APP #	TITLE	BUDGET REQ	FUND?	SCORE (MEDIAN)	Mean	SD	Low	High	Y	N	Resubmission	Previous CIRM Funding	Area of Impact
DISC0-14422	Engineering pluripotent stem cells for universally available, off-the-shelf T cell therapies	**$1,352,753**	**Y**	**90**	90	1	85	90	15	0	N	Y	Explores gene editing approaches that would create a platform to more easily produce universal CAR T cell therapies.
DISC0-14429	Identifying roadblocks to neural stem cell transplantation into human tissues.	**$1,551,394**	**Y**	**90**	89	2	85	90	15	0	N	N	Generates a comprehensive map of neural stem cell differentiation properties to inform development of cell therapies.
DISC0-14503	Characterization and applications of human blastoids for understanding early human embryogenesis	**$1,402,137**	**Y**	**90**	89	3	84	95	14	1	N	N	Use of blastoids to better understand human embryo development and address issues related to infertility.
DISC0-14448	Role of ataxin-3 polyadenylation site selection in ALS neuron toxicity and disease pathogenesis	**$1,514,416**	**Y**	**90**	87	6	70	90	12	3	N	N	Explores the role of ataxin-3 polyadenylation in ALS to better understand pathogenesis of the disease.
DISC0-14449	Modeling Retinitis Pigmentosa using patient-derived human iPSC organoids	**$1,612,617**	**Y**	**88**	88	3	80	90	14	1	N	N	Develops an organoid model of retinitis pigmentosa to understand pathogenesis and develop therapeutics.
DISC0-14405	Establishment of a novel approach to systematically study the dynamic organization of protein complexes in stem cells	**$1,515,601**	**Y**	**88**	86	4	80	90	12	3	Y	N	Develops a framework to study the complexity of multiple molecular interactions in cells.
DISC0-14357	Understanding Chemotherapy-Induced Peripheral Neuropathy Mechanisms using CRISPRi and Chemical Screens in Human iPSC-Derived Sensory Neurons	**$1,621,913**	**Y**	**86**	86	1	85	88	14	0	N	N	Studies the mechanisms of chemotherapy-induced toxicity to sensory neurons derived from iPSC.
DISC0-14460	Ex vivo fate mapping of human lung stem cell plasticity in fibrotic disease	**$1,625,998**	**Y**	**85**	86	1	84	88	14	1	N	N	Explores the role of lineage and plasticity of alveolar stem cell conversion to basal cells in lung fibrosis.
DISC0-14392	Harnessing vascular stem cells to grow and protect the human brain	**$1,625,998**	**Y**	**85**	86	3	80	90	12	3	N	N	Studies the role of vascular cell subtypes in development of the blood brain barrier.
DISC0-14350	The role of WNT and BMP signaling pathways in iPSC to iTenocyte step-wise differentiation for tendon repair	**$1,516,563**	**Y**	**85**	85	0	85	85	15	0	N	N	Explores methods using BMP and WNT signaling to generate tendon cells from iPSC for possible tendon repair strategies.
DISC0-14458	Overcoming barriers for airway stem cell gene therapy for Cystic Fibrosis	**$1,472,858**	**Y**	**85**	85	1	84	88	14	1	Y	N	Explores development of a cystic fibrosis gene therapy that targets the difficult to access airway stem cells.
DISC0-14424	Functional genomics to study cellular convergence across ASD risk genes in neurodevelopment	**$1,575,001**	**Y**	**85**	85	2	78	85	13	1	Y	N	Studies the functional effects of several ASD risk genes in a neural organoid system across multiple parameters.
DISC0-14521	hPSC-derived enteric ganglioids for cell therapy in gastrointestinal motility disorders	**$1,589,307**	**Y**	**85**	85	2	82	88	10	5	Y	N	Proposes the derivation, purification and characterization of enteric ganglioids derived from iPSCs for cell therapy.
DISC0-14447	Mapping the spatial and temporal responses of hESC-derived microglia to repeat mild closed head injury to identify therapeutic targets and mechanisms	**$1,555,140**	**Y**	**85**	83	8	60	90	10	5	N	Y	Studies the molecular response of microglial cells in a head injury model and tests effect of a possible therapeutic.
DISC0-14519	Defining the source of dysfunction in monogenic Intellectual Disability Syndrome neurons	**$1,500,337**	**Y**	**85**	83	6	75	92	8	7	Y	N	Explores how a loss of function of chromatin regulatory proteins leads to neuronal dysfunction in IDS.
DISC0-14366	Determining how age-specific heterogeneity of human hematopoietic stem cells and megakaryocyte progenitors contribute to thrombotic disease upon aging	**$1,536,000**	**Y**	**85**	82	7	65	89	8	7	N	Y	Studies the mechanisms that contribute to age-dependent dysregulation of blood stem cells.
DISC0-14514	An interactive data resource for hypothesis testing in stem cell single-cell gene expression and validation of the results with brain organoids	**$1,160,126**	**N**	**83**	85	6	79	100	6*	9	N	Y	
DISC0-14450	Investigating epigenetic reprogramming and cell extrinsic signaling events in the specification of human primordial germ cells	**$1,182,193**	**N**	**82**	81	2	75	85	1	14	N	N	

APP #	TITLE	BUDGET REQ	FUND?	SCORE (MEDIAN)	Mean	SD	Low	High	Y	N	Resubmission	Previous CIRM Funding	Area of Impact
DISC0-14499	Exploring pregnancy-associated systemic factors to rejuvenate aged stem cells - a new frontier in regeneration	**$1,543,645**	**N**	**82**	76	14	45	89	7*	8	N	N	
DISC0-14566	Immune cloaking of human stem cell-derived insulin producing cells for curative cell therapy without immunosuppression	**$1,192,586**	**N**	**80**	82	4	75	90	6*	9	N	N	
DISC0-14365	Cellular modeling of GATAD2B-associated neurodevelopmental disorder (GAND): Investigation of cellular and molecular anomalies affecting NuRD Activity	**$1,094,536**	**N**	**80**	81	2	80	85	1	13	N	N	
DISC0-14386	Interrogating Satellite Cell and Myofiber Defects and Repair in Human DMD using Single Nuclei/Single Cell RNA Sequencing of Muscle Resident Cells	**$1,559,931**	**N**	**80**	80	4	75	89	3	12	N	Y	
DISC0-14451	Using Human Neurons to Model Parkinson's Disease and Develop Therapeutics	**$1,542,234**	**N**	**80**	80	4	70	85	3	12	Y	Y	
DISC0-14342	Making of Geometry: Mapping the mechanics that shapes the human neural tube.	**$1,188,134**	**N**	**80**	80	2	75	82	0	15	N	N	
DISC0-14430	Decoding human symmetry breaking in 3D with optogenetics	**$1,191,101**	**N**	**80**	79	3	75	85	1	14	Y	N	
DISC0-14490	Developing a granulocyte macrophage progenitor-based immunotherapy	**$1,663,052**	**N**	**78**	78	5	70	85	2	13	N	N	
DISC0-14441	Treating non-healing ulcers by regulating outgrowth from cellular building blocks	**$1,567,884**	**N**	**77**	78	4	70	85	2	12	N	N	
DISC0-14377	Understanding the mechanisms and developing therapeutics for the neurodegenerative Parkinson's disease using human iPSCs	**$1,570,824**	**N**	**75**	77	8	70	92	5	10	N	N	
DISC0-14403	Plasticity and Endogenous Regeneration in Dental Injury and Repair	**$1,341,968**	**N**	**75**	77	4	74	90	1	14	Y	N	
DISC0-14457	Modeling and understanding alveolar hypoplasia in Down syndrome using iPSCs-derived alveolar type II cells	**$1,604,418**	**N**	**75**	77	2	75	80	0	14	N	N	
DISC0-14385	A novel population of autologous neural precursor cells for the treatment of brain injury following germinal matrix hemorrhage in preterm infants	**$1,510,035**	**N**	**75**	75	4	70	80	0	15	N	N	
DISC0-14470	Physiological and pathophysiological roles of eIF4G2, a putative regulator in translation initiation, in pluripotent and intestinal stem cells	**$1,739,760**	**N**	**75**	75	1	75	78	0	15	Y	N	
DISC0-14518	Investigating pediatric hematopoiesis in situ during steady state	**$1,513,522**	**N**	**75**	75	4	70	81	0	15	N	N	
DISC0-14581	Zika virus pathogenesis at single cell resolution: uncovering cellular mechanisms and therapeutic targets	**$1,605,342**	**N**	**75**	75	4	70	80	0	15	N	N	
DISC0-14587	Implementing a coupled system of integrative ML modeling and data validation in Alzheimer's disease	**$1,200,000**	**N**	**75**	75	1	70	75	0	15	N	N	
DISC0-14531	Stimulating a cartilage-resident progenitor population for regeneration in aging and OA	**$1,500,261**	**N**	**75**	74	3	70	78	0	15	N	N	

APP #	TITLE	BUDGET REQ	FUND?	SCORE (MEDIAN)	Mean	SD	Low	High	Y	N	Resubmission	Previous CIRM Funding	Area of Impact
DISC0-14567	The influence of human neural stem cells on autoimmune and regenerative function in mouse models of multiple sclerosis	**$1,549,209**	**N**	**75**	74	3	65	75	0	15	Y	Y	
DISC0-14346	Nodal Organoids to Dissect the Molecular and Cellular Architecture of Cardiac Pacing	**$1,598,398**	**N**	**70**	71	3	70	82	0	15	N	N	
DISC0-14533	Investigating the Role of Microglia in Autism Spectrum Disorder Using Patient-Derived hiPSCs in Culture and Cerebral Organoid Models	**$1,837,714**	**N**	**70**	70	1	65	70	0	15	Y	N	
DISC0-14456	Development of a synthetic cellular model of human embryonic Implantation	**$1,510,160**	**N**	**70**	68	7	60	80	0	15	N	N	
DISC0-14559	Machine Learning to Guide Design of CRISPR Engineered T Cell Therapies for Cancer	**$1,710,858**	**N**	**70**	68	8	50	84	0	15	N	N	
DISC0-14530	Engineered injectable pre-vascularized microporous implants for neural stem cell transplantation after stroke	**$1,605,001**	**N**	**65**	65	5	60	70	0	15	N	N	
DISC0-14543	Effects of antipsychotic drugs on early brain development and cellular function in schizophrenia compared to controls	**$1,571,057**	**N**	**65**	64	9	40	75	0	15	Y	N	
DISC0-14591	Phenotypic characterization of ALS and control iPSC-derived motor neuron and microglia differentiation via an automated stem cell culturing platform	**$1,181,250**	**N**	**65**	64	6	55	75	0	15	N	N	
DISC0-14510	Engineered nanotechnology for neural progenitor cell transplantation in Sanfilippo syndrome	**$1,605,000**	**N**	**60**	63	3	60	70	0	14	N	N	
DISC0-14504	Promoting differentiation of adult stem cells for the treatment of Multiple Sclerosis and other demyelinating diseases	**$1,920,000**	**N**	**60**	59	7	45	65	0	15	N	N	

*** Minority Report**

Application #	DISC0-14422
Title (as written by the applicant)	Engineering pluripotent stem cells for universally available, off-the-shelf T cell therapies
Research Objective (as written by the applicant)	Our goal is to develop new gene editing methods for producing universal, off-the-shelf, therapeutic T cells from induced pluripotent stem cells (iPSC) that can be applied to a range of diseases.
Impact (as written by the applicant)	We will develop new gene editing approaches to overcome the block to T cell development from iPSC that occurs when key genes are deleted to create universally, immunologically compatible chimeric antigen receptor (CAR) T cells.
Major Proposed Activities (as written by the applicant)	• Development of a gene editing strategy to generate mature CART cells from iPSC • Development of gene editing strategies to create universal T cells from iPSC for immunotherapy • Functional comparisons of T cells produced from iPSC by different experimental approaches
Statement of Benefit to California (as written by the applicant)	Approximately 60,000 Californians will die of cancer each year. While exciting successes have been reported using T cell therapy to cure blood cancers, many patients are unable to access this novel therapy and results for solid tumors such as brain cancers are still poor. Our goal is to produce an off-the-shelf universal T cell product from pluripotent stem cells to dramatically expand the reach of this promising therapy.
Funds Requested	$1,352,753
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." 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	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: 15	• The project aims to identify methods to produce functional single positive CART cells from iPSCs. Current limitations of allogeneic cell therapies produced from iPSCs include the requirement for a functional T-cell receptor (TCR) for positive selection; however, TCR expression must be eliminated to prevent graft versus host disease (GVHD) in cell-therapy recipients. Through stage-restricted expression of a helper TCR or CAR stimulation, the project aims to overcome this bottleneck. • The applicants will tackle two critical bottlenecks that face the field of iPSC-derived T cell immunotherapy. Both of these bottlenecks exist for iPSC-derived T cell products because the genetic manipulations required for production of CART from allogeneic sources of iPSC dysregulate the normal process of T cell differentiation. • The generation of T cells from iPSCs holds great promise, but current differentiation strategies prevent the generation of single positive cells due to the lack of positive selection or through CAR signaling that influences innate immune differentiation. • Because of high costs and the constraints imposed by clinical imperatives, access to promising therapies that use autologous (patient-specific) T cells is severely limited. Thus intense interest has developed in exploring allogeneic sources for an off-the-shelf product with universal applicability. Therefore, if successful, this project will have a major impact on the CAR-T therapy field. • If successful, the project would produce a platform that may be applicable to multiple CAR therapies and would allow the research community to advance iPSC-based T cell therapies. • The work addresses a significant bottleneck in the field. • This proposal addresses a very important clinically relevant area.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 15	• The proposed project has sound rationale. TCR expression and major histocompatibility complex (MHC) interaction are required for positive selection, and both molecules are disrupted in current iPSC cell banks for allogeneic T cell therapies. Introduction of transient signals through a helper TCR or a CAR may overcome these bottlenecks. • This project is based on sound rationale. The applicants will combine transient expression of a "helper" TCR to induce positive selection, with late expression of CARs in mature T cells to generate iPSC-derived non-alloreactive, mature, conventional CART cells. • iPSC-based T cell therapies hold great promise for cancer immunotherapy and the project may provide an improved platform to generate single positive CAR+ CD8+ T cells from iPSCs. • The preliminary data are supportive of the innovative approach. • The preliminary data are strong, especially with regards to the editing strategy. • iPSC-based T cell therapies hold great promise for cancer immunotherapy and the project may provide an improved platform to generate single positive CAR+ CD8+ T cells from iPSCs.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 15	• The applicant proposed three aims for this project. These three aims are coherently linked and should provide new insights on how to generate universal iPSC-T cell therapy. • Pitfalls and alternative approaches are appropriately addressed. • The timeline is appropriate. • This proposal builds upon a strong foundation of organoid platform development in the field. • The project is well designed. One potential limitation is that the requirement for NY-ESO-1 and HLA-A2 expression in stromal cells of the organoid platform is not mentioned. It is assumed that helper TCR expressing T-iPSCs will require recognition on stromal cells for positive selection.
No: 0	*none*
GWG Votes	**Is the project feasible?**

Yes: 15	• The lead investigator is an expert in the generation of T cells from iPSCs and has generated organoid platforms that allow productive differentiation to single positive T cells. A collaborator with experience in CAR design and clinical use of CART cells is included. Other team members provide technical support. • The proposed team is appropriate for this project and has the necessary expertise. • The team includes leaders in the field. • The resources and facilities are appropriate and sufficient for the proposed studies. • The proposed aims have a high likelihood of success and feasibility is strong.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• The generation of T-cell therapies that are agonistic to HLA would extend the applicability of these cell therapies for underserved populations. • Prior educational activities are described. • The applicant is committed to addressing diversity, equity and inclusion (DEI) challenges in the workplace. The applicant believes strongly that a diverse team and an inclusive environment is essential to accomplish the highest standards in research and education. • There is no plan to account for race, sex, or gender as variables in the production of T-iPSCs.
No: 0	*none*

Application #	DISC0-14429
Title (as written by the applicant)	Identifying roadblocks to neural stem cell transplantation into human tissues.
Research Objective (as written by the applicant)	We will generate a comprehensive map of human neural stem cell differentiation profiles that will serve as a reference for enhancing neural stem cell-based therapies.
Impact (as written by the applicant)	Our project will develop improved protocols for human neural stem cells differentiation, enhancing the fidelity, safety and robustness of future cell therapies.
Major Proposed Activities (as written by the applicant)	• Establish quantitative map of neural stem cell differentiations to serve as a reference. • Determine how radial glia neural stem cell differentiation is impacted by genetic or environmental perturbation. • Quantitatively compare neural stem cell differentiation in human and cerebral organoid model. • Determine mechanisms underlying neural stem cell differentiation in human developing brain tissue. • Compare neural stem cell survival in mouse brain tissue.
Statement of Benefit to California (as written by the applicant)	Californians asked CIRM to support stem cell research towards treatments for brain disorders by earmarking 35% of funds for central nervous system (CNS) projects. Cell therapy applications in the nervous system have the potential to address CNS disorders including epilepsy, stroke, and neurodegenerative disorders. However, neural stem cell transplantation into human brain tissue is inefficient and poorly understood. Our project directly addresses these limitations using a combination of genetic and cellular approaches.
Funds Requested	$1,551,394
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." 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	89
Median	90
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	• The project is designed to compare differentiation trajectories of radial glia after transplantation into adult brain, and identify factors that enhance survival post transplantation. This is a relevant and largely unexplored area of transplantation based brain repair. • The proposed project is intended to identify genes and pathways that control the fate of stem cells transplanted into humans with neurodegenerative conditions. While somewhat speculative, the proposed studies have the long term potential to help establish such therapies. • Human cortical development is not fully understood; the nature and fate of progenitor populations is not clear. Factors that would enable engraftment of neural progenitors into the adult brain are not clearly understood. • The applicant aims to better understand development of neural stem cells in the human. Gaps exist in our knowledge of human-specific cortical progenitors, particularly truncated radial glia. • Cortical organoids are widely used now. This study could be helpful to (i) the development of cell therapies and (ii) the fidelity of cellular modeling of human cortical development. • The potential impact is more in the comparison between in vitro and in vivo differentiation trajectories than in survival post transplant. • This is a relevant and largely unexplored area of transplantation based brain repair.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 14	• The study is based on a comparison of neural progenitor cells derived from pluripotent stem cells (PSC) to cells present in the developing human brain. Previous work has identified different classes of radial glia but has not established their precise role in cortical development. • Preliminary data using cortical slice technique indicate very different developmental potential of two types of radial glial cells. Applicant has developed powerful organotypic cell culture systems to analyze cell fate. • Yes. Background knowledge for assessing (i) the fidelity of in vitro models and (ii) tissue integration of cellular therapeutics is critical to successful use of PSC in research and therapy. • The technical plans and preliminary data in the proposal are very strong. The final goal, to identify specific regulatory genes and pathways is somewhat speculative. • The applicant provides good preliminary data that support successful execution of project. • The relevance of Aim 3 is not clear and does not model transplantation into tissue. • The project is based on sound scientific rationale.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 14	• The applicant proposes excellent experimental approaches using primary human tissue, stem cells, and lineage tracing. • The studies Aims 1 and 2 are largely descriptive, extend earlier studies by the applicant, and are likely to contribute to general knowledge in developmental biology. The unique focus on human tissues and mechanisms is a special advantage of these proposed studies. • Aim 3 attempts to identify molecular roadblocks to neural stem cell transplantation into the human brain, since transplantation of immature human neurons may offer therapeutic opportunities for neurodegenerative diseases. Access to human brain tissue is a key asset of the proposal. • Under Aim 3, the applicant will generate slice cultures of human tissue and study the differentiation and migration of co-cultured stem cells in this system. Transplanted cells will be isolated two weeks after the start of the co-cultures. From these cells, the applicants seek to identify potential sgRNAs for a CRISPR screen for factors influencing

	integration of cells into tissue. It is anticipated that this screen will point to candidate genes regulating survival and integration of transplanted cells. ● As an alternative approach, if the screen is unsuccessful, the applicant proposes to use fractionation of serum to identify candidate factors necessary for radial glial cell specification. Serum fractionation has been widely used to identify growth factors several decades ago and thus seems somewhat anachronistic now. ● The studies in Aims 1 and 2 are very well designed and uses unique material. The slice culture studies in Aim 3 may not adequately mimic a therapeutic transplantation. ● Cell engraftment into slices may not mimic cell therapy administration in vivo. ● The project plan and timeline demonstrate an urgency that is commensurate with CIRM's mission. ● Potential pitfalls are identified and alternative approaches presented. ● The discussion of pitfalls should be expanded.
No: 0	*none*
GWG Votes	**Is the project feasible?**
Yes: 14	● Newly identified genes might lead to therapeutic approaches to achieve success of stem cell transplantation in humans affected by neurodegenerative diseases. The success of this project is very difficult to predict. ● Well designed studies. The PI is an early career researcher with relevant experience and training. ● Aims 1 and 2 are very feasible. It's not clear if the studies in Aim 3 will truly model the clinical situation, but they still may be worth trying. ● The project is demanding and ambitious but feasible. ● This is a very qualified team with unique access to human tissue. ● The team has access to all the necessary resources to conduct the proposed activities. ● The budget is appropriate for the research proposed.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	● Yes. The human samples to be used in the proposed studies will come from a highly diverse population. ● Brain samples will be collected from a diverse patient population. Other aspects of DEI are not well articulated. ● The project plan and design adequately address and account for the influence of race, ethnicity, sex, gender, and age diversity. ● The project outcomes extend or validate the applicability of regenerative medicine discoveries to underserved populations, including underserved racial/ethnic communities. ● The applicant describes prior efforts or proposed plans for outreach, partnership, or educational activities to inform the development of DEI within the research project.
No: 0	*none*

