| | |
|---|---|
| **Mean** | 67 |
| **Median** | 65 |
| **Standard Deviation** | 4 |
| **Highest** | 75 |
| **Lowest** | 60 |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 14 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|

| | |
|---|---|
| **Yes:** 3 **No:** 10 | • Despite hundreds of genes being linked to autism, knowledge about convergent biological pathways that could be targeted therapeutically is scarce or even non-existent - and the applicant proposes to address this by investigating neurobiological convergence between two high risk autism loci, the 16p11.2 copy number variant (CNV), and Cul3.<br>• The applicants propose to create brain cortical organoid (BCO) models from iPSCs of male and female patients, accounting for sex and varying genetic background in which they will investigate impact of those two mutations on production of various neuronal classes using single-cell sequencing and proteomics.<br>• The application seeks to understand whether there are convergent mechanisms between two de novo variants associated with autism, a CNV (16p11.2) and mutations in CUL3. However, their approach focuses specifically on convergence between variants in just 2 loci. If successful, the knowledge gained will likely be incremental, and the project will likely have a limited to medium impact.<br>• The project has limited novelty. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 11 **No:** 2 | • The rationale for focusing on the two loci is based on (i) strong evidence of association with autism and (ii) the applicant's preliminary data implicating RhoA, a small GTPase which is a substrate of the KCTD13 (located in 16p11.2)-CUL3 complex.<br>• The preliminary data is compelling overall and supports the hypothesis that RhoA is upregulated following loss of function of KCTD13 and CUL3.<br>• No preliminary data are presented to justify proceeding with Aim 3. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 5 **No:** 8 | • The applicants have identified biological convergence between 16p11.2 and Cul3. They will now characterize convergence at the cell and circuit levels. However, while the proposed approach is fine for the single cell level, it is not the most appropriate approach for circuit levels because organoids lack the connectivity that exists in vivo.<br>• It seems that not all the required cell lines are yet available to the applicant. The applicant mentions the lines will be ready before the start of the project; this is potentially problematic if there is a delay.<br>• The project is designed to generate a large number of datasets: transcriptional profiles, chromatin accessibility and proteomics (Aim1) as well as functional datasets from cortical brain organoids derived from iPSC lines from patients with 16p11.2 deletion and Cul3 mutations. However, how these datasets will be utilized and interpreted, and whether (or how) the results will be meaningful or impactful, is unclear.<br>• Aim 3 (which is qualified as an exploratory aim) is not appropriately planned. The applicant proposes to generate many chimeroids (organoids from many cell lines mixed together). They provide minimal details on how they might do this, and overlook fundamental aspects such as scale and cost. The approach, along with the description of the hurdles it seeks to address, is also misrepresented, as it is much more difficult to grow cell lines together, and control their growth rate in an organoid as compared to 2D.<br>• Finally, there is a concern about whether the proposed sample size has sufficient statistical power, given the heterogeneity of cortical brain organoids and variability across batches. The applicant discusses statistical power for RNAseq, but not for the other proposed assays.<br>• Potential pitfalls identified and alternative approaches are presented for Aim 1, although they do not really address the fact that not all cell lines that they are proposing to use are available yet, and how they might move forward if they are not available by the start date of the grant. They do not identify any notable pitfalls in Aim 2, stating that the proposed techniques fall within their areas of expertise. There is only very limited discussion Aim 3 , where, as discussed above, many pitfalls are evident.<br>• The project plan is a list of experiments that could be done, without clear rationale for doing them. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 10 **No:** 3 | • Yes - the project is appropriately planned to deliver within the proposed timeline.<br>• Cell line availability is a potential concern.<br>• Aims 1 and 2 are likely to be achieved in the expected timeline. However, it is unclear if the project outcome will be sufficiently interpretable.<br>• The rationale, steps, and outcome of Aim 3 are not articulated in a logical manner. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 13 | • Partially yes - gender bias is taken into account. |

| | |
|---|---|
| **No:**<br>0 | • The team presents a compelling discussion for the need of diversity at many levels in autism research, but they only incorporate male and female lines in their study (and no ancestral diversity). |

| Application # | DISC0-15937 |
|---|---|
| **Title** (as written by the applicant) | Hematopoietic stem and progenitor cells serve as central hubs during perinatal liver inflammation |
| **Research Objective** (as written by the applicant) | Our proposal uncovers the role hematopoietic stem cells play in propagating devastating inflammatory diseases of the liver in infants and children. |
| **Impact** (as written by the applicant) | Our proposal will help determine whether hematopoietic stem cells can be targeted to reduce the morbidity of inflammatory liver diseases that occur in infants and children, like biliary atresia. |
| **Major Proposed Activities** (as written by the applicant) | • We will determine whether hematopoietic stem cells that reside in the developing liver can propagate liver inflammation in infants and children.<br>• We will develop new technology to track hematopoietic stem cells that reside in the developing liver to understand where they go and what they become. |
| **Statement of Benefit to California** (as written by the applicant) | Inflammation that occurs in the livers of infants and children can be life-threatening. Hematopoietic stem and progenitor cells (HSPCs) act as central hubs during this inflammatory process. Our research will shed light on the role of HSPCs during these devastating diseases and will identify potential strategies to target HSPCs and prevent the detrimental consequences that we observe in patients. |
| **Funds Requested** | $1,612,940 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 60