Application #	DISC0-14503
Title (as written by the applicant)	Characterization and applications of human blastoids for understanding early human embryogenesis
Research Objective (as written by the applicant)	Our work will yield an improved stem-cell based embryo model that we will explore with various omics approaches and genetic screens to gain insights into the pathways that control human embryos.
Impact (as written by the applicant)	An improved stem cell-based embryo model is a crucial step for in-depth studies of human development and will enhance our ability to understand and treat infertility and screen for new contraceptives.
Major Proposed Activities (as written by the applicant)	• To identify a method that enables robust formation of a stem cell-derived embryo model across diverse genetic backgrounds. • Assess the transcriptional, epigenetic and karyotype state of the embryo model. • Examine the paracrine signals and metabolomic regulation of the stem cell-derived embryo model. • Perform whole-genome and high-content genetic screens and in-depth dissection of gene-specific knockouts with the stem cell-derived embryo model.
Statement of Benefit to California (as written by the applicant)	In 2014, 21,018 in vitro fertilization (IVF) procedures were performed in CA, resulting in 7,230 live-birth deliveries and 8,793 infants born. This shows that IVF is inefficient, causing providers to transfer multiple embryos, which can result in multiple births and adverse health effects. Our work has the potential to develop an improved embryo model for studies of human development, infertility, and IVF approaches. The project will also employ six individuals to carry out the project, creating jobs within the state.
Funds Requested	$1,402,137
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." 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	89
Median	90
Standard Deviation	3
Highest	95
Lowest	84
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	• The project addresses a key knowledge gap in our understanding of the biology and application of stem cells, specifically in the area of preimplantation and implantation human development. • This is a key bottleneck in the field of regenerative medicine, as the ability to generate blastoids and perform genetic screens will have a major impact on our ability to study and potentially treat human diseases. • The project has significant potential for impact, as it could lead to the development of new therapies and treatments for a wide range of diseases and conditions. • Ultimately, the success of the project could have far-reaching implications for human health and well-being, making it a high-priority area of research. • Blastoids are a helpful model and as always with new models, questions about the optimal conditions remain. Aim 1 and 2 will add to that. Aim 3 will add possibly insights on upstream drivers. • Blastoids need a deeper biological understanding to enable downstream uses.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 14	• The rationale for the study is very sound, as others in the field have already accomplished similar structures and there is strong evidence to suggest that blastoids can be used to study human development and disease. • The project is highly relevant to human biology, as it seeks to better understand the early stages of human development and the genetic mechanisms that underpin this process. • Excellent plan to really explore blastoid development in a rigorous manner. • The preliminary data gathered by the researchers is extremely compelling and provides a strong foundation for the project. • The use of other techniques (e.g., inducible transcription factor) may help broaden the impact. • The proposal seeks to systematically apply their conditions and generate blastoids from over a dozen different PSC lines, followed by transcriptional and molecular profiling. Then they will perform secretome analysis and then test effects of candidate proteins on blastoid formation, and perform a knockout screen.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 14	• The study is extremely well-designed, with a logical progression of steps that build on one another to achieve the overall research objectives. • The project is divided into three main phases, including an optimization step to improve the efficiency of blastoid generation, the identification and characterization of the blastoid secretome, and the use of genetic screens to study gene function in blastoids. • The study design is carefully considered, with potential pitfalls and alternative approaches discussed in detail to ensure that the researchers are prepared for any challenges that may arise. • There is a clear sense of urgency in the timeline for the project, with the researchers aiming to complete the study within a relatively short time frame to maximize the potential impact of their findings. • All in place and logical design. • Highly rigorous.
No: 0	*none*
GWG Votes	**Is the project feasible?**
Yes: 14	• The project is highly feasible, with well-defined aims that are designed to be completed within the timeframe of the study. • Expertise of the PI and team is outstanding and will ensure success. • The research team and their collaborators are highly qualified and experienced, with the necessary expertise and resources to carry out the project successfully. • Lab has the required cell biology and molecular genomics expertise.

	• The project has been carefully budgeted to ensure that the necessary resources are available to support the research, including personnel, equipment, and supplies. • The team has access to the necessary facilities and infrastructure to carry out the project, including specialized equipment and expertise in stem cell research and genetic screening. • It seems ambitious to complete all experiments with the proposed number of lines.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• Big selection of representative cell lines. • Very strong. The applicant addresses diversity in the planning and design of the project with the variety of cell lines, and has described proposed plans for developing DEI within the project. • Multiple cell lines will be studied.
No: 0	*none*

Application #	DISC0-14448
Title (as written by the applicant)	Role of ataxin-3 polyadenylation site selection in ALS neuron toxicity and disease pathogenesis
Research Objective (as written by the applicant)	Here we will study the role of ataxin-3 alternative polyadenylation in the pathogenesis of ALS, and test if ASOs can reduce distal polyadenylation of ataxin-3 to rescue ALS disease phenotypes
Impact (as written by the applicant)	Our goal is to determine if ataxin-3 genetic dysregulation is a target for the development of therapies to treat ALS (Lou Gehrig's disease), frontotemporal dementia, and Alzheimer's disease
Major Proposed Activities (as written by the applicant)	• Assay ataxin-3 expression and function in motor neurons + cortical neurons derived from human stem cells for 3 ALS patients (who display a high-risk genetic alteration in ataxin-3) and 3 controls • Pinpoint the genetic alterations in the ataxin-3 gene most likely to account for high risk of developing ALS • Genetically modify a control human stem cell line to convert it to a high-risk ALS version by introducing the ataxin-3 genetic alteration • Assay ataxin-3 expression and function in motor neurons + cortical neurons derived from the genetically modified control line created in Activity 3 in comparison to its control (isogenic) counterpart • Develop a genetic therapy known as antisense oligonucleotides (ASOs) in human stem cells that is capable of reversing the effect of the ALS disease-causing genetic alteration in the ataxin-3 gene • Test if the most potent ASOs identified in Activity 5 can counter the ALS genetic risk factor in the ataxin-3 gene and thereby prevent ALS disease phenotypes in human stem cell-derived neurons
Statement of Benefit to California (as written by the applicant)	There are no highly effective therapies to treat ALS and a closely related disorder known as frontotemporal dementia (FTD), where patients show aggregation of TDP-43 protein. Here we will study if a genetic alteration in a specific gene predisposes individuals to developing TDP-43 protein abnormalities, which are a central feature of ALS, FTD, and Alzheimer's disease, and will determine if a genetic therapy directed at this defect could hold promise as a therapy for these devastating disorders.
Funds Requested	$1,514,416
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.” 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	87
Median	90
Standard Deviation	6
Highest	90
Lowest	70
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	12
(1-84): Not recommended for funding	3

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 application tests an interesting specific hypothesis, that alternative polyadenylation sites (APA) in the ataxin-3 (ATXN3) regulate TDP-43 functions and the neurodegenerative mechanisms leading to ALS. The studies are highly relevant for understanding and treating this devastating neurodegenerative disease. • TDP-43 has a critical role in pathology neurodegenerative disorders but it is far from clear how to attack this pathway therapeutically. • Therapies to slow TPD-43 dysfunction are not available presenting a large knowledge gap. Applicant will use patient derived iPSC lines to design and screen antisense oligonucleotides (ASO) that are able to shift ATXN3 polyadenlylations. • If successful, the genetic therapy could provide a treatment not only for ALS but other related disorders. • The approach is novel and could, while perhaps not curative, act as disease modulator. • Study addresses the potential for an ASO targeted on a newly identified candidate risk gene in ALS, potentially broader ramifications to a range of neurodegenerative disorders. • Potentially this project could open up a new approach to degenerative disease treatment, in particular ALS. • The applicants were not clear on what proportion of patients carry the variant - the therapy may address only a small minority.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 14	• The rationale for the project is scientifically and logically sound. It is based on convincing preliminary data obtained in cell lines, initial data obtained in human iPSC cells, and postmortem human material. • The project is based on the observation that aberrant accumulation of TAR DNA-binding protein 43 (TDP-43) is a pathological hallmark of many neurodegenerative disorders including ALS. • The rationale is well described and sound. Mislocalization of TDP-43 affects the transcriptome but potent modifiers of TDP-43 pathology are lacking. Genetic variants that affect alternative polyadenylation (APA) of the ATXN3 transcripts lead to transcripts with alterations that are also strongly associated with the accumulation of TDP-43 and risk of developing ALS. • Gain and loss of ATXN3 support the hypothesis. Post-mortem frontal cortex obtained from ALS/FTD patients show inversely proportional levels of phosphorylated key proteins. Knockdown in iPSC derived motor neurons leads to accumulation of TDP-43. • Role of TDP-43 in neurodegeneration is clear. • Preliminary genetic data on ATXN3 polyadenylation in disease are compelling, as are knockdown data in cells. It would be helpful to know what proportion of ALS patients and cell lines have this variant.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 13	• Using motoneurons and cortical neurons derived from human iPSC and the demonstrated expertise of the applicants with such cells represents a unique strength of the application. • The project uses human neurons derived from hPSC. Collaborators are expert in development of ASO, which will be aimed at modulating alternative polyadenylation site usage. Project will also use a variant and isogenic control. • iPSC protocols are established to generate motor neurons and cortical neurons. Screening approach is feasible. Applicants already identified and requested access to ALS iPSC lines from which iPSC derived neurons will be generated. ATXN 3 expression

	will be determined and a variety of appropriate assays will be performed to determine the functional significance of ATXN3 APA. ● Identification of candidate causal SNP variants regulating ATXN3 APA and validation by genome editing are well described. Analysis of non-neuronal derivatives is an additional strength. Proposed experiments for Aim 2 are well defined. ● A concern relates to the proposed studies evaluating the therapeutic potential of ATXN3 APA modulation. It is proposed to test the ASOs in iPSC lines from affected patients for their ability to reduce toxicity induced in two different ways and electrophysiological hyperexcitability. While interesting, three cell culture approaches may be overkill. A single test system may be sufficient. ● The key goal of these in vitro efficacy studies should be that they are a gating step for further development of ASO for clinical use. The cell culture studies are unlikely to be sufficient to move the ASOs into clinical testing. There is no discussion of relevant transgenic animal models. ● Targeting the ASO to motor neurons specifically is not addressed. ● How generally applicable will the results from a few cell lines with most extreme variants be to the general population?
No: 1	*none*
GWG Votes	**Is the project feasible?**
Yes: 14	● Highly achievable. Excellent discussion of pitfalls and and informative discussion of alternative approaches. ● PI is an experienced physician scientist with a long track record in neurodegeneration. Team includes experts in computational genetics and RNA processing and ASO technology. ● The proposed aims are logical and timelines are aggressive but potentially achievable. ● Aims are straightforward - use of cell culture models to assess role of ATXN3 polyadenylation variants and test ASO. Preliminary data on motor neuron differentiation seem limited. ● Alternative approaches are provided.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	● The applicants provide convincing descriptions of DEI related outreach to be conducted during the funding period. ● Well described. ● Candidate will access patient material from Hispanic/African American cohort; outreach not well articulated.
No: 0	*none*

Application #	DISC0-14449
Title (as written by the applicant)	Modeling Retinitis Pigmentosa using patient-derived human iPSC organoids
Research Objective (as written by the applicant)	The objective of this proposal is to develop a human retinal organoid model of autosomal dominant retinitis pigmentosa (adRP) to gain insights in pathogenesis and assess clinically relevant approaches to restore rhodopsin (RHO) protein function.
Impact (as written by the applicant)	Upon successful completion of this study, we will have established a disease-in-a-dish model and a novel therapeutic approach towards management of the devastating outcomes in RHO-associated adRP.
Major Proposed Activities (as written by the applicant)	• Recruitment of additional patients with RHO adRP due to copy number variation and RHO P23H mutation • Generation of additional iPSCs with adRP due to RHO mutations • Characterization of rod photoreceptor dysgenesis in RHO mutations • Studies into disease mechanism due to RHO mutations • Evaluation of small molecule NR2E3 inhibitor to restore phenotype • Evaluation of anti-sense oligonucleotides as a strategy to restore phenotype
Statement of Benefit to California (as written by the applicant)	Retinitis Pigmentosa (RP) leads to devastating visual impairment in millions of individuals in the US and in the state of California. Most individuals are legally blind by age 40. Thus, this results in a tremendous stress in the state of CA's resources. In addition, these disorders result in both a monetary and psychological stress on the family especially since the disorder often runs in families. Using patient's stem cells, we aim to better understand the disorder and identify new therapies.
Funds Requested	$1,612,617
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.” 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: 88

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	88
Standard Deviation	3
Highest	90
Lowest	80
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	• The project proposes advances the development and use of human stem cells in an organoid model for dominant negative/autosomal dominant Retinitis Pigmentosa (adRP). • The applicants propose creating a reliable human organoid experimental model of adRP by generating iPSCs from patients with RP to elucidate disease pathogenesis. They also propose developing a novel therapeutic approach: strategies to try to regulate protein expression of Rhodopsin (RHO) using small molecules and anti-sense oligonucleotides. • If the project is successful, the availability of a human cell model of RP would be impactful for the research community for both better elucidation of the disease and identifying potential targets for treatment. • Developing a human retinal organoid model of adRP would be a significant advance in the study of this disease, which is currently incurable and can lead to vision loss and blindness. • Currently there is a shortage of sufficiently reflective models for human retinal degenerations such as RP; most of the work has been done in rodent models. This limits pace of progress for understanding the diseases and development of therapeutics. • Human retinal organoids are a powerful tool for studying human eye diseases, as they can replicate complex retinal tissue architecture and function. • Insights gained from this study could help researchers better understand the pathogenesis of adRP, potentially leading to new diagnostic and therapeutic approaches. • This proposal represents a good application of disease modeling with patient iPSCs. • This indication is a good target for stem cell-derived treatments and gene therapy.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 14	• The proposal contains strong preliminary data built on a clear hypothesis. • The scientific rationale is sound; the strategies being proposed have been applied in other conditions. • The applicant has provided strong preliminary data supporting the feasibility of developing a human retinal organoid model of adRP. • The preliminary data is supportive and the prior and current experience of their team with working with 2D and 3D models using ocular tissues increases the probability of success. • The proposed experimental plan follows a logical sequence, building on the strong preliminary data to address key questions about the pathogenesis of adRP and potential therapeutic approaches. • One potential bottleneck in the development of a human retinal organoid model is the challenge of culturing and maintaining these complex structures in vitro. Another bottleneck is the need to accurately replicate the genetic mutations associated with adRP in the organoid model, in order to accurately model the disease.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 14	• The applicant has proposed a detailed timeline for each step of the experimental plan, indicating a clear understanding of the necessary time and resources required to complete the project. • The first aim (establishing and studying a disease model) and second aim (evaluation of potential therapies using the model) of the project are logically designed and sequenced. • The planning and timelines are appropriate and aligned. • The applicant has identified potential pitfalls in the development of a human retinal organoid model of adRP. • Pitfalls have been identified and the approach is multi-pronged. Regarding Aim 1: experiments are staggered, multiple organoids will be generated, and testing and quality checks are built in. Alternative editing strategies have been identified. Regarding Aim 2: multiple small molecule inhibitors identified for testing. For the antisense oligonucleotide approach of knockdown delivery, an alternative approach has been identified to enhance delivery. • Using gene-corrected cells could aid the applicants in benchmarking their assays.
No:	*none*

0	
GWG Votes	**Is the project feasible?**
Yes: 14	• The application stems from a great team with deep experience with retinal modeling. • The project team is appropriately qualified and staffed adequately. • The project is feasible within the timeframe. • The team has all necessary resources. • The applicants' approach should yield a good model for the disease. Gene-corrected isogenic lines are needed, as these are the gold standard for studying mutant cell lines.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• The researchers state that, where possible, they will try to include in patient recruitment an equal distribution of race, ethnicity, sex, gender and age. More specific details were not listed. • The applicants provide information about a few efforts to promote training opportunities and outreach, including mentoring and internship programs. • The proposal addressed the influence of race, ethnicity, sex, gender, and age diversity. • The application is not clear on prior efforts or proposed plans for outreach, partnership, or educational activities to inform the development of DEI within the research project.
No: 0	*none*

Application #	DISC0-14405
Title (as written by the applicant)	Establishment of a novel approach to systematically study the dynamic organization of protein complexes in stem cells
Research Objective (as written by the applicant)	We focus on human induced pluripotent stem cell (hiPSC) pluripotency and neurodifferentiation to develop a novel framework to allow simultaneous identification of multiple interactions between proteins and between proteins and the genome
Impact (as written by the applicant)	Our framework will allow high-throughput queries of the organization and functionality of proteins and shift the focus towards unprecedented, multi-dimensional studies of cellular complexity.
Major Proposed Activities (as written by the applicant)	• Establish two new complementary tools (Prod-seq and WhIp-seq) to catalog protein interactions, genomic binding and abundance. • Employ the new tools (Prod-seq and WhIP-seq) to study the polycomb group complex (PcG) members and their molecular interactions in hiPSCs. • Perform Prod-seq and WhIP-seq on hiPSCs-derived cortical neural progenitor cells (NPCs) and neurons. • Identify PcG configuration and interaction patterns during hiPSCs neurodifferentiation and validate the results by biochemical experiments and by comparing our findings to previously published data.
Statement of Benefit to California (as written by the applicant)	The successful completion of our project will pave the road towards new preventative strategies, treatments, and cures for diseases applicable to a variety of ethnic groups, and will therefore benefit the State of California and its highly ethnically diverse citizens. As we focus our efforts on induced pluripotent stem cells, our approach has the potential to advance the understanding of the physiology and disease using samples obtained from individuals from various genetic backgrounds.
Funds Requested	$1,515,601
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." 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: 88

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	86
Median	88
Standard Deviation	4
Highest	90
Lowest	80
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	12
(1-84): Not recommended for funding	3

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	• One of the key aspects that controls stem cell fate is the binding of various proteins to DNA at specific times, so the ability to monitor such interactions is very relevant to stem cell function in many diseases and tissues. • There is a clear need to better study protein/DNA interactions in stem cell models. • The technology proposed here could lead to an improved ability to interrogate cellular complexity, which can contribute to the understating of stem cell and regenerative biology. • The ability to easily and cost-effectively survey many molecular interactions is indeed a key bottleneck in understanding or preventing disease. • As a resource-generating project (or one that would facilitate resource-generating projects using the technology it establishes), it is straightforward to envision how someone with a hypothesis about protein binding events in stems cells would utilize this method to monitor their interaction and abundances. • Understanding causality is exceptionally hard. Showing that the proposed links in a chain of causality involve proteins (or nucleotides) that interact with each other is an important part of demonstrating causality. This tool will help add causal links to scientific research. • The aims related to polycomb proteins are relevant to stem cells: Polycomb Group (PcG) proteins are transcriptional repressors that epigenetically modify chromatin and participate in the establishment and maintenance of cell fates. These proteins play important roles in both stem cell self-renewal and in cancer development. • While the new techniques have potential to be impactful, their superiority to other existing approaches (such as Fluorescence Resonance Energy Transfer) is not clear.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 14	• The fundamental rationale that we need a more comprehensive identification of the interacting components of the cell is inarguable. Current protein interaction lists are too generic, limited, and full of false-positives to be useful in much of stem cell research. • It is difficult to find a molecular aspect of biology more relevant to disease than protein interactions, and thus new methods to monitor them broadly are widely relevant. • Lack of preliminary data was a key limitation in the prior application. In this revised version, the applicants provide additional experiments on molecules needed to develop this technology and prototype end-to-end test runs related to epigenetic marks. • The main concern from the previous review of this proposal was lack of preliminary data. The applicants now have preliminary data to support their rationale and approach. • The prior review included remarks about the "complexity" of the method which were not fully addressed in this resubmission. Those remarks may have been aimed at the issue of whether many labs in the future will consider utilizing this technology, given the extent to which tool molecules still to be optimized, rather than if the originating lab is capable of developing the technology. • Although lots of preliminary data were included in the revision, it is difficult to infer feasibility and scalability. • A major selling point of the technology (monitoring hundreds of proteins at multiple time points) has not yet been adequately tested, because there may be issues with using hundreds of antibodies to monitor interactions simultaneously. The extent to which one can monitor and reconstruct time-varying interactions is also untested. • The algorithm the applicants propose to use to detect protein complexes has been eclipsed by many superior alternatives that are available now. The applicants should consider this in their data postprocessing plans.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 14	• The combination of validation and application to a classic stem cell model and molecular system are appropriate. • Alternative protein binding systems that might be considered are accurately described.

	• One potential downside is that this is a methods development grant. At the conclusion of this grant the applicants may have a useful method that requires other researchers to move it forward. It was not clear how commercial aspects of the technology will be made available. • It is a technology driven project. If successful, the methodologies will allow for simultaneous identification and characterization of multiple interactions both between proteins within complexes and between DNA associated-proteins and the genome. This can be used to increase our understanding of stem cell regulation and differentiation more broadly.
No: 0	*none*
GWG Votes	**Is the project feasible?**
Yes: 14	• The timeline is aggressive, but feasible given the preliminary data shared since the last proposal. • Based on the preliminary data and expertise of team, the project is feasible. • Given the team's experience, it would be hard to claim that any other team is more qualified for this research. • The team is composed of researchers with complementary expertise. • The facilities are generally excellent and prior work on the basic method to be employed indicates they have access to all relevant facilities. • The methods are carefully described. • There are concerns that identified interactions might not represent real biology, and could instead be false positives or artifacts of the method. • Scalability may be of concern. Perhaps querying 100s of proteins as claimed in the application might not be possible, but even at a smaller scale, this technology would likely be informative and find niche applications.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• All experiments are conducted in two lines, which are African-American in origin, and one is female. However, because this study is not a cohort design the data that this project will generate is not intended to account for population differences. • In theory, yes; the authors point to reducing the cost of related assays as the main way to facilitate interaction studies in larger and theoretically more diverse populations. • The application provides adequate evidence from personnel history and educational activities that DEI is a substantive interest of the investigators. • This tool will be broadly applicable to all populations.
No: 0	*none*

Application #	DISC0-14357
Title (as written by the applicant)	Understanding Chemotherapy-Induced Peripheral Neuropathy Mechanisms using CRISPRi and Chemical Screens in Human iPSC-Derived Sensory Neurons
Research Objective (as written by the applicant)	The research objectives are to identify causal genes for chemotherapy-induced mitochondrial toxicity and neurodegeneration in sensory neurons and drugs that target this toxicity.
Impact (as written by the applicant)	These studies will open the possibility for genetic or drug targeting to prevent and treat drug-induced peripheral neuropathies and possibly neuropathies caused by disease or inherited.
Major Proposed Activities (as written by the applicant)	• Perform genome-wide CRISPRi screens in human iPSC-derived sensory neurons to identify critical genes and pathways for mitochondrial toxicity associated with microtubule targeting agents. • Perform a screen of 1600 small molecule drugs in iPSC-derived sensory neurons to identify drugs and therapeutic targets that attenuate the neurodegeneration induced by microtubule targeting agents.
Statement of Benefit to California (as written by the applicant)	California has a large population of cancer patients, many of whom suffer from debilitating adverse events from their cancer therapies. These studies seek to increase our knowledge about the causes of one common toxicity, peripheral neuropathy, and to identify potential genes and pathways that can be targeted for its prevention or treatment. The successful completion of these studies could improve the quality of life for cancer patients in California.
Funds Requested	$1,621,913
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." 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: 86

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	86
Median	86
Standard Deviation	1
Highest	88
Lowest	85
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: 14	• There is a great need to identify treatments for peripheral neuropathy for cancer patients as well as patients with other conditions (e.g. diabetes). • The overall goal is to use stem cell technologies to help find an effective treatment for chemotherapy-induced peripheral neuropathy. This is a serious and currently poorly treated condition many cancer patients suffer from. Successful identification of drug targets or drugs for the treatment of chemotherapy-induced neuropathy would represent a major achievement and benefit a large number of patients suffering from this condition. • Chemotherapy-induced peripheral neuropathy (CIPN) is a common, dose-limiting adverse event resulting from treatment with cytotoxic drugs. • The proposal is to perform a genome-wide CRISPR interference survey to identify novel genes and pathways involved in CIPN, and to screen a drug library for potential small molecule therapies. • It's important that we better understand the mechanisms of peripheral neuropathy.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 14	• The applicant aims to utilize iPSC-derived neurons in CRISPRi and drug screening studies to identify genes and targets to treat peripheral neuropathy induced by chemotherapy. • The proposed studies are well designed. The methodologies relevant to iPSCs reflect the current knowledge in the field of stem cell biology. Aim 1 is particularly meaningful and likely to generate interesting results. • The proposal includes valuable and interesting complementary approaches: a CRISPRi screen and a drug screen on neurons. • The model is based on generation of human sensory neurons from iPSCs and then stressed with chemotherapeutics. The rationale for the planned screens is appropriate.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 14	• In Aim1, a hiPSC-sensory neuron in vitro model of sensory neuropathy will be exposed to microtubule targeting agents, which are typically used in cancer chemotherapy. CRISPR-interference screen methods will then be used to identify genes associated with mitochondrial toxicity. Identified genes will then be validated with various functional and morphological methods. • In Aim 2, small molecule drug screening will be used to find potential drug targets among the genes identified in Aim 1. While the applicants will have access to automated screening technology, they could consider other more direct approaches reflecting recent developments in the biotech industry. • High-throughput screening of chemical libraries of new compounds or known drugs has been a standard approach in the industry and some academic institutions during the recent decade. However, more recently there has been a shift toward RNA drug technologies. E.g., siRNAs offer the potential to directly test the suitability of a putative drug target and they can be optimized for development as drugs. • The proposal is well designed, with appropriate pitfalls and alternative approaches identified. • Strong preliminary data.
No: 0	*none*
GWG Votes	**Is the project feasible?**
Yes: 14	• The feasibility of the screens is high and the preliminary data show that the applicant has several readouts for neuronal damage for screening and validation purposes. • The proposal is based on extensive preliminary data. These data provide a high degree of confidence that the proposed studies, particularly those in Aim 1, can be executed and will yield meaningful results. • The applicants have generated preliminary data showing the feasibility of their approach. • Strong team.

No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• The proposal upholds the principals of diversity, equity, and inclusion. • There are no concerns. • Chemotherapeutic toxicity affects individuals in all populations. If therapies are found that are effective, there might be differences due to genetic background, particularly in bioavailability and liver metabolism - but these issues are outside the scope of this application.
No: 0	*none*

Application #	DISC0-14460
Title (as written by the applicant)	Ex vivo fate mapping of human lung stem cell plasticity in fibrotic disease
Research Objective (as written by the applicant)	This proposal will design new models to study behavior that is specific to human lung stem cells, and screen for drugs that can target abnormal stem cells in fibrotic disease.
Impact (as written by the applicant)	This study will generate potential new therapeutic approaches to fibrotic lung diseases such as idiopathic pulmonary fibrosis.
Major Proposed Activities (as written by the applicant)	• Development of a combinatorial viral lineage tracing tool kit in mouse lung slices • Utilizing combinatorial viral lineage tracing in human lung slices to define human lung stem cell lineage trajectory after fibrotic injury • Identification of candidate compounds that can block abnormal lung stem cell differentiation • Development of fate mapping tools to study lung stem cells in diseased human tissue • Identification of candidate compounds that can reverse abnormal stem cell differentiation in the fibrotic lung
Statement of Benefit to California (as written by the applicant)	Our proposal will seek new ways to target stem cells in fibrotic lung diseases that affect Californians. We will generate new approaches to study lung stem cells that could serve as a platform to discover drugs that will improve stem cell health in lung disease.
Funds Requested	$1,625,998
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." 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	86
Median	85
Standard Deviation	1
Highest	88
Lowest	84
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	• This highly significant project will address a key knowledge gap in our understanding of a biology of human pulmonary alveolar stem cells and their potential to transdifferentiate into metaplastic basal cells that characterize idiopathic pulmonary fibrosis (IPF), a deadly fibrotic lung disease. • This is a very elegant proposal in which sophisticated genetic tools are proposed to complete a series of experiments, to define by lineage tracing the transdifferentiation of lung stem cells during lung injury. Preliminary data demonstrated that in humans, these lung stem cells have the potential to differentiate into basal cells. If it works, this can provide an important model to define lung stem cell differentiation and evaluate therapeutic interventions. • Interesting project addressing alveolar remodelling in IPF. Successful completion of this proposal will yield novel tools to lineage trace human lung stem cells ex vivo and provide a platform for drug discovery to reverse stem cell metaplasia in fibrotic lung disease • Novel lineage tracing analysis of lung fibrosis. High risk-high gain. There is no treatment for lung fibrosis.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 14	• Recent findings of the applicant's group suggest that endogenous human lung stem cells transdifferentate into metaplastic basal cells in situ after lung injury. • This project is based on a scientific rationale supported by a recent publication (where the PI is one of the co-authors) proposing a causal connection between a loss of human lung stem cells cells and an ectopic appearance of metaplastic basal cells in IPF lung alveoli. • Determining the origin of metaplastic basal cells in the human lung is crucial to designing approaches to reverse stem cell remodeling seen in IPF, but we currently lack the tools to perform fate mapping of human lung stem cells in their native cellular environment. • Applicants propose elegant method of labelling lung stem cells and basal cells in human lung slices. • Novel fate mapping platform to study the lineage trajectory of human lung stem cells ex vivo in lung slices derived from diseased human lungs. • There is some concern about novelty, mostly because a previous publication has demonstrated the basal differentiation. Here is more a development of new tools to study, in great ex vivo models, cell differentiation.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 14	• The project is appropriately planned and designed. Aim 1 will lineage trace the lung stem cells in the lung slices undergoing fibrotic transformation and define the transitional progenitor states during transdifferentiation in situ. Aim 2 will lineage trace metaplastic basal cells derived from IPF lungs to determine their capacity to differentiate back into human lung stem cells. • Experimental plans are reasonable and feasible. • There is some concern about the fact that the PI has not assembled a research team. • Personnel not clear.
No: 0	*none*
GWG Votes	**Is the project feasible?**
Yes: 14	• The project is feasible. • Builds on PI's existing strengths. • The specific aims are logical, realistic, and are likely to be achievable within the proposed timeline. The project carries high risk, but it also has high reward.
No: 0	• The main concern on the whole application is the use of PLCS. Can the cells survive the time required for infection and differentiation? There is a high possibility that they may not, making this a high risk-high reward proposal.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**

Yes: 12	• Fibrotic lung diseases have an increased prevalence in the underserved lower income groups perhaps due to smoking and exposures to toxic inhalants - known risk factors for the IPF. Available treatments are expensive, leaving the economically vulnerable population with little care. The project will address these areas of health disparity. • Applicants state that they will include specimen from donor cohorts that are sex-balanced and racially representative of the population of California.
No: 2	• There is no description by the PI of promoting inclusion and diversity or description of the needs for this in underrepresented populations. • Not well addressed.

Application #	DISC0-14392
Title (as written by the applicant)	Harnessing vascular stem cells to grow and protect the human brain
Research Objective (as written by the applicant)	The origins of brain vascular mural cells are unknown. This proposal will identify mural stem cells in the developing human and mouse brain and determine their impact on blood brain barrier formation.
Impact (as written by the applicant)	Brain vascular diseases can have profound impacts on long-term neurological function. This proposal will map the stages of mural stem cells to protect these cells and harness them for regeneration.
Major Proposed Activities (as written by the applicant)	• Determine the spatiotemporal dynamics of mural stem cells in the developing mouse and human brain. • Determine the cellular potential of human and mouse smooth muscle cells versus pericytes in the developing mouse brain. • Develop in vitro human neurovascular units (NVUs, organoids) to test the potential of human mural stem cells.
Statement of Benefit to California (as written by the applicant)	There are critical gaps in our understanding of blood vessel cells in the brain, which impact one of the most vulnerable patient populations. Preterm babies born before 30 gestational weeks are prone to developing brain hemorrhages. This condition affects approximately 12,000 babies a year in the United States, and survivors are prone to severe long-term neurological problems. This proposal will identify blood vessel stem cells to protect them and harness them for regeneration after injury.
Funds Requested	$1,625,998
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." 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	86
Median	85
Standard Deviation	3
Highest	90
Lowest	80
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	12
(1-84): Not recommended for funding	3

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	• The development of blood vessels in the human CNS is not adequately understood, and any attempt to understand hemorrhagic disorders in preterm infants lacks sufficient scientific foundation. • The findings will provide important background information for realizing a better understanding of hemorrhagic brain injury in preterm infants. • Application addresses the important medical issue of hemorrhagic brain injury in preterm infants. • If the development of neurovascular organoids from primary human cells can be advanced, this could provide a new model to assess neurotoxicity and test repair modalities. Baseline data on vascular development in the human brain will be a useful resource to the community. • Preterm birth often is associated with brain hemorrhage of unknown causation. A better understanding of the interrelationships of neural cells and blood vessels in the brain might help identify preventative measures. Possible future use of stem cells to repair the brain damage.
No: 1	• Not directly significant for the proposed preterm condition, but relevant for the basic underlying biology.
GWG Votes	**Is the rationale sound?**
Yes: 14	• The importance of the vascular environment on brain development and function is increasingly being demonstrated. Understanding lineage relationships of mural cells will enhance understanding and allow improved in vitro models of neurovascular development. • Establishment of the neurovascular unit is key to many aspects of brain function throughout life. Project might have significant implications beyond prenatal brain hemorrhage. • Preliminary data provide clues into cell lineage in both mouse and human, and defined cell surface markers to purify relevant populations. • The project aims to dissect cell lineages in brain blood vessel development using appropriate methodologies. The cellular composition of the vascular precursors in the human brain may be relevant to propensity to hemorrhage. Better articulation of a specific hypothesis regarding preterm hemorrhage would enhance the potential clinical relevance of the project but maybe we do not even know enough to advance a theory. • The rationale is sound for Aim 1 and 2; Aim 3 is a bit more vague and weaker.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 12	• Project is designed to produce important outcomes in three year time frame. • First aim will extend RNA-seq analysis, and use markers to localize mural stem cells. Second aim will define progenitor lineage in the mouse. Third aim will use organoids to model development of the neurovascular unit. This integrated approach should define ontogeny of mural stem cells and the neurovascular unit and provide important link between human model in vitro and in vivo model in mouse. • Overall the project plan is rational and defined in terms of stepwise analysis of mouse to human to in vitro models. Builds on a specific hypothesis that smooth muscle cells are evolutionarily conserved mural stem cells in the developing brain, and proposes appropriate experiments to test this. • Alternative approaches to spatial transcriptomics and lineage tracing are considered. • There is limited discussion of pitfalls and alternate approaches. This is particularly true for the experiments aimed at understanding human mural cell lineage relationships, where the transplant model into mouse brains is considered as risky but would be replaced by another risky transplant model. • Applicants could have considered existing in vitro models of the brain vasculature and blood brain barrier and explained what their study will add. • The title sets a rather ambitious stage with a focus on a path to help the thousands of prematurely born infants that are at risk due to insufficient vasculature development. The path towards this group of patients for regenerative medicine is one of the most complicated and this is not really addressed here nor the actual focus of the grant.

No: 2	*none*
GWG Votes	**Is the project feasible?**
Yes: 13	• Aim 1 is feasible and important background resource for the field. Mouse lineage tracing in Aim 2 is also feasible. • The project is somewhat ambitious but aims are within the scope of the laboratory's capabilities. • PI is an MD PhD with an innovative track record in this area of research. One collaborator is an expert in genetic manipulation the other a young scientist with a strong CV and appropriate experience. • PIs lab is fully equipped to carry out the studies. • While the underlying question is interesting and important in terms of fundamental understanding of vascular units in the developing brain, there is little direct connection to explaining preterm hemorrhage and neurological disease. • Needs more work on the human NVU model to be able to develop a robust system to assess mural progenitor lineage and function in developing human brain. • Rather overambitious and sometimes not convincing in its presentation. Models are very simplified and translation back to the proposed regenerative goal is not clear.
No: 1	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• Improving preterm outcomes would disproportionately impact underserved populations • Low socioeconomic status is associated with higher frequencies of preterm birth. • Applicants have new plans in place to approach Hispanic families who have experienced neonatal loss. • Applicant and co-investigators have plans to collect tissue and data from underserved communities. • The PI researches brain injury in preterm babies in their overall program and in their clinical practice. Incidence is higher in women of low socioeconomic status, often marginalized communities. To help these groups it is necessary to work with the community to access material after death in a sensitive manner. The PI is working now to reach out to Spanish speaking families and support groups. Strong outreach component.
No: 0	*none*

Application #	DISC0-14350
Title (as written by the applicant)	The role of WNT and BMP signaling pathways in iPSC to iTenocyte step-wise differentiation for tendon repair
Research Objective (as written by the applicant)	Development-inspired differentiation will enable efficient and specific generation of tenocytes that can repair tendon injury, restore dysfunctional tissue, and prevent long term effects.
Impact (as written by the applicant)	This study will eliminate heterogenous differentiation of pluripotent stem cells and will results in high yield and unified tenogenic phenotype.
Major Proposed Activities (as written by the applicant)	• Establish the mechanism by which WNT signaling regulates tenocyte differentiation. • Determine the fate of iTenocytes and their ability to regenerate rat Achilles tendon defect.
Statement of Benefit to California (as written by the applicant)	While tendon and ligament injuries affects all adults, many people belong to underserved communities that more often carry government-sponsored health insurances. Despite decades of research, there are no robust biological therapies for the tendon and ligament injuries. Cell therapy with the proposed treatment candidate may provide an efficient inexhaustible off-the-shelf cell source accessible to all.
Funds Requested	$1,516,563
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." 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	85
Median	85
Standard Deviation	0
Highest	85
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: 15	• This application proposes to develop iTenocytes from iPSCs for repair of tendon defects. The investigators will study the temporal requirement for WNT and BMP signaling during

	differentiation from iPSC to iTenocytes utilizing well-defined inhibitors of WNT and BMP signaling pathways. • There is a clear knowledge gap in the role of WNT and BMP signaling pathways in subsequent differentiation and development of tendons. Elucidating these mechanisms can lead to developing more powerful and specific differentiation protocols to be used in cell therapy applications. A robust method to derive clinically-relevant cells (tenocytes) for tendon and possibly ligament repair would have a significant clinical impact. • Tendon repair is a major issue in medicine. • There is a clear need to improve tendon repair approaches even though these injuries are not life-threatening. A similar strategy is being pursued for numerous cell replacement conditions. The approach proposed here may eventually be more easily (and locally) applied to patients. • The ability to make tenocytes would have considerable impact on regenerative medicine therapies.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 15	• The application holds high significance as there are no current treatment options for tendon damage. In terms of rationale, the involvement of WNT and BMP signaling in tenocyte differentiation is well defined. • Both aims provide good rationale and sound preliminary data. • The team has been successful using iPSC-derived mesenchemal stem cells to derive tenocytes with aid from lenti-viral transfection to increase expression of a key gene in this process plus applied mechanical loading. This proposal will aim to use small molecules that modulate WNT and BMP signaling. The approach is motivated with supportive preliminary data and may result in a therapy that is more scaleable and may have a more clearly defined regulatory path. • The role of WNT and BMP pathways in early stages of development is well established, but their role in late stages of sclerotome (SCL) to syndetome (SYN) and SYN to iTenocytes differentiation is not. The involvement of WNT and BMP in this process is supported by scRNAseq preliminary data.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 15	• The aims are well planned out and include in vitro and complementary in vivo components. • The preliminary data demonstrate the usefulness of an existing model to evaluate the efficacy of iTenocyte injections in damaged tendons. • The in vitro goals in Aim 1 are to derive iTenocytes using small molecule modulators of WNT (early inhibition) and BMP (late promotion) during the iPSC differentiation protocol. The preliminary data look promising in reducing undesirable subpopulations. RNAseq will be used to assess the homogeneity of the differentiation protocol. • In parallel, a bioreactor will be used to stimulate maturation of iTenocytes. Outcome measures will include gene expression analysis using qRT- PCR, RNAseq, cell orientation, and secretion of tenogenic matrix to the media. A limitation of this approach is that it is unclear how the application of mechanical loading in 2D will impact subsequent tenogenic phenotypes and function in 3D, because the cells will need to be released and seeded onto the collagen scaffold. Incorporation of loading may be more meaningful in the 3D system. • Aim 2 will test the hypothesis that iTenocytes will improve biological, mechanical, and behavioral outcome measures of rat achilles tendon defect repair. Spatial transcriptomics and scRNAseq of sorted iTenocytes will be used to examine their fate and differentiation status. • The in vivo studies in Aim 2 are comprehensive and will utilize imaging and RNAseq in combination with a panel of functional assessments to understand relationships between tissue repair outcomes and cellular properties. • One might have concerns that a focus on only two signaling axes (BMP and WNT) may be insufficient, but the preliminary data support this focus.