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

| | |
|---|---|
| **Mean** | 60 |
| **Median** | 60 |
| **Standard Deviation** | 0 |
| **Highest** | 60 |
| **Lowest** | 60 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 15 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 6<br>**No:** 8 | • The overall objective of this proposal is to define of how hematopoietic stem and progenitor cells (HSPCs) orchestrate perinatal liver inflammation (PLI) and then how this abnormal response propagates after birth to induce disease.<br>• The project addresses the effect of PLI on HSPCs and the immune mechanisms that might mediate long term liver damage in PLI. Understanding the mechanism of disease could lead to improvements in therapeutics or preventative interventions. If successful, it would advance the field's understanding of the immunological mechanisms and consequences of PLI. |

| | |
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| | • Ultimately, the goal seems to control abnormal response of HSPCs and to decrease abnormal immune state. The focus on myeloid cells (macrophages?) could also be better justified.<br>• The objective is interesting, but the broader impact is unclear. Do they intent to solve all the autoimmune disease? Are they focusing only on BA? They could define more precisely their field of interest.<br>• Understanding how fetal development can impact post-natal disease is a key objective. However, it is unclear how this proposal will achieve this goal.<br>• The path for potential translation from confirmation of the hypothesis to the development of stem cell-based or genetic therapies is not clear.<br>• Potentially useful knowledge, but it's not abundantly clear how this would advance regenerative medicine. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 5 **No:** 9 | • The rationale between liver disease and early HSCs maturation is difficult to follow, and the justification from the applicants not very clear. For example, biliary atresia is mentioned as a key example. However, the causes of this pediatric liver disease remain unknown, and the immune aspect is likely to be a consequence rather than a cause.<br>• The overall link between PLI, HSPCs and liver disease is difficult to follow when it comes to developmental biology, liver disease and human injury. This proposal is not about stem cells.<br>• BA is occurring first in the extra-hepatic region of the biliary tree. How can it influence HSPCs? This is unclear and needs to be better explained.<br>• The most important medical consequences and the central mechanisms underlying a potential long-term decrease in immune function due to perinatal exposure of hematopoietic stem/progenitors to an inflammatory liver environment are not sufficiently clear.<br>• Data focus on restricted propagation of a subset of myeloid cells as key to the "propagation" of perinatal liver inflammation by hematopoietic stem/progenitors in conditions in BA. The data indicate that the the cells protect against some of the inflammatory effects. However, mechanisms are not very clear beyond pointing to an already well-described chemokine/receptor pathway.<br>• The liver receives HSCs around 6-7 weeks and then maturation/amplification occur through a complex and yet not fully understood cross talk with liver cells. Fetal HSCs also migrate to the bone marrow between 14-20 weeks thus before any BA symptoms or other perinatal liver inflammation. Similarly, intrahepatic cholangiocytes are specified when HSCs are leaving the liver. This timing does not work with their hypothesis..<br>• The preliminary data support the role of the proposed cells in limiting pathology in PLI. I think the support for the contact model in Aim 2 is a bit less strong. There are concerns about the forward link to cell differentiation and the actual identity of what stem cells are being examined. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 5 **No:** 9 | • This project will dissect the immediate impact of inflammatory environment (mediated by a viral infection in the mouse model) on, especially, the myeloid hematopoietic lineages. It's not clear it will give meaningful results about the degree of, and basis for, long-term alterations in hematopoietic stem/progenitor cells due to perinatal exposure to diseased cholangiocytes in an inflammatory liver environment.<br>• The total absence of human models is a major limitation. There are now multiple cholangiocyte organoid platforms which could be used to validate some of their observations in the mouse.<br>• The entire project relied on animal models apart from Aim 2d where the applicant proposes to use spatial transcriptomic on human pediatric livers from patients with biliary atresia. Why not use this amazing resource first to validate some of their hypotheses? This is really the most exciting part.<br>• Some technical pitfalls are addressed. The bigger question of nailing down the mechanisms of altered long term function of hematopoetic progenitors is not considered.<br>• Aim 1 is reasonably designed. Aim 2 is significantly higher risk. Aim 3 is more descriptive but likely to be successful. There might be greater value in starting with the discovery in Aim 3 and building the mechanistic studies on those findings. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 9 **No:** 5 | • The team is qualified for the proposed work. The environment is outstanding. The budget is appropriate.<br>• The applicant team has great experience with the human disease and access to key tissue samples. Staffing and skills for the murine experiments are also fine. A reviewer notes uncertainty about the team's degree of sophistication in immunology. The major focus is restricted to subsets of myeloid cells, and the adaptive immune system is not |