	• The investigators plan to use a highly relevant animal model.
No: 0	*none*
GWG Votes	**Is the project feasible?**
Yes: 15	• The preliminary data as well as publications from the team is supportive of the proposal and demonstrates the ability to undertake all the technical biology, scRNAseq, and bioengineering aspects of the proposed studies. The genomics core at the institution is well-equipped to support those aspects of the proposal. • The investigator has the necessary expertise, and all methods are established. • There are no concerns regarding feasibility. • The applicant team is strong, and is pursuing a thorough phenotyping approach to characterize this pathway.
No 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• The proposal discusses that tendon injury afflicts all sexes and genders, and may disproportionately affect populations who are involved in physical and labor-based occupations. • While people of different races, ethnicity, sexes and genders suffer from tendon/ligament injuries, this disease has been shown to affect especially people from underserved communities such as African American and Latino older adults. • The application satisfactorily upholds principles of diversity, equity and inclusion. • The DEI plans are reasonable for this type of early pre-clinical study.
No: 0	*none*

Application #	DISC0-14458
Title (as written by the applicant)	Overcoming barriers for airway stem cell gene therapy for Cystic Fibrosis
Research Objective (as written by the applicant)	This research will allow the targeting of airway stem cells for long lived gene therapy for Cystic Fibrosis and for other airway diseases
Impact (as written by the applicant)	We will overcome the barriers to accessing airway basal stem cells for gene correction for Cystic Fibrosis (CF) and use a new gene correction strategy to correct >99% of all genetic changes causing CF
Major Proposed Activities (as written by the applicant)	• Determine the dosing and timing of detergent linked nanoparticles for accessing the airway stem cells • Package the gene correction cargo and perform delivery of this gene correction cargo into the stem cells • Perform successful gene correction of the mutated gene in the airway basal stem cells using the gene correction strategy
Statement of Benefit to California (as written by the applicant)	Cystic Fibrosis is one of the most common genetic disorders in the US. California is one of the U.S. states with the largest numbers of people living with CF at 2,386 people. These patients require lifelong, intense medical care both at home and in the hospital. Gene correction of this disease will have a major impact on these patients and their families and communities. It will also greatly reduce the cost of health care for these patients and for the state of California.
Funds Requested	$1,472,858
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." 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	85
Median	85
Standard Deviation	1
Highest	88
Lowest	84
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	• This project addresses a very important problem of delivering correcting gene therapies to CF airways. • Yes. Current therapies for CF only target 20% of individuals with CF. Gene therapy is an exciting possibility for these patients. • Development of an effective gene therapy for CF is an important goal. • The goal of this revised project addressing a major gap in the development and use of gene therapies is to develop an effective approach for treating CF, a disease that results from mutations in cystic fibrosis transmembrane conductance regulator (CFTR) gene. • Translatability to the in vivo system is questionable.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 14	• The project aims to develop a novel delivery system to try to target lung basal cells and penetrate the mucosal layer. • The project is based on a strong scientific rationale that transduction of cells thought to represent stem/progenitor cells of the lung airway epithelium with a copy of functional CFTR gene will restore the CFTR function and will result in a sustained alleviation of lung and airway pathology in CF. • Single nucleotide mutations of the CFTR gene are a logical target. In the present application, using a novel way to deliver gene editing systems has high potential. • Unfortunately, no discussion is provided on how sustained the proposed therapy is expected to be. While the cells are long-lived, the therapeutic effect from the gene transfer might not to be permanent. A discussion of potential clinical scenarios would be useful for assessing the scientific rationale of the project. • To address the sustainability of the therapy, the investigators could possibly take advantage of a functional Ussing Chamber assay that measures the short-circuit current as an indicator of net ion transport taking place across an epithelium proposed in Aim 3. For example, they could carry out a time course of the epithelial ion transport in transfected CF patient cells differentiated into air-liquid interphase cultures.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 14	• The project was scored highly at the previous round and here in the resubmission the applicants have addressed the major concerns raised by the reviewers. • The aims are appropriately designed to address the goals of the project. However, all the proposed work will be conducted in vitro in reductionist model systems. This is justified, given an early stage of study. However, with an eye for future clinical applications, the absence of an in vivo model makes the project risky. • There is a concern about the proposed model, which is far from reproducing what happens in the lungs of CF patients. It is very likely that the proposed experiments will work in this model, but in patients with an increase in mucus viscosity and extensive biofilms, it is a high risk. • Off-target effects should be better profiled with therapeutically relevant doses.
No: 0	*none*
GWG Votes	**Is the project feasible?**
Yes: 14	• The proposed experiments are well-designed. • Yes, the project is feasible, the applicants have a strong track record and the necessary expertise to deliver this project. • Strong pilot data and strong track record. • Still high risk, but current treatments for CF may make the lung epithelium healthier in CF patients and allow better transfection. Still unclear how long the effect will persist given lung epithelial turnover.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**

Yes: 14	• The applicants have well designed strategy to address the needs of diverse population affected by CF. • Because CF is a genetic disease that affects people of different races and ethnicities, the outcomes of the project will benefit a broad range of patients, including underserved communities. • This group is committed to supporting diversity and inclusion.
No: 0	*none*

Application #	DISC0-14424
Title (as written by the applicant)	Functional genomics to study cellular convergence across ASD risk genes in neurodevelopment
Research Objective (as written by the applicant)	Our objective is to enable scalable genetic screening to study how neurogenesis is impacted by risk genes implicated in human psychiatric disorders.
Impact (as written by the applicant)	We will develop and apply state-of-the-art genomic analysis to seek mechanisms and disease modifying solutions.
Major Proposed Activities (as written by the applicant)	• Identifying and validating efficient gRNA for gene editing • Identify risk gene effects in early neurogenesis
Statement of Benefit to California (as written by the applicant)	Mental health disorders are one of the most common health conditions faced by Californian citizens: 1 in 6 California adults have experienced some form of mental illness, and 1 in 24 have a serious condition that makes it challenging to carry out major life activities. Our work is to approach the basic mechanisms involved in these disorder-implicated genetic factors to seek potential solutions to help with these devastating conditions.
Funds Requested	$1,575,001
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." 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	85
Median	85
Standard Deviation	2
Highest	85
Lowest	78
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: 13	• Understanding the biological impact of mutations associated with autism spectrum disorder (ASD)/neurodevelopmental disorders (NDDs) in human-relevant models is critical to finding potential therapeutic targets. • Interaction of risk genes is likely to occur in ASD/NDD. Thus, approaches beyond single gene manipulation are critical and will yield new insights into gene networks. • Human organoid models have been shown to approximate human brain development and are, as of yet, the only approach to allow manipulation and robust readout. • Yes. The proposed project proposes a scalable genetic screen for risk genes implicated in human neuropsychiatric disorders that impact neurogenesis. • The proposed project could address one of the major bottlenecks in the fields of ASD and NDDs - evaluating the effect(s) of thousands identified risk genes/mutations that affect neurodevelopment. • This project nicely addresses a key gap in our understanding of iPSC that is relevant to human disease, specifically NDDs. • The genes to be studied are also associated with monogenic human genetic disorders. Thus, this project will be directly relevant to human disease.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 13	• Overall, yes. However, it could be argued that mutations occurring before or during the earliest stem cell stages will have a different impact on neurodevelopment than mutations occurring after dorsal/ventral fate has been established. The proposed perturbation (gene editing) will occur after organoids have been specified along dorsal or ventral fates. What is the relevance of the timing? • Yes. However, the significance of changes in expression profiles to development of NDDs remains elusive. • The preliminary data are compelling and supportive of the proposed project. However, the rationale for choosing these particular mutations is not entirely clear. • The applicant has laid out a very compelling rationale. Although there are multiple aspects of the project that are technically difficult, the applicant has a very clear and well-referenced background/rationale section that explains why their work is the next step. • The applicant also indirectly addresses the limitations of studying one specific type of iPSC-derived cell type at a time, which is a common approach. • The selection of genes to study has a very strong rationale, which is often lacking in similar studies. This increases my enthusiasm for the proposal significantly. • The applicant is working in a crowded field with this project, but they define a major bottleneck to discovery (specifically, the scalability of iPSC models for rare NDDs).
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 13	• The expertise of the PI is evident in the carefully arranged controls for each experiment and the justification for each particular of the experimental model (either from preliminary data or published literature). • I appreciate that, multiple times throughout the project plan, orthogonal techniques will be used to validate findings. • The future directions sections are an exciting look at next steps. • There is no question this is an urgent need that is commensurate with CIRM's mission. • Yes. the project is well thought through and well designed. The methodological approach is rigorous enough to produce meaningful results. • The revisions to the pitfalls section have been significant, and greatly strengthen the proposal as a whole. Comparing dorsal to ventral neuronal diversity is a very interesting, targeted question in the field and will be of interest to many researchers. • The high resolution imagining-based spatial transcriptomics work is especially well represented in the alternate approaches. • Some of the potential pitfalls are identified and alternative approaches described. However, this is not very exhaustive and at times quite general.

	• This is an ambitious project and I worry that it will take more than the proposed three years to complete. However, even finishing Aim 1 would be a major contribution to the field. • Cell proliferation seems to be the only morphological outcome - it is not clear how this outcome relates to the ASD/NDD phenotype. • The rationale that gene mutations will have a direct effect that is robust enough to be captured across variable organoids is not well supported, and no preliminary data are provided. • In Aim 1's studies the applicant will study 2-month-old and 6-month-old organoids. According to the applicant and a cited reference, progenitor genes expressed early show DD enrichment, while genes expressed later during maturation lean towards ASD. Are the 2- and 6-month timepoints designed to capture early versus late stages? The timing is not well discussed.
No: 0	*none*
GWG Votes	**Is the project feasible?**
Yes: 13	• The insight from Aim 1 will be limited although necessary for Aim 2 to assign cell identities . Aim 2 will add a new dimension to single cells sequencing and bulk data and will add spacial resolution to cellular processes that might be disrupted. • The applicant does not provide experiments that would directly test migration. • The applicant states that if effects are captured more ventrally this will somehow point to a hierarchy. This rational is not clear as defect in dorsal organoids could occur interdependently -i.e glial progenitor cells originate from both ventral and dorsal regions and could affect neuronal function in an non autologous way • The aims and experimental set up are well designed and, although ambitious, may be (tightly) achieved during the proposed project duration. • The outcomes are logical, and even if only a portion are able to be completed within the proposed timeline it will be a significant amount of data. • That's a lot of new trainees to have start in a lab at once, but based on Dr. Jin's record of successful project management in other areas, I don't have significant concerns about the proposed team. • I feel much more comfortable with the project after the new planning and design additions. • I was very concerned in the initial publication about the staffing for such a technically complex project, and are happy to see that Dr. Jin has recruited four graduate students, one post-doc, and two research technicians, an impressive growth in a short time.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• I am pleased to see that one male and female line will be used, and additional ethnicities will be included as allowed. It is a difficult project to do on multiple lines to adequately addresses diversity. • The applicants have addressed DEI very thoughtfully and appropriately. • Poorly developed. The proposal does not use organoids from different races, sexes, etc. The applicant suggests they will do so in future studies. • It is good future planning to have a diverse set of iPSC banked to allow replication or additional studies in additional lines. • The PI has one DEI pipeline project that they have initiated, a partnership with an existing institutional program. This is a very nice addition from the last revisions and increases my interest in the proposal. • A generic section about efforts is provided.
No: 0	*none*

Application #	DISC0-14521
Title (as written by the applicant)	hPSC-derived enteric ganglioids for cell therapy in gastrointestinal motility disorders
Research Objective (as written by the applicant)	The proposed aims will enable the generation, purification and characterization of enteric neurons from diverse hiPSCs and assessment of their efficacy for cell therapy in gastrointestinal motility disorders.
Impact (as written by the applicant)	This proposal addresses a significant unmet clinical need for a cell therapy approach for gastrointestinal motility disorders such as Hirschsprung disease, achalasia and gastroparesis.
Major Proposed Activities (as written by the applicant)	• Standardize the generation of enteric neurons from stem cells of diverse backgrounds • Developing methods to speed up the process of enteric neuron production from stem cells • Evaluating the ability of stem cell derived enteric neurons to survive in the host tissue and rescue the disease in mouse models
Statement of Benefit to California (as written by the applicant)	Gastrointestinal (GI) motility disorders are severe and common medical conditions resulting from dysfunction or degeneration of the enteric nervous system (ENS). The ENS is an extensive network of neurons inside the gut tissue that are responsible for local regulation of motility and digestion. This proposal is aimed at developing stem cell based therapies to replace the damaged or absent enteric neurons in motility disorders such as Hirschsprung disease, achalasia and gastroparesis.
Funds Requested	$1,589,307
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." 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	85
Median	85
Standard Deviation	2
Highest	88
Lowest	82
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	• This highly significant project addresses a knowledge gap in the application of human hPSC-based technologies to treatment of GI dysmotility diseases of the ENS, which currently do not have effective therapies. • Important area based on strong initial research. Addresses dysmotility diseases of the enteric nervous system (ENS), which currently do not have effective therapies. • The project is an application of stem cells to human disease, and also targets depletion of target genes to test their role in stem cell/ENS biology. • The project employs PSCs to generate patient-derived ENS grafts to address the bottleneck of having new stem cell based strategies to treat GI motility disorders. • Potential to help in gastrointestinal diseases, but also gain fundamental knowledge of the role of ganglia in the overall ENS. • Yes, defines and addresses a major bottleneck in the areas of GI biology and ENS integration. • Unclear whether the project will have a major impact on scientific knowledge in stem cell/regenerative medicine field; methods for ENS generation remain under review for publication.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 14	• The project is based on a strong scientific rationale that transplantation of functional hiPSC-derived ENS neurons would provide an effective treatment for incurable diseases of GI dysmotility. The team has already demonstrated that hPSCs (hESCs and hiPSCs) can serve as an abundant source of ENS neurons, and these results strengthen the project's scientific rationale. • Aim 1 is a rational extension to examine additional PSC lines, and feasible based on the published and preliminary work from this lab. • Aim 3 is risky but their preliminary data supports feasibility. If this aim is successful, it would be a significant step towards developing an effective cell therapy approach for treatment of GI motility disorders. • Figure 1 is novel and impressive. • The in vivo methods are very impressive, but remain unclear in terms of their surrogacy and standardization. • Aside from engraftment in Figure 7 shown, it is unclear why in vivo testing for proof of principle cannot be done first, and then optimize methods thereafter. At this point, it is unknown if the PSC derived ENS are functional vs. engraft. The number of cells required for engraftment is unknown, so it is challenging to interpret this. • The rationale for accelerated differentiation remains unclear. How rapid does this need to be? What is the lower and upper threshold if this is so precise and critical? Providing some context as to why this is unique vs. other integrating cell types in the neural lineage would be helpful in supporting such a claim. • If accelerated differentiation is critical, why is MOA so important in terms of moving the project forward? Some strategies are conflicting. • It is clear that cryopreservation is critical, but this seems to be best positioned once the ENS methods are optimized, and function is shown. Unclear why this is an immediate objective. • This proposal depends on ENS generation from hPSCs. This is difficult to evaluate given the data is being revised and collectively found in a paper under review.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 14	• The project is well designed and comprehensive in moving towards therapeutic approaches. • The project is appropriately planned and designed. • Since many of the GI dysmotility disorders are known to have an underlying ENCC defects, and the iPSC differentiation protocol includes an intermediate step of derivation of ENCCs, it is important to demonstrate that this protocol works with the iPSCs derived from patients affected by these disorders. It is suggested to incorporate these experiments in Specific Aim 1.

	• Aim 2.3 assesses effect of accelerated ENS differentiation on cell states. However, alternatives/strategies are not discussed if significant differences are found as this would raise questions regarding effectiveness of the process.
No: 0	• Clear and concise aims but acceleration and MOA of these accelerating molecules is unclear in terms of their use. • Functional integration of ENS in vivo seems the most challenging task and the single determinate of success. • Backcrossing of the mice would be transformative to this field. Unfortunately this is not a primary directive. • Pitfalls are provided; moving to analog generation upon failure of target is curious.
GWG Votes	**Is the project feasible?**
Yes: 14	• The proposed aims are logical and they are likely to be achieved within the proposed timeline. The revised application appropriately addressed issues raised by the reviewers of the original application and strengthened the feasibility of the project. • Extensive preliminary data and expertise of the lab support feasibility. • Very appropriately qualified and staffed project. The team has experts in the field and has access to all the necessary resources to conduct the proposed activities. • Budget seems suitable. • Timelines are adequate, although the number of iPSC lines and differentiation characterization is questionable in terms of optimization methods applied to all lines. • Aim 3 may not exhibit functional effects. This would then decrease enthusiasm for Aims 1 and 2.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• The project plan addresses racial, ethnic and gender influence on generation of functional enteric neurons from hiPSCs. Specifically, the investigators will establish standardized hiPSC-based methods for generation of enteric neurons that work consistently across iPSCs derived from different racial backgrounds and in both male and female cell lines. Toward achieving this goal, the team will take advantage of the available CIRM hiPSC lines derived from subjects of diverse backgrounds. • Employs iPSCs from a number of diverse lines. • The list of proposed iPSC lines and the intent is impressive and consistent.
No: 0	*none*

Application #	DISC0-14447
Title (as written by the applicant)	Mapping the spatial and temporal responses of hESC-derived microglia to repeat mild closed head injury to identify therapeutic targets and mechanisms
Research Objective (as written by the applicant)	We will generate an RNA activation map of human stem cell derived microglia activation states following brain injury to then test a new gene-edited microglia peptide delivery mechanism.
Impact (as written by the applicant)	Bottlenecks with the time and sex-dependent human microglia responses to repeat mild closed head injury and questions surrounding the delivery and efficacy of a trophic factor as a therapeutic.
Major Proposed Activities (as written by the applicant)	• Generate an RNA map of the human microglial response to repeat mild concussions at multiple post-injury timepoints on mice transplanted with microglia derived from male and female stem cells. • Use microscopy to histologically validate the microglia populations identified in Activity 1 express candidate genes from the early vs late and/or male vs female injury responses. • CRISPR edit male and female stem cell lines to append the sequence for a secreted trophic factor coupled to a reporter peptide at the end of the selected injury-response genes found in Activity 1. • Verify that the microglia generated from CRISPR editing are different than the parental lines as designed in Activity 3 (quality control). • Stimulate the CRISPR edited microglial lines in cell culture to confirm that the trophic factor/reporter peptide responds to injury and is secreted. • Perform repeat mild concussions on mice transplanted with the CRISPR engineered microglial cell lines and verify that the inserted trophic factor/reporter peptide is delivered to the injury site.
Statement of Benefit to California (as written by the applicant)	This research could benefit California by discovering genes that are specifically upregulated in microglia in response to brain injury. Editing a gene to deliver a trophic factor to the injury could be a pathway for treating concussion/closed head injuries or even more severe traumatic brain injuries (which effect >230,000 Californians/year and cost CA ~9.6 billion every year). This bottleneck could also open the door for stem cell based treatments of a variety of neurological conditions.
Funds Requested	$1,555,140
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.” 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	8
Highest	90
Lowest	60
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: 13	• Mild traumatic brain injury (TBI) is a prevalent, costly condition that is associated with considerable burden for society. There are limited treatments or disease modifying approaches available. • Microglia are a potential therapeutic target. While the application is focused on TBI, the approach could be extended to many other types of trauma, and potentially to chronic injury. • The project aims to define a transcriptional profile of microglia's response following TBI. This would contribute new knowledge and address an unmet medical need. • The effectiveness of BDNF as therapeutic is controversial and confusing. This project will provide a potential clarification of this issue. • Better understanding of how microglia respond to TBI at different timepoints may aid the development of treatments. • Many questions remain regarding the inflammatory process and its role after the TBI. • There is a need for new and better treatments for TBI.
No: 1	• I am concerned this model will not represent the biology of microglia in the human brain following TBI. That said, if the model is representative, these experiments could explain some human pathophysiology.
GWG Votes	**Is the rationale sound?**
Yes: 13	• Microglia activation is a known response to TBI. However, its impact on disease development/progression in the context of repeated TBI remains unknown. While the response of microglia to injury has been analyzed in animal models, characterizing the response of human microglia to TBI in an in vivo preclinical model is novel and addresses an important bottleneck. • Microglia have been shown to respond to injury in a highly plastic manner that varies with time since injury. Elimination of microglia prior to insult is protective against long-term pathological effects, suggesting a role of microglia in long-term sequelae from TBI. Additionally, long term pathology in humans following TBI has been linked to persistent activation of microglia. • As cause and effect are not resolved, it is critical to gain more insight into the dynamic changes of resident microglia in response to injury in a robust model system. • The applicant provides strong preliminary data on their mild TBI model, in which they transplant human microglia into a mouse, administer mild TBI, and then re-harvest the human microglial cells to characterize activation. • The project is well-motivated and based on sound rationale. • This project is relevant to TBI in humans.
No: 1	• I'm not convinced that characterizing the transcriptome of microglia after brain injury will be informative without corresponding pathological outcomes from the model. • A focus of the proposal is the potential differences between male-derived and female-derived microglia after xenotransplantation into mice. However, male and female microglial cells may not retain distinct physiology (if it exists) out of the context of the human brain.
GWG Votes	**Is the project well planned and designed?**
Yes: 13	• Sustained activation of microglia two months post-injury and transcriptomic data that are consistent with reports of dynamic microglia responses following brain injury are compelling. These support the characterization of the partially "humanized" microglia model in the context of TBI. • The lab has established a unique and highly reproducible model for mild TBI and has identified relevant endpoints for study. • The project is well planned and follows a clear logic.

	• Potential pitfalls are identified and alternative approaches presented. • The project plan and timeline demonstrate an urgency that is commensurate with CIRM's mission.
No: 1	• The power analysis appears adequate because it is based on the number of cells to be analyzed (which is 2,000 cells per replicate) However, the n=3 assumes that all animals behave the same, and that the transplantation procedures have no variability. • The power calculations lack discussion of multiple testing and are vaguely worded, e.g.: "detect a 30% difference in histological markers." Which histological markers, and how do they relate to scRNA seq results? • Identification of CHI-responsive promoters seems to be the most important outcome of Aim 1, but the project plan for Aim 1 does not include a clear method for identifying them.
GWG Votes	**Is the project feasible?**
Yes: 12	• Aim 1 is feasible - i.e., Figure 2 shows that cells harvested at 24 hours or 6 weeks post-injury and characterized using scRNA-seq show differential gene expression. The figure includes a representation of the observed and predicted timing of each gene response program. • The applicant has established the feasibility of Aim 2 by studying another disease indication using the proposed methods. They provide an example wherein they generated scRNA-seq guides to express reporter peptides in response to pathology (Fig 3). • The applicant will adapt the approach above, using CRISPR to generate reporter constructs with either an early or late response gene to drive expression of a mature, tagged BDNF. This is likely to work and will allow the applicant to examine the behaviors of the early- and late-responsive microglia. • As no behavioral studies are proposed, the number of animals used for the experiments seems adequate. • Will the human cells persist in mice? There are some feasibility issues, but this is expected for such a challenging study. • The project is feasible, as the injury model and process for microglia differentiation are already established in the applicant labs. • Yes; this is a very good team. • The team has access to all the necessary resources to conduct the proposed activities. • The budget is appropriate for the research proposed.
No: 2	• The team lead for bioinformatics may not have the requisite expertise or experience.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• Female subjects are 1.4 times more likely to suffer from TBI, yet the public focus has been on males acquiring TBI in sports. Experiments that consider sex-specific responses of microglia in TBI have not been conducted and will address this disparity. • The proposed studies will use one male and one female cell line. • TBI affects a diverse range of individuals. • TBI is common in underserved groups. • The applicant describes prior efforts or proposed plans for outreach, partnership, or educational activities to inform the development of DEI within the research project. • The applicant is the awardee of a CIRM COMPASS grant, and is involved in the CIRM Bridges program at their institution.
No: 0	*none*

Application #	DISC0-14519
Title (as written by the applicant)	Defining the source of dysfunction in monogenic intellectual disability syndrome neurons
Research Objective (as written by the applicant)	This study will use pluripotent stem cells derived from patients to determine why intellectual disabilities (IDs) caused by mutations in chromatin regulatory proteins lead to neuronal defects.
Impact (as written by the applicant)	Our study of intellectual disability (ID) syndromes will determine links between mutations and neuronal dysfunction
Major Proposed Activities (as written by the applicant)	• Our study of intellectual disability (ID) syndromes will determine links between mutations and neuronal dysfunction • Determine which ID syndromes show neuronal senescence in vitro • Identify the best culture method to recapitulate findings from ID syndrome brain data • Discover the primary trigger of neuronal stress and P53 activation • Perform loss of function study to identify primary response to defective chromatin regulation • Elucidate a potential role for neuronal activity in DNA damage and stress response in ID syndrome neurons
Statement of Benefit to California (as written by the applicant)	The project described here will bring great benefit from families suffering with Rett Syndrome. Our novel small molecules will be translated into drugs that have been shown to ameliorate symptoms of Rett Syndrome in neurons through modeling via human induced pluripotent stem cells. Rett Syndrome strikes 1:10000 live female births. In a State like California, this means thousands of families are suffering right now, with no treatment options available.
Funds Requested	$1,500,337
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." 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	6
Highest	92
Lowest	75
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	8
(1-84): Not recommended for funding	7

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	• Intellectual disability (ID) syndrome represents a large group of diverse disorders with no cures. Identification of a unified pathway would be an important contribution that would open new therapeutic approaches. • The connection between loss of function of specific proteins, dendritic-branching defects in neurons, and microcephaly is not understood. This represents a bottleneck in our understanding of intellectual disabilities (IDs). • The applicant presents a clearly defined hypothesis presented in the proposal. If this hypothesis is both correct and causative, this study will have a major impact in the field. • This is a very innovative study aiming to determine why (and how) IDs caused by mutations in chromatin regulatory proteins lead to neuronal defects. The hypothesis is that the DNA damage and neuronal senescence play an important role in the development of ID syndromes (e.g., Rett syndrome). • There is a very large gap in our understanding of the pathogenesis of neurodevelopmental disorders, and this project proposes a unifying hypothesis. • The project is relevant to IDs and could also provide insight into mechanisms of premature aging.
No: 1	*none*
GWG Votes	**Is the rationale sound?**
Yes: 12	• Yes. The applicant has previously identified a correlation between transcriptional aberrations, premature senescence, and induction of p53 in human induced pluripotent stem cell (hiPSC)-derived Rett syndrome neurons. Based on that, they now propose that premature senescence may be a cause of ID. • The applicant provides excellent explanations in response to previous GWG questions and concerns. They have revised their application accordingly. The rationale is sound and the new focus on IDs with microcephaly is strong. • The applicant provides compelling preliminary data from multiple models supporting their hypothesis. The current project is a logical next step and has been well considered and designed. • Previous GWG questions of the role of DNA damage have been addressed. • The rationale is clear. The question of causality is still an issue.
No: 2	• I continue to have some reservations about the causality stated in the hypothesis, but even if the hypothesis is not as broadly applicable as proposed, it is testable and will lead to interesting work. • Is there a particularly vulnerable neuroprogenitor population? If it also/instead affects neurons, why are there not continued neurodegeneration phenotypes for these patients? There is a period of regression for Rett patients, but not for the other syndromes like BOS or KAT6A/B. Do the neurons have premature senescence but then stop senescing so there's not further degeneration? • If the mechanisms presented such as telomere dysfunction cause neurologic dysfunction then why do patients with clear primary telomere dysfunction monogenic syndromes (such as mutations in TERC) have no neurologic dysfunction, even into adulthood?
GWG Votes	**Is the project well planned and designed?**
Yes: 12	• Yes. This is an ambitious project, but it is very well planned. The experiments are well designed and in a logical order. There are no unnecessary experiments, nor is anything major missing. However, the proposal does not provide a statistical analysis plan - i.e., power calculations, statistical tests/methods to be used, etc. • Overall, yes, but the choice of experimental controls is not always appropriate. • The timelines are not very clear.
No: 2	• It would have been very compelling to include a non-epigenetic disorder with a known mechanism of disease (there are many, including many with microcephaly), as opposed to Down syndrome, as a comparator.

	• Down syndrome is poorly understood and therefore doesn't serve as an appropriate negative control here (though it is another interesting line of inquiry: see Krivega et al AJHG 2022 *Consequences of chromosome gain: A new view on trisomy syndromes).* • I would suggest including something like a tubulin-associated microcephaly, for which the mechanism is well-described. • There are still a limited number of pitfalls and alternatives presented. It may be helpful to think about both scientific and technical pitfalls for each of the proposed sub-aims. • For example, the alternative approach for Aim 2 states that the hypothesis may be wrong but the experiments are worth doing. This is not an alternative approach nor an alternate hypothesis. • There is a lack of statistics and power calculations.
GWG Votes	**Is the project feasible?**
Yes: 13	• This is a very ambitious project, but the team is well suited and equipped to execute it successfully within the proposed timelines. • The team and collaborators are suited for this work. They may consider adding someone with clinical genetics experience. • The timeline for the project - i.e., what will happen when - is not clearly presented. A Gantt chart, or a timeline clearly showing a plan for year 1, 2, and 3 would be highly beneficial. • Yes - the team has good in vitro systems to study development of cells derived from patient iPSCs. • I have no concerns about resources at the PI's institution.
No: 1	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• Yes - the applicants have paid a lot of attention to DEI while designing their study. Undeserved populations will benefit from this study. • There is a very strong proposal vis-a-vis diversity in the cell lines. • The cell lines to be used account for race and sex. • The PI does not report any formal DEI efforts, such involvement in a pipeline program, etc. However, the PI does mentor students who are first generation scientists.
No: 0	*none*

Application #	DISC0-14366
Title (as written by the applicant)	Determining how age-specific heterogeneity of human hematopoietic stem cells and megakaryocyte progenitors contribute to thrombotic disease upon aging
Research Objective (as written by the applicant)	Our research will determine how aging of human blood stem cells leads to dramatic increases in disorders of platelets, cells that normally prevent bleeding but form harmful clots when dysregulated.
Impact (as written by the applicant)	Our findings have the potential to inform prevention and mitigation strategies of bleeding and clotting disorders that contribute to significant morbidity and mortality in minorities and the elderly.
Major Proposed Activities (as written by the applicant)	• To determine how characteristics of platelet precursor cells are altered upon human aging. • To determine how different pools of human platelet precursors change functionally with age. • To test how age-related inflammation and clinically approved drugs alter the function of human platelet precursors. • To determine the differences between how blood stem cells from young and aged individuals differentiate into platelet precursors. • To determine the effects of inflammation and pharmacological intervention on the differentiation of aged human blood stem cells into platelets and platelet precursors.
Statement of Benefit to California (as written by the applicant)	Our research will benefit California and its citizens by informing improved and personalized strategies to reduce the detrimental impact that bleeding and clotting disorders have on human health, in particular minorities and the elderly. Californians are among the millions of people who take daily platelet-modifying drugs. Understanding age-related dysregulation of platelet production by human blood stem cells will be hugely beneficial for individual health and financially impactful for society.
Funds Requested	$1,536,000
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." 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	82
Median	85
Standard Deviation	7
Highest	89
Lowest	65
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	8
(1-84): Not recommended for funding	7

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	• Aging is known to affect frequency of thrombotic disease, for example, thrombocytopenia and thrombocytosis are affected by age. Age dependent changes in platelets arise from changes in activities of their progenitors, specifically hematopoietic stem cells (HSCs) or precursor megakaryocytes. This proposal aims to understand how changes in megakaryopoiesis lead to a drastic increase in platelet-related disorders upon aging. • Completion of this project should provide more baseline data on human megakaryopoiesis and its changes with age. This will inform future applications to understanding, diagnosing and treating thrombotic diseases. This project is related to the hematopoietic stem cell field. • This work could open up a new understanding of platelet and megakaryocyte biology.

No: 1	• Achievement of the applicants' aim to provide a putative explanation for and path towards mitigating age-related platelet dysregulation would benefit public health.
	• The goal of this proposal is to provide an actionable understanding of how age-related changes to megakaryopoiesis leads to a drastic increase in platelet-related disorders upon aging. Completion of the experiments will reveal mechanisms of age-specific platelet production dysregulation that can be targeted to mitigate age-related thrombotic disorders.
	• The proposal is ambitious and overly broad. The applicants wants to answer a number of questions:
	  ◦ Why dysregulated megakaryopoiesis is increasingly prevalent with age
	  ◦ Why some individuals produce too many platelets while others produce too few
	  ◦ Why some individuals respond to certain platelet-modifying drugs while others do not
	  ◦ Why platelet-modifying therapeutics may be differentially effective depending on age
	  ◦ Whether age-specific megakaryopoiesis originates from aging HSCs or by cell-intrinsic changes to megakaryocyte progenitors (MkPs)
	  ◦ How age-related inflammation influences megakaryopoies
	  ◦ What cell types and molecules may be targeted to achieve control of platelet production
	  ◦ More focus, perhaps on just one of these questions, might make for a stronger application.
	• The logic supporting the statement that "Understanding age-related dysregulation of platelet biology will be hugely beneficial for individual health and financially impactful for society" does not hold. Just because platelet biology changes with aging does necessarily not mean that understanding that process will be hugely beneficial.

GWG Votes	**Is the rationale sound?**
Yes: 13	• The work is motivated by strong and intriguing findings in mice. • The project builds on the team's published work in mice showing that whereas HSCs functionally decline with age, MkPs gain expansion capacity upon aging. Further lineage tracing data revealed a non-canonical pathway from HSCs directly to megakaryocytes in aging mice, which could help explain the differences in platelet behavior with age. • The preliminary data in the mouse that is built on functional assays and lineage tracing is strong. There is some preliminary data from human bone marrow samples that MkPs are heterogeneous and that heterogeneity changes with increasing age. It is not clear whether this results from the same mechanism as in mice, but this will be explored further here. • Little is known about megakaryopoiesis in aging humans in terms of the relationship between declining HSCs and increasing production of platelets. Exploring this in more detail as proposed here should help determine the sources of heterogeneity with age.
No: 1	• The applicant's claim that distinct differentiation paths during hematopoietic ontogeny are novel does not account for prior work in this field. The authors should consider literature related to the "Waddington landscape."

	• Aim 1.1 seems unfocused, without a clear description of how the data will be used, what hypothesis will be tested, and how this aim will advance the overall proposal. • The applicants should justify the use of mice as a model for aging platelets, given their short lifespan. • The proposal contains unsupported claims that do not lend credibility to the rationale. For example, the claim that platelet dysregulation contributes to Alzheimers is unproven.
GWG Votes	**Is the project well planned and designed?**
Yes: 13	• The different aims of the project are carefully planned and designed. Aim 1 will use molecular techniques to assess cell surface markers and RNAs in purified progenitors from young and old bone marrow samples to describe heterogeneity with age. The applicant has already done this in mice successfully and will compare mouse and human data. • More traditional lineage tracing methods could be incorporated.
No: 1	• The applicant may have overestimated the ease and generalizability of comparative transcriptomics. • It is unclear what components of Aim 1.2 will be performed in mice.
GWG Votes	**Is the project feasible?**
Yes: 13	• The aims are clear, build on existing data and can be achieved in the proposed timeline. This is a strong proposal, with good preliminary published data in mice and some preliminary data in humans. It addresses an important issue with considerable clinical relevance if it does indeed find new pathways to modulate platelet production with aging. • There is extensive description of the potential pitfalls and alternative approaches to be used, which is a real strength of this proposal. The timelines are appropriate and the material and technologies are in hand and ready to implement. • Importantly in Aim 1.2, the applicants will move from descriptions of molecular changes with age to assessing functional differences with age. The applicants will test whether old progenitors make more platelets in vivo by transplanting into immunodeficient mice and in vitro in various platelet assays. These studies should be feasible because the applicants have done these sorts of assays before and provide preliminary data.
No: 1	• Some of the aims as stated are feasible, but overall the project is too ambitious.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• Platelet disorders span a number of races, demographics, and populations. Understanding the basic biology of aging platelets could help personalize therapies and benefit diverse populations. • The bone marrow samples will be obtained from individuals of diverse backgrounds. The applicants will deliberately track race, ethnicity, sex, gender, and age diversity as well as HLA type, for all samples whenever possible. This will be baseline data for future studies, because it is known that frequency of diseases vary by sex and race. However, the applicants acknowledge that the sample size in this initial study will not necessarily reveal all underlying variables. • Excellent DEI activities from the PI and team, through the PI's training record and institution affiliations.
No: 0	*none*

Application #	DISC0-14514
Title (as written by the applicant)	An interactive data resource for hypothesis testing in stem cell single-cell gene expression and validation of the results with brain organoids
Research Objective (as written by the applicant)	We are building a "virtual molecular microscope" where anyone can quickly visualize a very recent, high-throughput molecular assay, single-cell RNA-seq, and spatial gene expression studies
Impact (as written by the applicant)	Currently, a lot of data has been published, including hundreds of datasets on the cerebral cortex alone, but it takes hours to convert the datasets and look at them. Our websites will reduce this to seconds.
Major Proposed Activities (as written by the applicant)	• Find and convert existing single-cell gene activity datasets published over the last few years and add them to a centralized web resource. • Create the first "spatial" dataset of the cerebral cortex, one that shows gene activity in single cells but on sections of human brain tissue, not just the cells in isolation • Upgrade the web resource such that users can combine the data from Activity 1 (isolated cells) with Activity 2 (spatially arranged cells) and can compare the different datasets
Statement of Benefit to California (as written by the applicant)	California is a hub for stem cell research, partially thanks to CIRM. Our new data repository and data analysis tool will allow stem cell researchers, many of which who are based in California, to save a lot of time when looking at datasets. Our website will share these datasets with the world. Finally, the project has a big potential to attract more research funding from other sources to California. Hopefully, there will ultimately be health benefits to Californians thanks to this tool.
Funds Requested	$1,160,126
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.” 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	85
Median	83
Standard Deviation	6
Highest	100
Lowest	79
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	6*
(1-84): Not recommended for funding	9

* See Minority Report below

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	• By analyzing the single cell expression data, the project is able to provide new insights into the biology of stem cells and their application in regenerative medicine. • The project uses human stem cells and human cortex, which is highly relevant to human biology and disease, as these cells have the potential to treat a wide range of diseases and disorders. • The identification of a gap in knowledge related to the lack of a unified platform for single cell expression data is a significant contribution to the field, as it addresses a key challenge that researchers face in analyzing and interpreting complex data sets. The development of such a platform has the potential to accelerate research in the field of stem cells and regenerative medicine, as it will allow researchers to more easily access and analyze data, and to make more informed decisions about their research. • By addressing this challenge and providing new insights into the biology of stem cells and their role in the development of the human cortex, the project has the potential to accelerate the discovery and development of new stem cell-based therapies, provide new insights into a wide range of human diseases and disorders, and enable the research community to formulate and test novel hypotheses in the field of regenerative medicine. • While the project is largely descriptive in nature, the massive amount of data that it is analyzing has the potential to lead to the emergence of new themes and connections that have not been made before. • Insofar as brain organiods and spatial transcriptomes are used as readout of stem cell conditions or engineered stem cells, this project is relevant to more robustly phenotyping the effects of stem cell engineering, which is a long-standing issue in developing therapies (stem cell-related and otherwise) for brain diseases. • The software component of what's proposed would have a major impact over time, given the reach of this group and popularity of their current work. • The applicants provide letters of support from nine investigators, both in CA and around the world, who have found previous interactions with this group's tools beneficial and look forward to the functionality offered by the proposed database. • In terms of the use of spatial transcriptomics, there is no universal repository for the data, and consequently no easy way to display or analyze the data across experiments. However, the spatial transcriptomics work proposed here is likely not a breakthrough on its own. Another group studying the developing human brain has posted a pre-print using similar approaches, which indicates that the field is likely not far behind in generating the type of data proposed in Aim 2. • Aim 2 has already been done by others.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 13	• The proposed project is based on sound scientific rationale, as it addresses a key challenge in the field of stem cell research related to the lack of a unified platform for single cell expression data. • The project's focus on comparing human cortical development datasets is reasonable and has the potential to expose major similarities and differences in the biology of stem cells and their application in regenerative medicine. • The project's focus on studying human cortical development is particularly significant, as this is an area of research that is currently underrepresented in the field of stem cell research. • The authors make a convincing case that there is a lack of standardization for spatial transcriptomics data and (to a lesser extent) adequate visualizations for those datasets. Addressing the lack of standardization is a first step to addressing the somewhat lesser gap of adequate visualization tools. • An integrated database would be a useful tool for many researchers. • Prior experimental and computational work from this group has been of high quality, so there is little reason to doubt this proposal will accomplish its aims. There is a lack of direct preliminary data, but this is likely due to the proposed transcriptomics approaches being very standardized. Direct preliminary data is not strictly needed for software development.
No:	*none*

1	
GWG Votes	**Is the project well planned and designed?**
Yes: 11	• The research proposal is well planned and designed to give meaningful results, as it is divided into three distinct components that will be executed in a logical sequence. The first and second component of the proposal will involve the collection and processing of a large amount of single cell expression data, while the third component will involve the integration of these data into a unified platform for analysis. • The first aim will involve collecting available data. The applicants are in a good position to accomplish this given their connections to several groups generating data and their prior software tool experience. • The second aim has already been performed by another group and is an obvious approach to managing spatial transcriptomics data. • For the third aim, in theory developing a visualization and imputation tool for single cell distributions in spatial transcriptomics would be of value. However, the applicants may encounter challenges with cell type identification. The applicants propose to establish fixed cell type definitions, but this approach does not account for the expectation that users may have unique definitions. To maximize utility of this resource, it will be important to allow for flexibility in definitions, clustering parameters, and imputation algorithms. A potentially more useful approach would be to allow users to supply their own cell type definitions and imputation algorithms which can be applied to all data in the resource. • From a software development perspective, this proposal does not explore alternative approaches is the usual manner, which would be to get user feedback on mockups and iterate the platform. • The timeline is in line with CIRMs mission and the proposal's focus on analyzing human cortical development datasets has the potential to make a significant contribution to the field of regenerative medicine. • The timeline is short in terms of gathering and processing a large number of datasets. • In terms of urgency, Aim 2 does not have the same long-term potential as the software work. It may have been included to strengthen the link to stem cell work, and in essence this work has already been done.
No: 3	• The proposal does not discuss potential pitfalls and alternative approaches. • The proposal has a goal of understanding how laminar and areal cell markers are structured within cortical organoids. However, the proposal does not describe how the three aims are related to this goal.
GWG Votes	**Is the project feasible?**
Yes: 14	• The proposed project is highly feasible, as the project aims are well-defined and are expected to be achievable given the expertise of the team. • The project team consists of investigators who bring unique and complementary expertise to the project. This includes expertise in single cell genomics, stem cell biology, and bioinformatics, which are all critical to the successful execution of the proposed research. • The project team has also demonstrated a track record of successfully executing similar projects, which further enhances the feasibility of the proposed research. • In terms of budget, the proposed funding is appropriate for the scope of the project, as it will cover the cost of collecting and processing a large amount of single cell expression data, as well as the cost of integrating and analyzing this data. • Aim 1 relies on human persistence above all else, access to brain tissue for aim 2 appears to be secure, and the software tools for aim 3 are available. Overall, the team has all necessary resources to complete this project. • Given the likely non-uniqueness of Aim 2, the applicants should consider removing this aim and focus on getting their more unique software available on a faster timeframe, ideally with a few iterations incorporating user feedback. • For software development on a new data type, there is benefit to iteration. Getting input from those using spatial transcriptomics data, in terms of what these users would want to see in a software tool, could be helpful.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes:	• Data from diverse populations can be incorporated into the database.