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| | considered. Addition of a member with a complementary immunological focus could potentially could be strengthened the team.<br>● The evidence that PLI drives the human disease are limited. However, such mechanisms will always be difficult to study in human, since the symptoms of the disease become apparent after progression. Aim 2d could address this problem, but it is too limited and might not have an impact on the project. This part should come first and be used to generate hypotheses.<br>● The preliminary data are definitely convincing regarding the importance of the proposed monocyte in the context of a type of infection. However, this model might not be an entirely accurate reflection of the human disease, since there is no evidence of this type of infection in these patients.<br>● The timing of infection is problematic. Indeed, the infection needs to be done when HSPCs are still in the liver.<br>● The data on hepatoblastoma are not incredibly relevant. Did they find any interaction between HSPCs and cholangiocytes? There is now single cell data on the human fetal liver. Such info could help to support their hypothesis.<br>● The work proposed by the team is routinely done in the current environment. So, there is no doubt that mouse experiments are feasible. The spatial transcriptomics experiments might be more complicated.<br>● I think Aims 1 and 3--while ambitious--are achievable. I worry that Aim 2 will require significant optimization and is only currently formulated in a preliminary way.<br>● As laid out the project is feasible, but not as impactful as one might wish. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 12<br>**No:** 2 | ● The applicants recognize the importance of this aspect and include diversity/gender in their experimental design.<br>● This is a very basic research project, but it does address sex and age thoroughly and will use appropriate community level selection for patient samples. There is no restriction on the application of the outcomes. The applicant has provided a description of outreach efforts.<br>● Sufficient attention is paid to DEI principles to be okay for an early stage discovery project. Human specimens come from a population that reflects the diverse population of the applicant area. The project is not discriminatory, but it provides no special applicability to underserved populations as presented. |

| Application # | DISC0-15657 |
|---|---|
| **Title**<br>(as written by the applicant) | Targeting neurodevelopmental and genetic mechanisms of cerebral palsy |
| **Research Objective**<br>(as written by the applicant) | New insights into hypoxic and genetic CP mechanisms including biological and epigenomic outcomes will be gained using a novel stem cell-based brain organoid model |
| **Impact**<br>(as written by the applicant) | We will address roadblocks in the CP field with human iPS cells to make cortical organoids to model CP in a dish with hypoxia, gene editing, or both, to catalyze new clinical approaches |
| **Major Proposed Activities**<br>(as written by the applicant) | • In Aim 1 we will optimize and expand the CP in a dish human brain organoid model<br>• In Aim 1 we will also define epigenomic mechanisms of CP-related hypoxic injury<br>• In Aim 2, we will make hIPSC and then organoids with NTNG1, SYNE1, and AHDC1 patient-specific genomic genomic variants<br>• In Aim 2, we will also define the roles of NTNG1, SYNE1, and AHDC1 genomic variants in hypoxic sensitivity and epigenomic mechanisms<br>• In Aim 3, we will make hIPSC and organoids with CREBBP, TUBB3, and MAPK8IP3 patient-specific genomic variants<br>• In Aim 3, we will determine the impact of CREBBP, TUBB3, and MAPK8IP3 genomic variants on brain organoid development and epigenomic mechanisms |
| **Statement of Benefit to California**<br>(as written by the applicant) | Our proposal will use innovative stem cell technologies to strongly advance knowledge about CP through a new model we call CP in a dish. More than 100,000 people in California have CP with many health and societal impacts, yet CP research has been very limited. Our work will address that gap to benefit California. For example, the new knowledge from our studies and its model system can be used directly to try to develop fresh clinical approaches to prevention and treatment of CP. |
| **Funds Requested** | $1,573,659 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: --

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

| | |
|---|---|
| **Mean** | -- |
| **Median** | -- |
| **Standard Deviation** | -- |
| **Highest** | -- |
| **Lowest** | -- |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 14 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 5 **No:** 8 | • The causes of cerebral palsy (CP) are multifaceted and poorly understood. This project seeks to understand the genetic and environmental (hypoxic) contributions to CP. using stem cells and human brain organoids.<br>• The applicants propose to combine human brain organoids and CRISPR gene editing to model CP in a dish - while interesting, this actually does not really address the above question, nor is it an innovative approach.<br>• The project promotes the CP in a dish idea, which seems to be just the idea of using organoids with specific mutations. Thus, my enthusiasm for innovation here is low. It does not define or address a major bottleneck in CP research and is generic in its goals.<br>• The project has some difficulty defining a specific knowledge gap and instead proposes multiple omics methods to profile mutations and environmental conditions associated with CP. This comes across as a one-size-fits-all approach to mutation profiling, which would be better served by a Perturb-seq-style assay instead of making each mutation a sub-Aim.<br>• If successful, there is good potential for linking specific mutations to organoid phenotypes. It's unsure if these phenotypes are truly representative of CP patients, and a stronger case would need to be made for this kind of translational impact.<br>• The applicants state they will address the gap related to prevention or treatment strategies for cerebral palsy (CP), and CP mechanisms. However, the proposed study does not actually address this gap. At best, it will provide some new insights into potential mechanisms. It is a big leap and overstretching the significance of the study to link the latter with prevention and treatments. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 0 **No:** 13 | • CP is a large unmet medical need. However, modeling such a complex condition in cortical organoids is questionable. How will white matter (important in CP) be modeled in these organoids?<br>• For Aim 1, the investigators propose an interesting study of hypoxia on organoids. However, there is an extensive literature on hypoxia and stem cells. How will organoids improve upon what we already know from hypoxia studies in stem cells and neurons? For example, although it is not presented in the application, a major issue in CP are structural problems related to white matter. Do the organoids develop white matter tracts? Could those be measured for structural changes upon CP-related perturbations?<br>• Aims 2 and 3 seem very similar. There is very little hypothesis-driven science here; each Aim is just three different point mutations with similar omics readouts. This is a bit of a fishing expedition and sets the project up for purely descriptive findings with little work to establish mechanism.<br>• There is good preliminary data for the hypoxia modeling, although the RNA-seq analysis shown seems very rudimentary. For the CRISPR gene editing, the investigators show the ability to do knock-in, but not in a CP context, and without any indication that the proposed readouts post-CRISPR knock-in will yield high-impact results. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 1 **No:** 12 | • Aim 1 is hypothesis-driven and explores the impact of hypoxia on growth, morphology, apoptosis, select markers, gene expression and transcription. Aims 2 and 3 do not seem hypothesis-driven (or well differentiated) and propose to each profile 3 point mutations in different genes using omics readouts.<br>• Although some pitfalls are provided, they are superficial in their presentation. For example, from Aim 1: "Our combination of both focused and global, unbiased epigenetic and transcriptomic studies balances risks and increases the ability to find new targets by global genomics analyses." How does qPCR and RNA-seq balance risk? These are established, routine assays for many labs.<br>• Yes - but its seems that Aims 2 and 3 are quite similar. The effects of hypoxia on stem cells are quite well described already - what is so different about organoids?<br>• Potential pitfalls are identified, but the strategies to overcome them are not very clear. In addition, all possible pitfalls seem to be very "unexpected". |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 7 | • Yes - but it's not innovative and has low translatability.<br>• The work is feasible, but I am unsure that the project will yield insights into CP.<br>• The proposed team is appropriately qualified and staffed.<br>• Yes, the project is well supported by the institution's stem cell facility. |

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| | ● The budget is appropriate. |
| **No:** 6 | *none* |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 12 **No:** 1 | ● The applicants state they will use stem cells to derive brain organoids from as much of the full range of diversity as possible. However, this scope of this effort is not clearly defined. It seems the applicants plan to address DEI, but at this stage they did not explore the available resources well enough to make meaningful plans.<br>● This part seems to be recycled from a different grant concerning cancer and contains a statement about cancer health disparities in for rural residents and communities of color.<br>● Good outreach work is described. |

| Application # | DISC0-15974 |
|---|---|
| **Title** (as written by the applicant) | MADR transgenic and somatic transgenic manipulation of human iPSCs and emergent lineages |
| **Research Objective** (as written by the applicant) | We propose to translate our MADR transgenesis approach to human iPSCs, including a suite of tools. Further we will create high-grade brain tumor models with this toolset. |
| **Impact** (as written by the applicant) | The ease of genetic manipulation of human stem cells and the study of high-grade brain tumors in children and adults |
| **Major Proposed Activities** (as written by the applicant) | • Generation of MADR human iPSCs<br>• Characterization of MADR transgenesis in iPSCs<br>• Validation of inducible transgene expression with MADR<br>• Characterization of vMADR transgenesis in iPSCs<br>• Generation of high-grade somatic transgenic cerebral organoid models of brain tumors using MADR |
| **Statement of Benefit to California** (as written by the applicant) | The genetic manipulation of human cells is necessary for many of cell therapies, from the repair of cardiac tissue to the creation of CAR-T cells for cancer. Current methodologies are technically challenging, inefficient, and non-modular. Our MADR approach obviates many of these issues and many of the safety concerns surrounding transgenesis. We propose to make a MADR genetic toolset which would benefit all of the patients in California that could require such modified cells. |
| **Funds Requested** | $1,536,599 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: --

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

| | |
|---|---|
| **Mean** | -- |
| **Median** | -- |
| **Standard Deviation** | -- |
| **Highest** | -- |
| **Lowest** | -- |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 15 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 2<br>**No:** 12 | • The proposal addresses a technical aspect for some, but not all, applications. It's not addressing a major bottleneck.<br>• Transgene insertion into iPSCs is not a major bottleneck.<br>• This grant does not address a key knowledge gap, rather it is a grant that aims to produce better methods to genetically engineer hiPSCs, as opposed to lentiviral and CRISPR approaches. |