14	• Yes, the applicant described prior efforts or proposed plans for outreach, partnership, or educational activities to inform the development of DEI within the research project. • Given the number of samples and datasets that will be incorporated, the project outcomes extend or validate the applicability of regenerative medicine discoveries to underserved populations, including underserved racial/ethnic communities. • Theoretically, in the future this project could extend or validate the applicability of regenerative medicine discoveries to underserved populations, including underserved racial/ethnic communities, but intrinsically it does not. • While brain tissue from only three sources will be used in Aim 2 and these tissues are rare, none of these brain tissues are stated to be from diverse subjects. • Most of the comments supposedly related to DEI made by the applicant are not relevant to the proposal itself. For example, stating that the availability of the resource on the internet is a DEI benefit does not support DEI principles in this project. • It would be great to have searchable annotation of the genetic background of samples in the proposed resource, and this was not proposed. • The diversity of the application more broadly will simply be a reflection of the diversity present (or not) in the single cells and spatial transcriptomics field. The authors say they will gather the 100 "best" studies for inclusion, but do not describe how the best studies will be defined. Perhaps if the applicants established some rigorous dataset selection criteria, diversity could be a component of those criteria.
No: 0	*none*

MINORITY REPORT

If an application receives a Final Score of 1-84 and 35% or more of the scientific members of the GWG recommend an application for funding, then a minority report is provided that summarizes the perspective of those scientific members.

This application was scored by all 15 GWG panelists. Nine panelists scored 79-84, five panelists scored 85-90, and one panelist scored 100. The majority of the 15 panelists indicated that the proposal addresses a significant unmet need in the curation and visualization of single-cell and spatial transcriptomics data. The majority of the panelists also agreed that the proposed work is likely feasible given the applicant team's strong track record. The panel was divided on whether the project was well planned and designed. The majority of reviewers felt that the application should better incorporate user feedback and input into the design of the software tool. Reviewers who recommended this application for funding noted the applicants' strong track record and CIRM's prior investment in the platform that will be enhanced as part of this application. Reviewers who recommended this application for funding highlighted the potential for this project to address significant gaps in data aggregation and the overall utility of having a searchable database with visualization capabilities.

Application #	DISC0-14450
Title (as written by the applicant)	Investigating epigenetic reprogramming and cell extrinsic signaling events in the specification of human primordial germ cells
Research Objective (as written by the applicant)	This project will identify the progenitors that get specified to primordial germ cells (PGCs) in humans, and map the epigenetic reprogramming and signaling events that are key for this cell state transition.
Impact (as written by the applicant)	Due to a lack of access to early human embryos, the project will employ 3D gastruloids to impact our understanding of primordial germ cell specification and the mechanisms involved in their emergence.
Major Proposed Activities (as written by the applicant)	• Identify the progenitors that specify primordial germ cell-like cells (hPGCLC) in humans • Confirm the identity of hPGCLC progenitors and understand the role of epigenetic reprogramming in the specification of hPGCLCs • Map spatial regulation of hPGCLC specification
Statement of Benefit to California (as written by the applicant)	Primordial germ cells (PGC) are the founder cells that differentiate to sperm or egg, and dysregulation in this germline specification results in infertility, a disorder that affects 10% of adults. Despite its significance, our understanding of how and where PGCs are specified 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	$1,182,193
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." 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	81
Median	82
Standard Deviation	2
Highest	85
Lowest	75
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: 12	• There is a bottleneck in the field of infertility related to our inability to define origins of germ cells in the human embryo. This project will use 3D human embryo models and multiomic approaches to define primordial germ cells (PGC) progenitors and the pathways that are involved in establishing this lineage. • The project's potential impact extends beyond fertility treatment. It could also have implications for genetic research and disease modeling. • If successful, the project has the potential to generate germ cell precursors and actual human germ cells. • Currently, the human germ cell precursor is unknown, which limits our ability to study it. • The project addresses a major bottleneck in the field of fertility treatment.
No: 2	• I have some concerns about the validity of the gastruloid model and how relevant the findings will be to real human embryos.
GWG Votes	**Is the rationale sound?**
Yes: 13	• The rationale for the project is based on existing literature, which highlights the importance of germ cells in human reproduction and the challenges in generating functional human germ cells in the lab. • Additionally, the investigator has presented promising preliminary data that supports the feasibility of the project and the potential for generating human germ cells from induced pluripotent stem cells (iPSCs). • It is known that primordial germ cell (PGC) progenitors can arise in vitro in stem cell derived epiblast/amnion structures. There is some knowledge of transcription factors involved. The applicant's focus is on epigenomic factors, which is a competitive angle in the crowded field of infertility research. • The applicant has already used gastruloids to form early amniotic sac-like structures and developed scMAT-seq technology that allows measurement of DNA methylation, DNA accessibility and transcriptome from single cells - a very nice technology. The applicant posted a paper in BioRixv on this approach, showing that PGC progenitors emerge from the epiblast and have a distinct epigenomic phenotype. • The applicant presents great preliminary data.
No: 1	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 11	• The molecular characterization, lineage tracing to find progenitors and spatial mapping studies are well-structure. • The description of planned epigenomic analyses is vague and the plans for interpretation are unclear. • The proposal is based on strong preliminary data, appropriate stem cell models and strong expertise in multiomics approaches to single cell analysis. Further technology development to provide spatial multiomics in gastruloids and other cell systems is intriguing but probably needs to be a separate grant. • Most of the project components are in place and ready to go. However the spatial multiomics technology requires considerable technical development and seems beyond the scope of this proposal. • To address potential pitfalls, the study incorporates multiple complementary approaches, including transcriptomic analysis, CRISPR gene editing, and functional assays. • Pitfalls and alternative approaches are described where appropriate. The technologies proposed are state of the art but generally within the expertise of the group. • The study design is strong, with a focus on optimizing approaches. • The project plan and timeline demonstrate an urgency that is commensurate with CIRM's mission.
No: 3	*none*
GWG Votes	**Is the project feasible?**
Yes: 13	• The project employs complex technologies to a simple system. The PI has the track record on the tools, so this is likely doable.

	• The proposed aims and expected project outcome logical and likely to be achieved within the proposed timeline. • The proposed team is appropriately qualified and staffed. • The team has access to all the necessary resources to conduct the proposed activities. • The budget is appropriate for the research proposed. • Aim 1 is an extension of the applicant's work in BioRixv and is feasible. It will provide important baseline data. • Aim 2 will test how important histone marks are for the induction of PGC-like cells, and is a more complex aim. • The lineage tracing with barcoding will require careful analysis. The other experiments are also important but require careful application of approaches like CUT&tag to quantitate histone marks. Given that the number of PGC-like cells that develop in the gastruloids is quite small, this aim is challenging. • Aim 3 is a technology development aim and seems out of scope for this grant. Achieving all three aims in the time frame of the grant is ambitious. • The PI has strong background in multiomics and has developed the scMAT-seq technology required for this study. Additional partners bring required expertise in gastruloids and optogenetics, respectively. This could be considered a team grant.
No: 1	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• Sex will be taken into account with the analysis of the development of germ cells from both male and female stem cell lines and at different ages. • A variety of male and female cell lines (20) will be used. • The influence of race, ethnicity, sex, gender, and age diversity is addressed. • The PI is heavily involved in DEI-oriented activities. • The applicant describes prior efforts or proposed plans for outreach, partnership, or educational activities to inform the development of DEI within the research project.
No: 0	*none*

Application #	DISC0-14499
Title (as written by the applicant)	Pregnancy-associated systemic factors to rejuvenate aged stem cells - a new frontier in regeneration
Research Objective (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 (as written by the applicant)	The study will address the longstanding knowledge gaps related to the mechanisms of pro-regenerative impact of pregnant systemic milieu and female muscle stem cell senescence
Major Proposed Activities (as written by the applicant)	• Isolation of female pelvic muscle stem cells from diverse population of young and old donors • Comparison of genetic, epigenetic and phenotypic signatures of young and old female pelvic and non-pelvic muscle stem cells • Learning how pregnancy-associated systemic factors impact aged female muscle stem cells' genotype, phenotype, and regenerative potential
Statement of Benefit to California (as written by the applicant)	The proposed studies bridge longstanding knowledge gaps regarding aging of female pelvic muscle stem cells and whether pregnancy-associated factors could be used to mitigate functional decline of these cells with age. 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	$1,543,645
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." 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	76
Median	82
Standard Deviation	14
Highest	89
Lowest	45
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	7*
(1-84): Not recommended for funding	8

* See Minority Report below

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	• This proposal aims to elucidate the pathogenesis of pelvic floor disease, which is not currently well understood. • Regeneration and stem cells appear to play a role in the disease process. The proposed experiments will likely add novel and important mechanistic understanding. • There is a clear lack of treatment options for pelvic floor diseases. • The proposal focuses on the potential therapeutic use of stem cells for these conditions, with a focus on factors that contribute to disease regression. • The proposed research addresses two major gaps in the field – understanding and treatment. • This project will address an important knowledge gap in our understanding of the biology and regenerative capacity of women's pelvic floor muscle stem cells. • Pelvic floor disorders represent a major public health issue, particularly in older women; these disorders are associated with significant morbidity and a diminished quality of life. • Outside of surgical intervention, effective therapies for this disorder are currently unavailable, at least in part, because of our poor knowledge of pelvic floor muscle biology. This project will attempt to overcome this bottleneck by elucidating the properties and regenerative capacity of pelvic floor muscle stem cells (MuSCs). • The idea of pregnancy as a physiological state of parabiosis is extremely interesting and novel. Understanding how hormonal alterations affect stem cells and regeneration will reveal basic mechanisms of muscle function. • This application is relevant to human biology and especially for pelvic floor muscle disorders, which affect healthy aging, social life, and psychology. • Understanding how aging affects pelvic human MuSC function will be important for women's lives all over the world. • The reduced capacity of limb MuSCs during aging has been studied extensively, especially in animal models. However, almost nothing is known about pelvic MuSC function. Given the important socio-economic impact of the pelvic floor disorders, the proposed research addresses a major gap in the field. • The proposed work will have a major impact on scientific knowledge in the stem cell field – both for pelvic muscles and for the effect of hormones on stem cell function. • I have some concern related to the limited description of the mechanistic studies (RNAseq, ATACseq and DNA methylation assays) and how these will be conducted.
No: 2	*none*
GWG Votes	**Is the rationale sound?**
Yes: 12	• The project is based on a solid rationale supported by current literature and extensive preliminary data. • The proposed rationale by which factors associated with pregnancy might be of benefit is innovative and scientifically plausible. • The project is supported by very strong preliminary data. These include: an established animal model, data from cadavers, ability to culture pelvic MuSCs, and the broad array of established lab methods to be utilized in the project. • The project is built on the idea that pelvic floor muscle MuSCs can be activated and induced to proliferate, differentiate, and contribute to the pelvic muscle regeneration in response to various cues, including systemic factors elevated in pregnancy. No strong evidence in support of this hypothesis is presented in the application. • 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 applicant's hypothesis that hormonal regulation during pregnancy affects MuSC behavior and function. • The study showing accelerated cell cycle entry measured by higher EdU incorporation) of pelvic MuSCs following mechanical stimulation in pregnant compared to non-pregnant rats, is intriguing. • There are some weaknesses in the preliminary data: • The investigators appear to have forgotten to include Figure 4, which contains key information about the state of the MuSCs and their time to division. • Figure 5B presents a graphical representation of KEGG pathways but it is not clear which genes are up or down and what comparisons are specifically made.

	• The downregulation of the collagen 5 isoforms is very interesting in Fig. 5, but this is not enough to claim downregulation of MuSC quiescence. Moreover, HeyL downregulation (as a direct Notch target) is not significant, while Hif1a is known as an interacting factor to Notch and not necessarily upstream of it, at least not in muscles. In contrast, there are numerous other genes (MyoD, Myf5, Pax7, among others) to demonstrate quiescence.
No: 2	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 8	• The project is very well planned. The preliminary data strongly support the overall experiment and design. • The established animal model for this particular disease is important and of great significance. The ability to collect the relevant human cell type is well described. The established cell culture model is a major strength. • Single cell experiments are well described and designed. • The proposal includes adequate discussions of pitfalls. Alternative approaches are well described and appear reasonable. • The proposed isolation of hMuSCs is a great method and will efficiently allow isolation of cells from young and old muscles, including pelvic muscles. • The experiments monitoring hMuSC function in vitro are well planned. Time-lapse microscopy will allow the applicant to investigate division dynamics and is feasible. • Including additional markers for cell sorting, especially for aged MuSCs is appropriately considered since these cells are expected to be in lower numbers. • Although the investigators include gene expression and epigenetic studies under Aim 2 on the Specific Aims page and in the timeline, there is no description of these in the Research Plan. However, under alternative approaches, the applicant proposes to use these multi-omics analyses to identify specific regulatory interactions (such as transcription factor/target interactions through motif analysis), as well as larger-scale changes in chromatin state.
No: 6	• I have some concerns with the molecular approaches and the feasibility of identifying specific systemic factors that are present during pregnancy and affect MuSCs. • The identification of systemic pregnancy-associated factors that regulate the state of PFM MuSCs from serum of pregnant women is an unrealistic task. Without a high-throughput functional assay on the defined MuSC cell population, Aim 2 will be a “fishing expedition” without much chance for success. • Aim 3 is dependent on aims 1 and 2, and thus is unlikely to be successful.
GWG Votes	**Is the project feasible?**
Yes: 12	• The project is supported by strong preliminary data. • The timeline appears appropriate. • The team is highly qualified. The investigator is a leader in the field. • All necessary resources are available. • The budget is appropriate. • The planned characterization of MuSC properties is well defined and logical. • The planned collaboration agreement will provide the necessary specimens from a variety of cadavers, allowing a wide range of ages to be investigated. • The planned collaboration with another research group will provide excellent muscle stem cell resources. • Single cell RNAseq, ATACseq and DNA methylation assays are not the easiest to perform. The applicant will benefit if they bring an expert on those assays in their team.
No: 2	• It is unlikely that the goals of the proposed specific aims could be achieved within the proposed timeline, due to the deficiencies in the rationale and experimental plan. • The PI is a clinician and a professor of obstetrics and gynecology with extensive expertise in surgical reconstruction of the pelvic floor. They also have research experience in the field. • The additional four key personnel have relevant experience to take part in the project. However, these investigators will mostly function in an advisory role.

	• The only member of the team who will do "wet lab" experiments is a single technician or a postdoc to be hired. Given the extent of the work proposed for this study, the staffing is insufficient.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• The project addresses race, ethnicity, and age diversity appropriately. • The project will also benefit underserved communities as these communities are also disproportionally affected by the disease. • Prior efforts and plans are described. • Pelvic floor disease primarily affects women. It has been historically studied in Caucasian participants; as a result, the benefits of past research have not been the same in different ethnic and racial populations. The applicant team is committed to applying the results of their study to diverse populations. • The biospecimens to be used for the proposed studies will represent the full diversity of people in California. • Most of the studies in animal models will be performed in male animals, due to their larger muscles - which provide more cells. • Given that males and females have different muscle regeneration kinetics, and that hormones could also affect this kinetics, this proposal is uniquely positioned to address issues that have never been studied before. • Including samples from participants from non-Caucasian patients will bridge a current scientific gap.
No: 0	*none*

MINORITY REPORT

If an application receives a Final Score of 1-84 and 35% or more of the scientific members of the GWG recommend an application for funding, then a minority report is provided that summarizes the perspective of those scientific members.

This application was scored by all 15 GWG panelists. One panelist scored 45, seven scored 60 to 82, and seven scored 85 to 89. The great majority of the 15 panelists indicated that the proposal met criteria 1 (has the necessary impact and significance), 2 (has a sound rationale), 4 (is feasible), and 5 (upholds DEI). The panel was divided on whether the application met criterion 3 (has a strong project plan). Reviewers who supported funding thought the project was well designed and described, with the proposal including appropriate studies and adequate discussion of pitfalls and alternative approaches. They were impressed by the preliminary data, established animal model, established cell culture system, ability to isolate relevant cells (hMuSCs) from human cadavers for study, resources, and applicant team.

Application #	DISC0-14566
Title (as written by the applicant)	Immune cloaking of human stem cell-derived insulin producing cells for curative cell therapy without immunosuppression
Research Objective (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 (as written by the applicant)	We test a novel molecule to reduce immune activation upon transplantation of allogeneic stem cell derivatives cells that can lead to alleviation of disease symptoms by improving graft health
Major Proposed Activities (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 • Test the immune cloaking of edited human cells when combined with immune cells in culture as well as in mouse models
Statement of Benefit to California (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	(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." 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	82
Median	80
Standard Deviation	4
Highest	90
Lowest	75
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	6*
(1-84): Not recommended for funding	9

* See Minority Report below

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	• Immune suppressive drugs are currently needed for allogeneic stem cell therapy to prevent immune rejections. This project aims to establish immune cloaking human stem cell lines via gene knockout of a DNA modifying factor. If successful, this project will have a big impact on stem cell therapy because authentic immune cloaking human stem cell lines can be differentiated into many cell lineages, such as neurons and cardiomyocytes. All of these cells may be used in the clinic for treating diseases. • The goal is to develop an effective strategy for obtaining induced pluripotent stem cell (iPSC)- and embryonic stem cell (ESC)-derived allogeneic hypoimmune or immune evasive insulin-producing pancreatic beta cells. These cells would continue to be surveilled and removed from the system if they undergo undesirable transformations but will not require a long-term immunosuppression for the graft's survival and function. • The applicants will take advantage of a recent finding showing that a DNA modifying factor plays an important role in regulating interactions between beta cells and immune cells in vivo in mouse models; these interactions normally trigger an immune rejection and elimination of the beta cells. ◦ On the basis of these results, the applicants hypothesize that deficiency of this DNA modifying factor will prevent immunologic rejection of transplanted allogeneic human beta cells. • The applicant team is well rounded and has assembled a fantastic group of consultants with expertises in human ESC differentiation, immunology and islet transplantation.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 14	• The project is based on a sound scientific rationale. • The applicants present solid data supporting their hypothesis, and the plan to delete the DNA modifying factor is logical and innovative. • There is evidence from multiple groups, including the applicants' team, that it is possible to obtain large quantities of functional insulin producing cell clusters (IPCs) in vitro from human iPSCs and ESCs using a defined stepwise protocol; these IPCs reverse diabetes in animal models. • The applicants demonstrate that they can differentiate normal human ESCs into IPCs and they can transplant these clusters into animals. • Published results from a research group and data from applicant show that interruption of the DNA modifying factor addressed in this proposal leads to immune evasion in the context of an autoimmune attack on pancreatic beta cells. • A 2021 publication demonstrated that knockout of the DNA modifying factor addressed in this application in beta cells reduced expression of inflammatory genes needed to activate immune cells in mice. If this finding holds true in human beta cells, removal of the DNA modifying factor may allow these cells to avoid rejection by T cell-mediated autoreactivity. ◦ However, for beta cell therapies, other cell types including T cells, NK cells, and macrophages may also mediate allogeneic rejection, and the applicants have not addressed this possibility.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**

Yes: 12	• The project is well-planned and designed.
	  ◦ For the first aim, the investigators will generate knockout human PSC lines to test the role of the DNA modifying factor in the stem cell-derived IPCs. They will use an established differentiation protocol to obtain the IPCs, and will measure DNA modifying factor function during and at the end of fate specification into the endocrine lineage. Transcriptomic, functional, and metabolic assays will be carried out to characterize the cells generated from the edited lines.
	  ◦ For the second aim, the team will ask if a deletion of the DNA modifying factor from the iPSC and ESC lines may lead to resistance of the IPCs derived from these lines to allogeneic immunity. To this end, the knockout IPCs will be exposed to immune cells in vitro and in vivo to determine their ability to activate T cells.