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| | • Transgenesis into iPSCs is widely done with a variety of different approaches including a recently published and highly similar technique.<br>• This is a minor technical improvement and quite similar to other techniques.<br>• Overall, this project does not address any significant knowledge gaps. It is already possible to genetically engineer human pluripotent cells, and the proposed work is mostly incremental improvements over existing approaches.<br>• It is not clear how introducing trangenes into neural organoids is transformative. There may be some utility in modeling brain cancer with this approach, but not in a patient specific way. It would not be possible to introduce a panoply of mutations easily one at a time. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 7<br>**No:** 7 | • The proposal is scientifically sound. I have no doubt that this group can do what they propose.<br>• The rationale is perfectly sound. The preliminary data shows success at introducing single copy transgenes into rodent brains using in vivo electroporation. This relies upon using mouse lines with recombinase target sequences at the ROSA26 locus. This is an existing and well-used approach.<br>• The applicants have already made a human iPSC line that harbors specified sites integrated into these cells, though it is unclear where this has been integrated and whether or not this locus expresses in a variety of differentiated lineages.<br>• All very straight-forward and feasible except the need.<br>• A panelist expressed issues with Aim #3. To make a model of "patient-specific" brain tumors, it will likely require multiple mutations to be made. It seems like the team is planning to just put in one mutation for each organoid model. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 5<br>**No:** 9 | • Aim 1 is described as a refinement, though the advance over the prior MADR paper seems very incremental and very technical. Aim 2 is a new delivery approach for the cassette and a useful alternative to other techniques. Aim 3 is jumping to a tumor organoid model and utilizing the cassette for known tumor drivers.<br>• The project is well planned and designed but not super impactful. Other techniques already exist.<br>• The approach is reasonably well designed.<br>• Gene expression may prove a problem; the appropriate promoter would need to be chosen, and the gene of interest would not be under the control of the endogenous elements.<br>• They will have to choose which isoform/splice variant to flip in. This can be problematic for genes with multiple isoforms or genes that have other elements in the introns that regulate expression. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 10<br>**No:** 4 | • They have the right expertise and have a really great paper showing that this works in vivo in mice.<br>• No feasibility concerns.<br>• The application presents a rather shallow discussion with limited alternatives presented. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 12<br>**No:** 2 | • If better models are created, better therapies would likely follow for all patient populations.<br>• Without providing detail, the applicant mentions that the host institution provides care for all patients without need to pay.<br>• There is not much depth to the DEI section other than brain cancer affects a wide range of people, and this work could make better models of disease.<br>• It would be great to include a diversity of iPSC genetic backgrounds when they create the "landing pad" cell lines.<br>• No specifics are provided regarding what will be done in the project regarding DEI principles. |

| Application # | DISC0-15893 |
|---|---|
| **Title** (as written by the applicant) | Expanding in vitro fertilization technologies via direct meiosis induction |
| **Research Objective** (as written by the applicant) | This will allow stem cells to be used as new therapies to address more types of patient infertility. |
| **Impact** (as written by the applicant) | Many populations are unserved or underserved by existing assisted reproductive technologies. We seek to eliminate bottlenecks to serving those populations. |
| **Major Proposed Activities** (as written by the applicant) | • Bioinformatics dissection of the problem<br>• Testing of the solution in human cells<br>• Animal validation of fertility applications |
| **Statement of Benefit to California** (as written by the applicant) | This will create new technologies that will enable a wider range of couples to have biological children. This will reduce the cost and medical burden of fertility treatments and make them more accessible. |
| **Funds Requested** | $1,335,834 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

**Final Score: --**

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

| | |
|---|---|
| **Mean** | -- |
| **Median** | -- |
| **Standard Deviation** | -- |
| **Highest** | -- |
| **Lowest** | -- |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 14 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
|---|---|
| **Yes:** 6<br>**No:** 7 | • The project aims to address a significant knowledge gap in the field of in vitro gametogenesis (IVG), specifically focusing on the optimization of the latter stages of gamete maturation and direct meiosis induction, which is crucial for the development of functional reproductive cells.<br>• The project identifies a major bottleneck in the development of IVG technologies, which is the efficient and scalable induction of meiosis in vitro. Addressing this could significantly advance the field.<br>• If successful, the project could advance IVG development by providing a scalable solution to generate gametes in vitro, potentially expanding fertility solutions to a wide range of underserved patient populations.<br>• Identifying factors responsible for and/or inducing iPSCs to enter meiosis would increase our understanding of cell division. |

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| | • Defining the epigenetic and transcriptional trajectory governing human meiosis is critical for understanding the basic mechanisms of germ cell development. Induction of haploidy would benefit development of human IVG and treatment of infertility.<br>• A major issue in in vitro fertilization (IVF) is cost. That will not be addressed. While the Team argues that harvesting eggs is a significant cost, the huge price tag is the hormones necessary to create an environment amenable for embryo implementation and then to keep a pregnancy.<br>• Pushing iPSCs into meiosis could lead to viable germ cells.<br>• Producing functional reproductive cells from iPSCs would have a major impact on human health.<br>• Driving in vitro meiosis and generation of haploid cells is a very complex and difficult goal. However, this application does not address many critical aspects of IVG and meiosis and thus is unlikely to succeed. This project is unlikely to have any meaningful impact on human IVG due to vague rationale and poorly planned experimental approach.<br>• This application has a very low chance of being successful based on the proposed experiments.<br>• It would be surprising if the expression of a couple of factors in hiPSCs caused cells to enter meiosis. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 1<br>**No:** 12 | • Infertility is a significant public health issue that affects millions of people worldwide (estimated that 10% of couples in the USA have issues conceiving).<br>• The project is highly relevant to human biology and disease, as it addresses infertility—a significant and growing concern in human health. By aiming to develop new IVG technologies, the project could provide solutions for individuals who are currently underserved by existing fertility treatments.<br>• The project is grounded in a clear scientific rationale, aiming to address the challenge of direct meiosis induction (DMI) in the context of IVG. The proposal outlines a multi-faceted approach, including the creation of a bioinformatics model of meiosis and optimization of DMI efficiency in various cell types, which is supported by current scientific understanding.<br>• The rationale that simple overexpression of some (non-identified yet) meiotic factors in somatic cells or iPSCs will drive in vitro meiosis and haploidy is weak. Germ cells in vivo develop not in a vacuum, their growth and maturation is intricately supported and guided by a somatic cell niche that in turn develops within gonads of an organism.It would be surprising if the expression of a couple of factors in hiPSCs caused cells to enter meiosis.<br>• Preliminary data, such as the estimated pseudotime from single-cell data of spermatogenesis and activation of predicted targets during spermatogenesis, appear to support the project's approach.<br>• There is little preliminary data. It appears that various published expression data will be re-analyzed to identify candidate genes for the hiPSC mis-expression analysis.<br>• Preliminary results in figures 1 and 2 are of poor quality and it is unclear how these data were generated. There is a lack of solid preliminary data demonstrating the feasibility of aims.<br>• The proposal might benefit from a more thorough presentation of preliminary data to establish a stronger case for the likelihood of success, particularly in demonstrating the efficacy of the proposed gene targets in inducing meiosis.<br>• Experimental details are poorly explained.<br>• it is likely that the proposed experiments cannot be accomplished.<br>• Few details on how experiments will be performed in Aims 1 and 2.<br>• Aims 1 and 2 are not likely going to be successful based on the proposed experiments. Aims 3 and 4 rely on the successful completion of Aims 1 and 2. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 1<br>**No:** 12 | • The project is methodically structured with clear objectives and specific aims, such as creating a bioinformatics model of meiosis and optimizing DMI efficiency in various cell types, which are designed to yield meaningful results in the field of IVG.<br>• The research plan includes a multi-modal approach and considers a range of mammalian species, suggesting that potential pitfalls in the methodology have been considered and alternative approaches are in place.<br>• The experimental plan is very vague based on the simplistic assumption that identification of some master regulators of meiosis will be sufficient to produce mature, haploid human gametes The application does not address how the meiotic progression will be monitored and ploidy of the resultant cells will be assessed.<br>• One goal is “to identify and directly target the gene expression program responsible for driving meiosis.” This one goal is likely to take many years and the work of many individuals. It is very unclear if the above goal is obtainable.<br>• Low chance of success. |

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| | • Experimental details are poorly explained.<br>• The proposal does not adequately address many critical aspects of IVG and meiosis, making it unlikely to succeed in filling the significant knowledge gap it aims to address.<br>• The team does not appear to have expertise in assisted reproductive technology or meiosis.<br>• The project's aims are aligned with CIRM's mission to advance stem cell and genetic research, and the timeline provided suggests a sense of urgency in achieving the project's goals. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 3<br>**No:** 10 | • The project's aims are clearly defined and logically structured, focusing on creating a bioinformatics model of meiosis, optimizing DMI efficiency, and validating DMI for IVF applications. These steps are methodically planned to build upon each other, suggesting a logical progression that could feasibly be achieved within the proposed timeline.<br>• The team appears to have a strong background in the relevant fields of in vitro gametogenesis and bioinformatics, suggesting they are well-qualified to undertake this project.<br>• The ambitious nature of the project, particularly the leap from bioinformatics modeling to practical IVF applications, may present significant challenges within the timeframe. The proposal could benefit from a more detailed timeline that accounts for potential delays in transitioning between research phases.<br>• Most experiments are unlikely to produce expected results. Aims 2, 3 and 4 are highly dependent on the success of a previous aim and thus unlikely will be even started. Verification of key stages of meiotic progression and ploidy is critical bot not planned.<br>• Major potential problems in driving meiosis in vitro are not addressed and the feasibility is uncertain.<br>• The proposal could provide more information on the specific expertise of team members in the critical areas of meiosis and IVF technology to fully assess the team's capacity to address all aspects of the project.<br>• The PI has relevant expertise in CRISPR/Cas 9 gene editing techniques, but no prior experience in biology of germ cells, IVG and IVF technology.<br>• There is no experience in relevant methods.<br>• The host institution provides modest resources and facilities for molecular biology and cell culture studies. Insufficient resources and oversight to support human IVF and animal reproductive studies planned in this project.<br>• The proposal mentions the use of the CIRM IPSC database for testing DMI in a diverse sample of patient-derived cells, indicating access to critical resources for the project's success. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 9<br>**No:** 4 | • The proposal mentions the use of the CIRM IPSC database for testing DMI in a diverse sample of patient-derived cells, indicating access to critical resources for the project's success. Otherwise not much detail provided.<br>• Will use the CIRM iPSC database to “test DMI in a diverse sample of patient-derived cells across age, sex, and ethnic backgrounds.”<br>• Disagree that the “medical and financial costs of IVF reside primarily on female patients….”<br>• Very unclear. Cost may be less, but this is not discussed in the context of DEI.<br>• Vague suggestion that “scientist-led seminars to review and update knowledge on disparities within the fertility and IVF fields” will be performed.<br>• Not many details in the application to judge DEI. Some general discussion of high IVF costs but how the proposal will lower that is unclear. |