	• The experimental layout is appropriately planned and designed to give meaningful results. The animal model chosen is ideal for this study. One concern is about the feasibility of isolating and expanding the auto reactive T cell clones from banked T1D samples. It is not certain that a sufficient number of cells could be harvested from peripheral blood to test autoimmunity in experimental settings. • In their research plan, the applicants do not describe the approach they will use to knock out the DNA modifying factor in hPSCs. It was challenging to assess how they will achieve adequate knock-out efficiency or what steps they will take to avoid off-target editing in stem cells.
No: 2	• A main concern is that knock-out of the DNA modifying factor might only protect cells from autoimmunity and not from allorejection. The applicants acknowledge this is a potential issue and plan to include additional genetic modification to study the effect of deleting the DNA modifying factor in this context, but this seems insufficient to overcome this potential challenge.
GWG Votes	**Is the project feasible?**
Yes: 12	• The milestones are clearly defined and logical. • This is an early-stage discovery project, and it carries a high risk, but also a high reward. Given the known multifactorial mechanisms of allogeneic graft acceptance and rejection, it is unlikely that modulation of a single gene would provide a long-term protection from the allo-immune attack and completely solve the problem. Thus, the proposed aims might not be fully achievable within the proposed timeline. • The applicants acknowledge the high risk of the project in the potential pitfalls section, and describe a plan to investigate combinations of genetic modifications in addition to knocking out the DNA modifying factor to determine if the DNA modifying factor provides an immune evasive effect in other contexts. • The lack of one gene may not be enough to prevent immune rejection, and combinations may be needed. • The proposal lacks human-specific data, which makes it challenging to assess feasibility. • The team could benefit from an expert in genome editing. The lack of this expertise is apparent in the limited details regarding the approach the applicants will take to modulate their target gene or how they will control for potential off-target editing.
No: 2	• It is questionable if the applicants will be able to efficiently generate human beta cells lacking the DNA modifying factor. • The applicants have not shown that 1) they can perform gene editing in hPSCs and that 2) their gene edited cells can differentiate into beta cells as efficiently as normal cells. Published studies indicate that lack of the DNA modifying factor addressed in this approach also inhibits mesoderm and blood differentiation. Therefore, the applicants should not assume that its deficiency will not affect endoderm and beta cell differentiation.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• 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 a stem cell core at a major research institute that recruits patients with type 1 diabetes to collect material for cellular reprogramming. The investigators will also prioritize lines with diverse HLA subtypes. • There is no discussion of the influence of race, ethnicity, sex, gender, and age diversity for these studies. The sex of the cell lines or the mice used for transplant is not particularly relevant at this stage of the project.
No: 0	*none*

MINORITY REPORT

If an application receives a Final Score of 1-84 and 35% or more of the scientific members of the GWG recommend an application for funding, then a minority report is provided that summarizes the perspective of those scientific members.

This application was scored by all 15 GWG panelists. One panelist scored 75, eight panelists scored 80 to 84, and six scored 85 to 90. The majority of the 15 panelists indicated that the proposal addressed a significant need, had the potential for impact, and was based on a sound rationale. The panel was divided on whether the application contained sufficient preliminary data to indicate that beta cells lacking the DNA modifying factor could reliably be generated. Reviewers in the minority who recommended the application for funding felt that the applicants provided adequate data to support this approach. Reviewers who recommended the application for funding also noted that the applicants appropriately acknowledge the risks and potential pitfalls of their proposed approach, and felt that completion of this project would advance the field despite these risks.

Application #	DISC0-14365
Title (as written by the applicant)	Cellular modeling of GATAD2B-associated neurodevelopmental disorder (GAND): Investigation of cellular and molecular anomalies affecting NuRD Activity
Research Objective (as written by the applicant)	Human and animal models of NuRD-deficiency will identify NuRD-subtype function in context of neurogenesis. Multi-omic studies will identify/quantify molecular and cellular changes in NuRD-deficiency.
Impact (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 (as written by the applicant)	• We will generate GAND induced pluripotent stem cells (iPSCs) with inducible expression of HA-GATAD2A to see if GATAD2B's paralog can function to correct the cellular phenotypes seen in neural precursor cell (NPC) growth and cortical laminar marker assays. • 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. • 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. • GAND-iPSCs will be used to generate cerebral organoids and using immunohistochemistry will determine if NPC and cortical neuron subtypes are generated and co-express cortical laminar markers. • GAND-iPSCs will be grown as cerebral organoids and that will undergo single cell RNA-seq and ATAC-seq to determine what genetic pathways are dysregulated in NPCs and neurons with NuRD deficiency. • Cortices from mouse models of GAND will undergo undergo single cell RNA-seq and ATAC-seq to determine what genetic pathways are dysregulated in NPCs and neurons to inform and confirm human IPSC data.
Statement of Benefit to California (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 induced pluripotent stem cells (iPSCs) and mouse models to study the epigenetic dysregulation found in NuRD deficiency and many other NDDs. We hope to identify abnormalities in NuRD-deficiency that can be applied to many other NDDs, while also fulfilling CIRM'S goal of understanding brain disorders.
Funds Requested	$1,094,536
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." 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	80

Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	1
(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: 12	• The project focuses on the role of NuRD in different subtypes of neurodevelopmental disorder (NDD) and is relevant to stem cell application to human disease. If successful, the project will have a moderate impact on the basic knowledge of stem cells and their direct application to the clinic. • Neurodevelopment disorders (NDD) represent a disease entity with limited treatment options. The underlying disease mechanisms are not well understood. The proposed experiments will use stem cells for disease modeling of an NDD. • GAND is a genetic syndrome with a high impact on the patients. • It is highly likely that the detailed and comprehensive experiments will provide novel insights into functional mechanisms associated with GAND. • Ultrarare disorders are often neglected by academia and industry. Therefore, this project is well aligned with CIRM's mission. • NuRD appears to also play an important role in neuronal development and differentiation. Novel insights in this area might be gained, as well. • The applicants will test the hypothesis that NuRD activity plays an important role in human neural precursor cell (NPC) proliferation, differentiation, and corticogenesis. • The proposal has a high likelihood of providing important insights into disease mechanisms and cellular processes.
No: 1	*none*
GWG Votes	**Is the rationale sound?**
Yes: 11	• The rationale for the project is sound, but the goal of the project is not very clear. In general, this proposal is not very clearly written. • The overall justification for the overall study design, disease focus and experimental approach is very well developed. • The proposal is supported by strong preliminary data. • All proposed approaches are supported by preliminary data. • If successful, the proposal will provide comprehensive insights into cellular mechanisms and the role of NuRD signaling. • The rationale is weak. The relevance of mouse models to either human embryonic development or human disease is highly questionable. The proposal does not acknowledge variability between human organoids or species-specific differences. • Protein complexes are important in many different aspects of cell behavior - the applicant needs to focus this proposal on a specific cellular process. Also, it will be hard to jump from mouse to human. • Yes, but the applicant raises my concern by not acknowledging key limitations.
No: 2	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 10	• The applicants provide extensive descriptions and details on the proposed experiments. All aspects are well discussed and as mentioned above, supported by strong preliminary data. • This project is well planned. However, although the applicants mention the number of cell lines they plan to use, their sources are not clearly defined. Furthermore, statistical analyses and power calculations are not clearly presented.

	• The proposal discusses in great detail potential limitations and alternative approaches. These are well justified and appropriate. • The timeline is consistent with the CIRM mission. • The basis of the hypothesis tested in Aim 1, that developmental phenotypes due to 2B-NuRD deficiency can be ameliorated by exogenous expression of GATAD2B or repression of one of its target genes (ARX), is unclear. Direct comparison between iPSC-derived human organoids and mouse embryonic samples is flawed. • While some attempts to identify possible pitfalls and possible solutions have been included, these are not very clear. • The variability of iPSC lines is not addressed.
No: 3	• There is too much complexity in the system. The study overview is simple; the applicant will study gain- and loss-of-function of one gene.
GWG Votes	**Is the project feasible?**
Yes: 11	• Yes, the project is logically designed, but the timelines are very tight (although achievable with a competent team). • The proposed experiments are ambitious but can be achieved in the proposed timeline. • The team is highly qualified. • All necessary resources are available. • The budget is appropriate. • The project is feasible and the budget is appropriate. • This is feasible, but percent efforts currently given for the investigators might not be sufficient.
No: 2	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 10	• Yes, although it is very difficult to apply DEI to ultrarare disorders. • The proposal addresses the influences of race, ethnicity, sex, gender, and age diversity. • The proposal is likely to benefit a diverse and underserved population. • The applicant addresses outreach, partnership, or educational activities. • The response is very generic. • The proposal does not include precise validation of the applicability of regenerative medicine discoveries to underserved populations, including underserved racial/ethnic communities. A general statement about ensuring as diverse a trial population as possible is not sufficient. • Not very strong.
No: 3	*none*

Application #	DISC0-14386
Title (as written by the applicant)	Interrogating Satellite Cell and Myofiber Defects and Repair in DMD using Single Nuclei/Single Cell RNA Sequencing of Muscle Resident Cells
Research Objective (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 (as written by the applicant)	These studies will elucidate satellite stem cell and myofiber defects in Duchenne and Becker Muscular Dystrophy (DMD/BMD) and determine efficacy, mechanism and toxicities of exon skipping and AAV-gene therapy.
Major Proposed Activities (as written by the applicant)	• Continue bioinformatic analysis of existing DMD snseq/scRNAseq dataset. • Perform biopsies and prepare and bank new muscle tissue, PBMC and expanded satellite and T cell populations in culture and determine dystrophin expression. • 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. • Perform pilot experiments involving TNC/EGFR competitor interaction to follow up snRNAseq data identifying increases in TNC expression in DMD versus healthy muscle. • Collect and snRNA/scRNA sequence and analyze GT biopsies relative to snRNAseq reference set. • Clone dominant TCRs and screen for specificity to dystrophin micro-dystrophin or exon skipped dystrophin peptide antigens.
Statement of Benefit to California (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	$1,559,931
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.” 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	4
Highest	89
Lowest	75
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	• DMD contributes significantly to morbidity and mortality. Recent advances in the treatment of DMDs improves the outcome. However treatment success is not uniform and with a number of treatment modalities now available, treatment selection still remains challenging. The proposed experiment will further characterize the disease process and changes associated with treatment. This is of great significance. • The proposal investigates an important topic that is highly relevant to human disease. Single cell available data from human patients are currently limited. The investigators have acquired snRNAseq data from several dozen human muscle biopsies, which is an invaluable source of information to begin to understand disease severity in DMD patients. • DMD is a heterogeneous disease from a disease severity and outcome perspective. The obtained data will provide novel and valuable new insights into the disease mechanisms including the regenerative potential of muscle cells. • The project will provide novel and potentially significant insights into the disease variability. Clinically most important it might address variability in treatment response and might lead to more targeted treatment. • Expanding the cohort of human samples from diverse patients, which is also linked to clinical data, along with matched blood and muscle derived cell resources is powerful. • The investigators have optimized and previously used a scRNAseq for human DMD/BMD/healthy samples. Thus, it is highly likely that they will be able to add more information to their existing database. • The dataset that will become available in the course of this study to associate disease progression with molecular signatures of human SCs in untreated and repaired (AAV-treated and/or exon-skipping treated) samples. • Important potential in DMD treatment if satellite cells can be reactivated. • Although the work proposed here is not innovative per se since other groups have used the same method for human cells, the large number of human samples, including patients with and without generic therapy, distinguish this study from previous ones. • Strictly speaking, this proposal is not a stem cell or gene therapy proposal. It is focused on snRNA-seq or scRNA-seq analysis of muscle samples. These samples were from human muscle biopsies and will provide some valuable information from these experiments. • This proposal is similar to current funding mechanisms available to the investigators which resulted in a powerful dataset. The current CIRM application aims are almost identical to these funding mechanisms with the difference of increasing the human sample number by about a dozen samples. • This project may not address a major bottleneck in the treatment of DMD. The use of snRNA-seq or scRNA-seq to analyze human cells is very common these days. • This project is not a hypothesis-driven project, but will generate large amount of data. It is unclear whether this project will have a major impact in the DMD or stem cell field even if the applicant successfully performs all the aims. • It is not clear that the data available will enable the research community to formulate and test novel hypothesis in the future.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 11	• The rationale is sound. The application is well supported by current literature evidence. The proposed experiments are well described and the analysis plan is well presented. The proposed methods are state of the art. The ability to collect tissue biopsies is a strength of the proposal. • Using single cells from DMD patient's muscle cells for RNA-seq analysis is sound.

	• The preliminary data are very supportive for all aspects of the proposal. Aim 1 and 2 are well supported. Aim 3 applies this to samples obtained under treatment and will utilize similar approaches as the previous aims. • The obtained data have a high likelihood of providing novel insights into the cellular heterogeneity in DMD. • The preliminary data is strong. • The assumption that by increasing the sample number, it will result in robust discovery of more rare and currently hidden cell types and more robustly observe gene expression differences is not supported by data.
No: 3	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 10	• All aims have a high likelihood of obtaining novel and important insights. Aim 3 is likely to generate important insights. • Limitations are well described and alternatives are presented appropriately. • The timeline is appropriate the commensurate with CIRM mission. • All of the aims are appropriately planned and designed. • Access to 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. • Addressing the immunological barriers and other toxicities in some patients allows evaluation of biopsies post-treatment to assess durability of treatment and will be helpful for realizing the full promise of genetic therapies in DMD. • Making the dataset available to the research community will be helpful to allow other researchers that do similar studies to compare results. Given that different investigators use various isolation methods that might influence result interpretation, having a human DMD atlas is a good starting point. • Increasing the number of cells might alter the specific molecular profiling of these cells and therefore interfere with data interpretation. Preliminary studies to demonstrate that this does not happen using analysis of current human data (from the existing human samples) would have been useful. • It is unclear if all human samples will be collected in proposed time as this relies on availability of patient muscle biopsy tissues. It is vague if all the proposed biopsies can be isolated and analyzed on time. • Pitfall and alternative approaches are not well investigated. The researchers rely on their facilities and resources but they do not describe technical issues with expanding the cells.
No: 4	*none*
GWG Votes	**Is the project feasible?**
Yes: 12	• The project team is assembled from leaders in this field with many years of experience. The scientific environment is excellent, and the team has all the necessary resources to conduct the proposed experiments. • The team is highly qualified. • This team is qualified to do the proposed research. • All necessary resources are available. • The budget is appropriate. • There are no concerns that the proposed timelines can be achieved. Patient recruitment is always of some concern however the infrastructure and resources appear very supportive. • I have no major concerns about the feasibility of this project. Minor concern is whether they can recruit sufficient number of patients within this time frame. • The proposed project is very similar to the ones already funded by other groups. The additional recruitment and analysis of DMD (n=3), BMD (n=6), and healthy individuals (n=3) are definitely important since the more human samples the merrier, but it does not constitute a major innovation compared to their previous and current studies. • Not very novel but will provide useful database.
No: 2	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**

Yes: 14	• The project addresses and accounts for the influence of race, ethnicity, sex, gender, and age in the context of the disease. • The project is applicable to underserved communities. • Outreach, partnership, or educational activities are addressed. • Collection of biopsies from a wide range the of mutations, age, disease progression, and ethnic and socio-economic backgrounds is mentioned, however, this is something that cannot be guaranteed and relies on availability of patient muscle biopsy tissues. • The proposal relies on availability of patients and is difficult to expand on that. Nevertheless, the investigators have taken all appropriate measures (including a full-time community liaison) to provide patient outreach and expand the potential of new patients.
No: 0	*none*

Application #	DISC0-14451
Title (as written by the applicant)	Using Human Neurons to Model Parkinson's Disease and Develop Therapeutics
Research Objective (as written by the applicant)	We will use iPSC-derived neurons as a model to understand mechanisms of Parkinson's disease (PD) and explore therapeutics.
Impact (as written by the applicant)	There are many challenges for finding a cure for PD, due to the lack of effective therapeutic targets. The success of proposal will help understand a potential target better and develop therapeutics.
Major Proposed Activities (as written by the applicant)	• Characterization of disease relevant phenotypes in iPSC-derived neurons. • Evaluation of the disease-modifying activity of the compound series. • Reveal the pathogenic mechanisms PD.
Statement of Benefit to California (as written by the applicant)	About 500,000 Parkinson's disease (PD) patients are currently living in the U.S, and approximate 1/10 of them live in California. An effective treatment for PD is desperately needed. We will identify a therapeutic candidate and understand the cellular pathways of this target with the hope to treat PD. This study is closely relevant to public health of the state of California and will greatly benefit its citizens.
Funds Requested	$1,542,234
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." 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	4
Highest	85
Lowest	70
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	• The project makes use of induced-pluripotent stem cell (iPSC)-derived neurons to model Parkinson's disease and examine new therapeutics, thus advancing our understanding of

	the biology of Parkinson's disease and creating avenues for new disease-modifying therapies. ● The project aims to address an unmet need in PD research with a human neuron model of PD based in iPSC-derived patient neurons. ● The proposed studies explore the role of a specific protein in the pathophysiology of Parkinson's disease and the possibility to influence this protein pharmacologically for the treatment of the disease. Confirmation of this protein as drug target can have significant impact on research for disease-modifying therapies of Parkinson's disease. ● The project will advance biological understanding and provide a platform for drug screening. ● It is important to be able to model PD in order to develop better treatments. ● Compound and mechanism are clear strength.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 13	● PD is a common (1% over 60- and 4% over 80-year-old) neurodegenerative disease with still limited treatment options. The resubmission is specifically focused on a protein as a target in the disease. Reducing this protein is proposed as a therapeutic strategy. ● The proposal is an extension of earlier (in part CIRM-funded) studies by the applicants, in which they identified a protein as a critical player in Parkinson's pathophysiology. It makes sense to further pursue this research and to attempt full characterization of the role of the protein. ● The proposal is based on previous data by the applicant suggesting that a pathway represents a common denominator underlying different types of PD. ● The previous findings regarding the protein are compelling. Lacks preliminary data in validity of cell model both in terms of neurons generated and the ability to detect disease-relevant pathology. ● It is not clear whether pathology is recapitulated in the iPSC neurons. ● Biological age of the induced cells is not clear. ● The project focuses on one pathway. It is not clear how generally translatable the findings will be to all PD patients.
No: 1	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 11	● The proposed mechanisms and compounds influencing them will be tested in in vitro models of Parkinson's disease (patient-derived iPSCs) and Drosophila models. To move the approach to clinical utility, it will be unavoidable to show efficacy in mammalian in vivo models. ● The project is well planned but the experimental model lacks sufficient preliminary data. ● It is stated that "The small molecules discovered in this study are predicted to be suitable for oral uptake, be free of toxicity, and pass the blood-brain barrier." These are the key questions. Why are the applicants not addressing them? A statement of belief does not suffice. ● Response to previous critiques is very minimal.
No: 3	● There is some concern regarding the neural model in Aim 1; the fly addition has not addressed this issue. While it is suggested as common denominator, it is still not entirely clear how generally translatable the findings would be to PD patients. ● It is not clear at what stage the compounds would act and be most beneficial. The applicants may need the suggested companion diagnostic.
GWG Votes	**Is the project feasible?**
Yes: 13	● Team and environment very good. Preliminary iPS derived neurons and models available. ● The studies to achieve the stated objectives are well planned and likely to generate meaningful results. ● Compound is already identified. ● The team is well positioned to study the proposed pathway in PD. ● The team has access to the necessary resources and has proposed an appropriate budget.

	• The project is feasible to conduct, but it is unclear whether it will generate the data expected. The first milestones are to culture and derive neurons and to examine their phenotypes. This is required for the project to be successfully executed and very risky to initiate a project before this is firmly established. Better preliminary data is needed to show the ability to detect disease relevant pathology.
No: 1	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• The project outcomes could be applicable to underserved populations. The applicant has described prior efforts in outreach and DEI activities. • Partially so. But the proposal lacks diversity in the cell lines included.
No: 1	• Little diversity.

Application #	DISC0-14342
Title (as written by the applicant)	Making of Geometry: Mapping the mechanics that shapes the human neural tube
Research Objective (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 (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 (as written by the applicant)	• We will map out the forces involved in neural tube closure • We will systematically test how the neural tube responds to physical variations in their environment, such as substrate stiffness • We will uncover the molecular mechanisms involved in coordinating forces in response to variations in physical features.
Statement of Benefit to California (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	$1,188,134
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." 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	2
Highest	82
Lowest	75
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	• The proposal addresses a key knowledge gap in our understanding of the origins of neural tube defects in early human development and how to intervene to prevent them. The project would add important baseline data on neural tube development not available from any other human system. • The proposal outlines a plan to understand the mechanical control of human neural tube folding in vitro and whether it can be harnessed to understand neural tube defects. • However, it lacks sufficient preliminary data and a plan to further understand molecular and genetic mechanisms. How will the proposed platform be utilized to produce new therapeutics for neural tube closure related diseases?
No: 2	• The project will use stem cell models to understand mechanical aspects of neural tube formation in humans. • Yes. The human cell model will address difficulties in monitoring biomechanical forces in intact embryos, and will address species differences in the biomechanics of tube formation. • The project will fill gaps in our knowledge of mechanisms of neural tube closure. Neural tube disorders are still amongst the most common birth defects.
GWG Votes	**Is the rationale sound?**
Yes: 12	• The project has the potential to address the important question of how gene action translates into morphogenetic forces in this system. • The team have published some excellent results recently using their model of neural tube closure. The images are fairly convincing, but the discussion of neurons is confusing. • It is difficult to relate outcomes from the biophysical studies directly to neural tube malformation. • Yes. It is known that the process of neural tube closure involves complex mechanical folding and fusion processes that can go wrong and cause developmental defects. There are good tools available now to be able to monitor mechanical changes in tension, elasticity, etc. in living cellular structures and the development of a reproducible stem cell based model of neural tube in humans is important. Preliminary data presented here and in the paper published in Nature in 2022 are very compelling. • The rationale is sound, but the proposal doesn't cite published literature that overlaps with goals of the project (i.e., self-organizing cerebral structures). • Preliminary data are underdeveloped but point to interesting and relevant directions. No animal model data are presented that corroborate the applicant's in vitro model. No data are shown on known genetic defects of neural tube closure in their model system.
No: 2	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 12	• It's difficult to understand how measurement of substrate stiffness can be related to the environment in the embryo. • Alternative approaches to modulate the stiffness of hydrogels are proposed. • The possibility that integrins other than B1 might be involved is acknowledged. • The project will improve our understanding of the role of biophysical forces in the model, but it is not clear how key outcomes will influence our thinking around failure of neural tube closure. It is unfortunate that the authors did not explore the effects of specific genes or environmental factors on mechanobiology. • Studies with b1 integrin have predictable outcomes. • The system is well established and robust. • The applicant does not describe how they would modify their model system if studies indicate that known pathways in neural tube closure are not involved in the way that the model system mimics this process. Thus, the proposal does not address whether this system is relevant to the human embryo - an ongoing issue with all stem cell embryo models. • The project is well designed except no in vivo validation is proposed. The project relies essentially on an in-vitro model based on ectodermal derivatives. • Potential cytotoxicity of the photo-initiator on the stem cells and an alternative approach was not addressed.