| Application # | DISC0-15674 |
|---|---|
| **Title** (as written by the applicant) | Identification of metabolic pathways that mitigates hyperglycemic insult to fetal heart |
| **Research Objective** (as written by the applicant) | We will discover nutrients/metabolites that make the heart more healthy. |
| **Impact** (as written by the applicant) | When the mother is diabetic, the fetal hearts are immature and defective. Our product can be a preventative therapy for the fetuses from mothers with diabetes. |
| **Major Proposed Activities** (as written by the applicant) | • Identify metabolic enzymes that are responsible for cardiac maturation defect under hyperglycemia<br>• Identify nutrients/metabolites that mitigate cardiac maturation defects under hyperglycemia<br>• Predict chemicals that potentially mitigate cardiac maturation defects using artificial intelligence<br>• Confirm the nutrients/metabolite candidates to improve cardiac maturity and function<br>• Confirm the chemical inhibitors for the target metabolic enzymes to improve cardiac maturity and function |
| **Statement of Benefit to California** (as written by the applicant) | When the mother is diabetic, the babies have much higher chance of developing heart disease and anomaly. There is a clear demographic disparity in California in how many women and how many babies are affected. Our products are nutrients/metabolites that are expected to be low cost, thereby reachable to underserved communities. |
| **Funds Requested** | $1,522,702 |
| **GWG Recommendation** | (1-84): Not recommended for funding |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

**Final Score: --**

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

| | |
|---|---|
| **Mean** | -- |
| **Median** | -- |
| **Standard Deviation** | -- |
| **Highest** | -- |
| **Lowest** | -- |
| **Count** | 13 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 13 |

## KEY QUESTIONS AND COMMENTS

Proposals were evaluated and scored based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| GWG Votes | Does the project hold the necessary significance and potential for impact? |
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| **Yes:** 6<br>**No:** 6 | • The overall objective of this proposal is to define mechanisms by which metabolism can induce congenital heart disease (CHD) which represent one of the leading cause of infant mortality.<br>• Genetic anomalies account for less than 10% of CHD and there is a strong association with gestational diabetes. This is an interesting hypothesis which remains to be fully demonstrated. |

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| | • This research initiative aims to leverage a human induced pluripotent stem cell (iPSC) line to delve into the intricate process of cardiomyocyte (CM) maturation.<br>• A notable incongruence arises from the project's misleading title, which reads, "Identification of metabolic pathways which mitigate hyperglycemic insult to fetal hearts." The research does not involve the direct use of fetal hearts; rather, it employs human pluripotent stem cell (hPSC)-derived CMs as the primary subjects. The discrepancy between the project's title and its actual methodology warrants attention, as it may inadvertently mislead readers.<br>• The proposal states that it will define and address how in utero hyperglycemia may be implicated in CHD and potential development of preventable therapy for diabetic mothers. However, the experimental design does not address directly CHD and it remains unclear how results of stem cell differentiation to CM in vitro could prevent CHD in children.<br>• The core focus of this proposal lies in the investigation of metabolic pathways aimed at enhancing the maturation of CMs. It is imperative to underscore that this approach, while crucial for advancing our understanding of CM maturation, is not novel. The pursuit of optimizing metabolic pathways to bolster CM maturation has been previously explored and documented, as evidenced by the paper https://pubmed.ncbi.nlm.nih.gov/34039977/. While the proposal aligns with the broader objective of addressing the challenges associated with mature cell generation from stem cells, it is crucial to acknowledge the existing body of knowledge in this specific domain. The referenced paper serves as a testament to the pre-existing discourse on the subject, necessitating a comprehensive review of the current proposal's unique contributions in light of the established literature.<br>• Transition from stem cells to fully matured cells represents a substantial challenge. A notable instance is observed in the generation of mature CMs, where achieving characteristics such as the utilization of fatty acids as an energy source, a heightened mitochondrial mass, well-defined sarcomere structures, and augmented contraction force remains a formidable bottleneck. The mature CMs are deemed more conducive to disease modeling.<br>• Using an in vitro system to validate such hypothesis will be challenging.<br>• Given that scientists have already developed methods for generating mature CMs from stem cells, the necessity of this project is open to question. |
| **GWG Votes** | **Is the rationale sound?** |
| **Yes:** 2<br>**No:** 10 | • This project is relevant to human disease (cardiac diseases).<br>• It is possible that CM maturation can be enhanced through manipulation of metabolic pathways.<br>• This proposal lacks compelling preliminary data. A glaring inconsistency arises in the text, where the authors reference their preliminary data in Figures 2, 3, and 5. Strikingly, the proposal fails to include any corresponding data for these figures, creating a significant discrepancy that undermines the credibility of the document.<br>• In the context of Aim 1, there is a conspicuous absence of preliminary data demonstrating proficiency in efficiently infecting CMs with the CRISPR screening library and, equally crucial, maintaining proper representation of the sgRNA library. The absence of such foundational evidence raises concerns about feasibility and validity of Aim 1, leaving a notable gap in the proposal's scientific rationale and experimental foundation.<br>• The link between diabetes and CDH is relatively indirect and might not be entirely causal.<br>• The link between CDH and functional maturation of cardiomyocytes is not clear.<br>• It is unclear if the proposed approach of modulating glucose concentration during CM differentiation in vitro can be used to study in utero hyperglycemia and CHD. |
| **GWG Votes** | **Is the project well planned and designed?** |
| **Yes:** 2<br>**No:** 10 | • The project is divided into three Aims. Aim 1 will perform CRISPR screens to identify metabolic enzymes in CMs. Aim 2 will use a machine-learning-approach to screen nutrients/metabolites potentially involved in CDH. Aim 3 will validate factors identified in Aims 1/2 using cardiac organoids.<br>• The experimental plan will primarily study the effect of glucose modulation in the culture/differentiation media on maturity of CM.<br>• The experimental plan needs to be better articulated. Aim 1 and 2 are two different projects. There is little link with Aim 3.<br>• Description of experimental design and approaches is vague, making it difficult to evaluate if expected outcomes will achieve the end goals of each Aim.<br>• Figures are missing and mixed up.<br>• Aim 1 will transduce early immature CM with the lentiviral libraries for tens of thousands of sgRNAs targeting thousands of metabolic enzymes and thousands of sgRNAs targeting hundreds of solute carrier proteins (transporters) and evaluate their impact on CM maturation. There is little detail presented on how many replicates/experiments/treatment groups are planned. Also, no information on how the end results of each sgRNA will be measured and conclusions made. |

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| | • Aim 2 is extremely complicated and looks like an entirely new project.<br>• The rationale for Aim 3 on validating factors during 3D differentiation in cardiac organoids as opposed to 2D in the Aim 1 is unclear. Is 2D suboptimal compared to the 3D? Why not just conduct the Aim 1 using 3D differentiation?<br>• Aims 2 and 3 depend on the success of a previous aim(s) suggesting that if a previous aim fails or does not produce expected results, then Aims 2 and/or 3 will not be able to start.<br>• This proposal is not appropriately planned. Aim 3 is dependent on Aim 1 and 2.<br>• In the 'Sex as a Biological Variable' section, the PI included irrelevant content about mouse fetuses and Xist mRNA, which lacks relevance to the core focus of this proposal.<br>• The inclusion of unrelated information not only detracts from the proposal's coherence but also raises concerns about the thoroughness and attention to detail in crafting a well-structured and focused research plan. |
| **GWG Votes** | **Is the project feasible?** |
| **Yes:** 2<br>**No:** 10 | • The applicants have broad expertise in CDH and the link with metabolic disorders. They have performed a large amount of work in animal models in the past. The switch to hPSCs is interesting and they are developing important expertise. However, some knowledge accumulated in the past might be difficult to transfer to in vitro models.<br>• Performing genetic screens in hPSCs is challenging, as CRISPR/Cas9 can be toxic for undifferentiated cells, while its expression is strongly silenced during differentiation. So part 1 is high risk and will be difficult to achieve especially as there is no plan to express CRISPR/Cas9.<br>• It would have been incredibly useful to show that low glucose conditions increase the expression of the reporter, since Aim 1 and 2 strongly relies on this system.<br>• There are more efficient protocols to generate CMs with high functionality. The increase in function in low glucose is limited.<br>• Insufficient preliminary data is provided that would demonstrate feasibility<br>• Most experiments are feasible but unlikely to be achieved within the proposed three year timeline.<br>• Aim 1 feasibility is a concern giving that a huge number of candidate enzymes and transporters will be targeted.<br>• Aims 2 and 3 are dependent on identification of enzymes and transporters in Aim 1. Verification of expected edits for each sgRNA must be performed by more robust and up-to-date approaches, such as whole genome sequencing.<br>• Concerns about the feasibility are:<br>  ◦ Viral Infection in CMs: The ambitious inclusion of a CRISPR library with tens of thousands of single guide RNAs (sgRNAs) raises questions about the practicality of viral infection in CMs. The onus lies on the applicant to furnish preliminary data illustrating their capability to execute viral infection while preserving the sgRNA composition within the infected cell library.<br>  ◦ Reliance on the sole readout in Aim 2 prompts concerns about its adequacy as a comprehensive indicator of CM maturity. The field acknowledges the multifaceted nature of CM maturation, and utilizing a single marker may not capture the intricacies of this process adequately. It is imperative to address these concerns by either justifying the exclusive use of the proposed marker, or incorporating additional markers to provide a more comprehensive assessment of cardiomyocyte maturity. Failing to do so risks compromising the robustness and depth of the research in Aim 2.<br>• It is advisable for this team to consider incorporating an expert proficient in CRISPR screening. Given the intricate nature of the project, particularly concerning the viral infection of CMs with a CRISPR library containing a substantial number of sgRNAs, having an experienced CRISPR specialist on the team would enhance the overall technical capabilities. This addition would contribute valuable insights into optimizing the experimental design and ensure the successful execution and interpretation of the complex CRISPR screening methodology, potentially addressing challenges and optimizing the efficiency of the project.<br>• They may not have sufficient resources for CRISPR screening in CMs. |
| **GWG Votes** | **Does the project uphold the principles of diversity, equity and inclusion (DEI)?** |
| **Yes:** 10<br>**No:** 2 | • The applicants clearly defined the importance of diversity but the experimental plan could consider this aspect in more detail.<br>• They plan to use different hPSC lines for research. There is no mention about race and ethnicity of their lines.<br>• Cardiac disease impacts underserved racial/ethnic communities.<br>• The applicant did not describe prior efforts for outreach activities to inform DEI development.<br>• Human ESCs/iPSCs used in this proposal do not appear represent diverse race/ethnic/gender/age groups. |