	• The alternative approach using liposomes to release calcium ions to crosslink hydrogels is incomplete as it does not address how this can be spatially controlled.
No: 2	*none*
GWG Votes	**Is the project feasible?**
Yes: 11	• The applicant has provided strong validation for their model and the proposed experiments. • Team has expertise in bioengineering and hydrogel technology. • The labs have requisite instrumentation and shared facilities will support the project. • The experiments are well described and largely achievable. The investigator has a lot of experience in quantitative assessment of cell behavior in response to mechanical signals. The investigator has extensive experience in biomechanics and cell behavior. • A co-investigator provides expertise in tunable hydrogels. The groups work closely together and have already patented this system for studying neural tube formation and function. • Understanding mechanical forces driving neural tube folding and how adhesion signals involved and expected project outcome are logical and likely to be achieved within the proposed timeline. If the proposal is modified to increase its relevance and impact to human biology and disease, its timeline may need to be altered. • The team have access to all necessary resources to conduct the proposed project.
No: 3	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• The project would extend or validate the applicability of regenerative medicine discoveries to Hispanic population since they are at higher risk of neural tube defects. • The cell lines to be studied will include groups at higher risk of neural tube defects during pregnancy. • Hispanic women are more prone to pregnancy with neural tube defects. • Increased knowledge of how neural tube defects arise has potential impact on underserved populations. • The PI and the group are very active in outreach to Hispanic students and other groups - good involvement in mentorship, etc. • The applicant plans to engage and recruit undergraduate students from underserved backgrounds. • Both the PI and Co-PI proposed plans for further participation in STEM outreach events.
No: 0	*none*

Application #	DISC0-14430
Title (as written by the applicant)	Decoding human symmetry breaking in 3D with optogenetics
Research Objective (as written by the applicant)	We will learn about how the human embryo establishes the basic roadmap for developing the rest of the body.
Impact (as written by the applicant)	30% of human pregnancies are lost in early development and we do not know why. This affects both natural pregnancies and pregnancies from in vitro fertilization (IVF).
Major Proposed Activities (as written by the applicant)	• 3D printing human stem cell based embryo models • Understand how the orientation of early embryonic tissues influences embryo development • Determine how chemical signals are read by embryos • Define the minimal information required for proper development
Statement of Benefit to California (as written by the applicant)	The citizens of California will be benefited from the development of widely applicable, cutting edge tools for tissue engineering which will substantially advance the field. Understanding how the human body builds itself will improve fertility treatments and will increase equity for those receiving these treatments. The students trained in this project will work in biotechnology firms across the state, contributing to the economy and building the regenerative medicine-focused workforce.
Funds Requested	$1,191,101
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." 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	79
Median	80
Standard Deviation	3
Highest	85
Lowest	75
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	• The bottleneck to be addressed is the desire to generate highly reproducible stem cell embryo models. The applicant will use 3D printing to control the number and arrangement of cells in a "stembryo," and will provide local, optogenetically controlled signals to promote symmetry breaking in a reproducible manner. This is certainly an attractive model in principle. • This project comprises basic discovery of technical aspects that drive formation of 3D "stembryo" structures. • The project addresses fundamental questions related to symmetry breaking. • The applicant aims to address a technological bottleneck by actively manipulating the environment and cell numbers in blastoids. • The project has the potential to enable more precise and efficient experimentation in blastoids research. • If successful, the project has the potential to significantly impact the research community. • If successful this system could become available more widely and could advance the field of modeling early embryo development.
No: 1	*none*
GWG Votes	**Is the rationale sound?**
Yes: 12	• Both cell composition and signal interpretation by cell are relevant questions in embryology. I have some concern about how the findings will translate to actual post-implantation embryos. • The overall rationale is sound and is based on extensive published work in the field defining stem cell based systems, studying gastrulation and defining signaling pathways. • If feasible, the use of 3D printing to control cell number and spatial localization of stem cells to form reproducible aggregates is a good idea. Optogenetics is the expertise of the PI and is a good addition to the system. • However, there are only minimal preliminary data to show that the applicant can make embryoids in the 3D printer and keep them alive. Further, these "stembryos" are not necessarily blastoids or gastruloids.
No: 2	• The rationale for the project is sound, as it is using state-of-the-art technologies to interfere with the formation of the pre- and post-implantation embryo. • The project aims to build on previous research in the field of blastoids, which is an important area of study for understanding early human development. • The preliminary data supporting the project are promising, but more direct data showing successful blasoid formation and manipulation would strengthen the proposal. • The project has the potential to significantly advance our understanding of human biology, particularly the biology of early stages of human development. • The proposed research has the potential to generate new technologies and approaches for studying complex biological systems, which could have broad applications in the field of medicine.
GWG Votes	**Is the project well planned and designed?**
Yes: 11	• Yes. The tools exist and, overall, the aims are well structured. • A foundational experiment is missing. • The project design is clearly articulated and well thought out, with a sound methodology for manipulating blastoids. • The proposal describes in detail the steps involved in the project, including collection and analysis of data, as well as expected outcomes and potential implications. • The design of the project is sufficiently rigorous, with controls in place to ensure that the data collected is valid and reliable. • The proposal includes alternative approaches to the study of blastoids, demonstrating a thorough consideration of potential challenges and roadblocks. • The project timeline suggests a sense of urgency and is realistic in terms of the scope of the proposed research. • The overall concept is strong, the optogenetic expertise of the PI is a strength, but I have some uncertainty about the overall 3D printing model. There is a lack of alternate approaches presented.
No:	*none*

3	
GWG Votes	**Is the project feasible?**
Yes: 12	• There are no strong preliminary data to support the use of 3D printing, but all other aspects of feasibility are in place. • The project is feasible, but the applicant has many technical issues to overcome. The PI has extensive bioengineering and optogenetics expertise, but limited background in human embryonic stem cell cultures or developmental biology. • The PI's institutional colleagues can provide advice, though they are not listed in the proposal.
No: 2	• The proposed aims of the project are feasible and have the potential to yield meaningful results in the timeframe proposed. • The project team has the necessary expertise to carry out the proposed research, with experience in relevant areas of study and access to cutting-edge technologies and techniques. • The proposal demonstrates a thorough understanding of the resources necessary to carry out the project, and the team has access to those resources. • The budget proposed is appropriate for the scope of the project, with a clear breakdown of expenses and a well-justified request for funding.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• Generally, yes, though the applicant's response is very basic. • The proposal addresses the influence of race, ethnicity, sex, and age diversity. • Different stem cell lines could be used to better represent diversity. • The description of efforts or plans for outreach, partnership, or educational activities to inform the development of DEI within the project needs to be expanded.
No: 1	• The applicant's response is minimal.

Application #	DISC0-14490
Title (as written by the applicant)	Developing a granulocyte macrophage progenitor-based immunotherapy
Research Objective (as written by the applicant)	Our study aims to overcome the limitation in expanding and genetically engineering immune cells by developing a stem cell-based approach to harness the power of immune cells for cancer treatment.
Impact (as written by the applicant)	The stem cell-based approach could be used to develop off-the-shelf immunotherapies to treat cancers and other immunological diseases.
Major Proposed Activities (as written by the applicant)	• Optimizing conditions for the expansion of the stem cells that give rise to immune cells • Generating immune cells with enhanced antitumor activity from stem cells • Assessing the antitumor activity of engineered immune cells in a cancer model
Statement of Benefit to California (as written by the applicant)	Cancer is one of the leading causes of death in California and worldwide. Successful completion of this study will lead to the development of a novel immunotherapy for cancer treatment. This novel immunotherapy will be developed here in California and could benefit tens of thousands of cancer patients living in California.
Funds Requested	$1,663,052
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." 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	5
Highest	85
Lowest	70
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: 14	• The project aims to define factors necessary for optimal ex vivo expansion of granulocyte-macrophage progenitor (GMP) cells as well as genetic manipulations that may augment

	or enhance macrophage-based cell therapies, which is relevant to human biology and disease. • The project identifies the isolation and expansion of large numbers of macrophages, the lack of proliferation of macrophages, as well as genetic manipulation of terminally-differentiated macrophages as major bottlenecks and limitations for macrophage-based immunotherapies. • If successful, the project would provide a strategy/platform to expand large numbers of macrophage progenitors with or without genetic manipulations, including the expression of chimeric antigen receptor (CAR) molecules and other effectors or genetic edits for immunotherapy. • The applicant proposes new techniques for the differentiation of macrophages from progenitors in cord blood. • It has not been possible to make macrophages on a scale suitable for cell therapy applications. If successful, the proposal will lead to generation of human macrophages in large quantities for use in cancer therapy. Macrophages may also find application in the context of tissue repair. • If successful, the proposal will lead to generation of human macrophages in large quantities for use in cancer therapy. • Potentially using engineered CAR from GMP, if shown to be superior to other CAR modified cells, along with M1/M2 switching. • Unclear, little information is given about the need for alternative therapies and whether use of GMP expansion would be superior or fit a specific void in current experimental therapies. • Aim 1 is particularly appreciated. There was less enthusiasm for Aims 2 & 3.
No: 1	*none*
GWG Votes	**Is the rationale sound?**
Yes: 13	• The rationale of expanding progenitor cells with genetic manipulations, differentiating into macrophages, and utilizing this for therapy has a sound rationale. Macrophages contribute to anti-tumor activity through phagocytosis, antigen presentation, and cytokine secretion, which can also potentiate the adaptive immune response. • The project addresses the expansion problem through a novel mechanism to propagate GM precursors derived from hPSC on a large scale. This also enhances the capacity to perform genetic modifications to create CAR-MACS. • Ability to produce macrophages at large scale could impact research and therapy for a number of disorders, given their key role in inflammation and repair. • The project is relevant to cancer immunotherapy. Methods to generate macrophages for cell therapy may show promise as single agent drugs or in combination with modulation of the adaptive immune system. • The preliminary data related to mouse and human GMP expansion is compelling. The applicants show that the medium can support the expansion of GMP cells and that these cells are transcriptionally and functionally similar to isolated GMP cells. The applicants also show that CAR+ GMPs show increased phagocytosis of target leukemia cells, which has been shown previously for CAR+ monocytes. • Strong preliminary data using mouse and human cell expansion factor, preliminary data on genetic engineering. • Strengths in existing data on methods for expanding GMPs in culture from ES/iPS cells. • Unclear whether these are HSC-like cells or GMPs. No progenitor assays are performed or suggested. • How are the proposed objectives complementary or related and how will they be applied to each other? There is no tie in from expansion improvements to CAR generation to M1 vs. M2 switching approaches. • The screens are based only on 5-7 day factor addition. It is surprising that this works, and no changes in cell density or batch conditioning is required given previous work for other primitive hematopoietic cells. What drives the long term culture and production of these cells? Is this self-renewal of GMPs alone? It seems difficult to understand despite the results. The cellular mechanisms are unclear. • The competition assays to narrow down targets using probe vs. normal compound is unclear. Little explanation is provided, and this is not common practice. In general, this target deconvolution is over-simplified and will be challenging. Unclear why this is being

	done for one inhibitor, but not equally for another inhibitor, as incorporated in pitfall mitigation. Abandoning target deconvolution to analog generation is not pitfall mitigation. • It is unclear how RNA response and affinity mass spec results from inhibitor target deconvolution are to be complemented or contrasted. • How will aim 1 optimization be used for the battery of trial and error KO lines being generated in Aim 2? Will KO GMPs require different factors or have changes in growth and response? The diverse source of GMPs to be used have not been detailed nor the number of experiments and if human heterogeneity will affect the results. The mouse system is more homogeneous. • Is there an established degree of polarization to M1 vs. M2 believed to be optimal? Can polarization be detrimental? If not only GMP are affected, and these are HSC-like cells, are the polarization factors likely to affect expansion or lineage development? These possibilities are not discussed. • Figure 1 does not show other conditions for in vitro expansion of GMPs, thus the fact that GMPs in culture are the most like de novo GMPs is not surprising. The FACS sorted population phenotype is for primitive cells, and thus HSCs. Unclear how "GMPs" are being targeted here. • In vivo data in mice is impressive. Phenotype of resulting cells vs. just GFP would provide evidence that these are GMPs or progeny. Details of how long these are were cultured prior to in vivo introduction is not provided. • Figure 4 is impressive from an engineering of cells point of view, but non-B cell leukemic cells or any cells lacking CD19 expression are required as control for specificity. Otherwise, the meaning of this data is unclear.
No: 2	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 10	• Each aim provides for improvement and refinement of macrophage production technology towards a clinical product. Genetic manipulation to lock in macrophage polarization state is interesting and could markedly expand potential therapeutic applications. • The applicant addresses possible alternate mechanisms of chemical inhibitors and alternate approaches, alternate mechanisms for enhancing antitumor activity, and other approaches to macrophage polarization. • The potential pitfalls are identified and there are appropriate alternative approaches proposed but without significant detail. • The timeline is appropriate. • In Aim 1, a screen of compounds that may enhance GMP proliferation appears relevant. However, identification of interacting proteins for the current inhibitors appears duplicative. The IC50 and interactions of one inhibitor is known. Also, another inhibitor is known to impact a specific signaling pathway but there is no hypothesis of interacting proteins based on involvement in this signaling pathway. • In Aim 2, two of the genetic modifications made to the macrophage therapy to enhance the efficacy are all related to improving T cell functionality. However, there are no plans to test the efficacy of such alterations in the macrophages in combination with T cells, either in vitro or in vivo. • In Aim 3, macrophages will be injected into mice and tumor-infiltrating macrophages will be recovered and analyzed for phenotype. However, this proposal assumes the macrophages will traffic to the tumor and infiltrate. No data exists to suggest this will be the case.
No: 5	• Provided pitfalls. • Timelines adequate, but no synergy or optimization between aims is being deployed. Thus, direct result generation is likely possible as proposed. • Connection between aims is unclear. Functional characterization of the cells needs more detail. • Little connection or synergy between aims. • Aim 2 and 3 have no additional value. • Moving to analog generation upon failure of target is curious.
GWG Votes	**Is the project feasible?**

Yes: 14	• The aims are likely to be achieved within proposed timeline, the team is appropriately qualified and has access to all the necessary resources. • The aims are straightforward and the preliminary data indicate that they are achievable. • PI is a leader in the application of small molecule technology to stem and progenitor cell expansion; great collaborators in chemical biology. • Small molecule screens are a strength. • The facilities and resources are sufficient for the proposed activities. • The budget is appropriate. • Budget seems suitable. • The investigator has significant expertise in signaling that contributes to hematopoietic cell multipotency, but there is a lack of sufficient cell therapy expertise. • It might be a good idea to engage a physician involved in cancer immunotherapy or hematology to help with preclinical development. • Aim 1 could be improved with better characterization of the differentially expressed genes by gene ontology or gene set enrichment to common pathways that may enhance GMP expansions.
No: 1	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• Sources of GMP will be derived and compared between races among women, from low incidence to high incidence of cancers. This is hoped to determine expansion or functional differences based on donor origin towards future applications. • The proposal aims to perform GMP expansion from cord blood of individuals representing diverse communities and the applicant has performed over 50 such expansions to date. The applicant will study diversity in the factors listed above as variables related to GMP expansion. • The applicant will evaluate the protocol for GMP across groups from diverse genetic ancestries. The study plan will ensure the technology is effective across genetically diverse backgrounds. • Through the applicant's institution there is excellent opportunity for engagement of underrepresented groups in research. • There is no specific evidence that the project outcomes would enhance the applicability of cell-based immunotherapies to underserved populations. • There is a weak description of the educational activities to inform DEI within the research project, largely leaning on institutional resources.
No: 0	*none*

Application #	DISC0-14441
Title (as written by the applicant)	Treating non-healing ulcers by regulating outgrowth from cellular building blocks
Research Objective (as written by the applicant)	We propose to leverage the therapeutic potential of cell spheroids to prolong cell viability and engraftment, which is further enhanced by degradable hydrogels, to combat non-healing wounds.
Impact (as written by the applicant)	The major bottlenecks addressed include improving cell survival, reducing delays in treatment by using allogeneic cells, and restoring lost therapeutic potential of cells by formation into spheroids.
Major Proposed Activities (as written by the applicant)	• Define the preferred design space for engineered hydrogels and spheroid formation to maximize secretion of natural, therapeutic biomolecules. • Correlate changes in hydrogel degradability with the secretome. • Test the bioactivity of the secretome on inflammation, vascularization, and innervation. • Test the role of hydrogel degradation on wound healing in partial thickness wounds of healthy donors. • Test the role of hydrogel degradation on repair of full thickness non-healing wounds in a model of type 2 diabetes. • Assess potential differences in therapeutic potential between iPSC-derived and bone marrow-derived MSCs for treating non-healing wounds.
Statement of Benefit to California (as written by the applicant)	Chronic, non-healing wounds are common in patients with diabetes. California is home to the largest number of diabetics in the nation; non-healing skin ulcers affect one in every 100 Californians, and there is presently no FDA-approved drug treatment. Using a combined cell- and biomaterials-based approach, we propose an innovative strategy to combat non-healing wounds. The materials can be manufactured in California, providing jobs for qualified employees and revenue for the state.
Funds Requested	$1,567,884
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." 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	78
Median	77
Standard Deviation	4
Highest	85
Lowest	70
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	2
(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: 9	• The goal of this project is to develop a new effective strategy for the treatment of chronic wounds using bioengineered spheroids. These spheroids will be composed of hiPSC-derived mesenchymal stromal cells (MSCs) and endothelial cells (ECs) embedded in a biodegradable hydrogel. • Non-healing wounds represent a significant clinical challenge. If successful, this project has the potential to have an impact on wound healing, especially for diabetic patients. • If successful, this project will provide a way to treat non-healing ischemic ulcers by using MSCs and hydrogels. The chance of success is unclear since similar approaches have been proposed in many published papers. • This is an important clinical area. • Overall, yes, but the proposal is not very novel; responsiveness of the proposal to CIRM's priorities/needs is not clear.
No: 4	• This project does not address a key knowledge gap in the stem cell field. The use of MSC, ECs and biomaterials for treating diseases is not novel and has been reported in many papers. • The rationale for use of iPSC-derived cells in this project is not clear. Both MSCs and ECs are easy to get from primary sources and they are expandable. • I don't think this project addresses a key knowledge gap in stem cell field. The use of MSCs for wound healing has been reported.
GWG Votes	**Is the rationale sound?**
Yes: 11	• The rationale is that degradation of an engineered, synthetic hydrogel will (i) improve the pro-angiogenic and anti-inflammatory effects of transplanted iPSC-MSC/EC spheroids, (ii) enable endothelial cell sprouting and (iii) result in wounds' vascularization, epithelialization, and healing. • Yes. The rationale is based partly on work from the applicant group showing that heterotypic spheroids of MSCs and ECs with tunable VEGF and PGE2 secretion cause improved healing in a human skin-equivalent model. • The applicant has shown that (i) trophic factor secretion from MSC spheroids is tunable and (ii) MSC spheroids in fibrin gels promote wound healing. • This project is based on sound rationale. The applicant will combine MSCs, ECs and a hydrogel to treat wounds. • The project's scientific rationale is supported by the applicant's preliminary results. • This project is clearly significant and relevant to human disease (wound healing). • Overall, yes, though my enthusiasm for this proposal is low as the approach is not novel and several other groups are trying similar approaches. After decades of intensive research in this field, there is very little progress. • Hydrogels can help stability of cell grafts; this is not particularly novel.
No: 2	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 11	• This is a good, clear proposal. My only critique is related to the skin explant model. It seems that diabetes is not well-modeled here - it would be valuable to include studies modeling diabetic wounds in human skin. • The aims are logical. The first aim will allow the team to refine the composition of PEG-4MAL hydrogels to select optimal hydrogel degradability for the second aim. However, the proposed DRG studies, while important, currently appear premature. • In Aim 2, it is not clear how the studies of wounded ex vivo human tissue explants in the first phase will inform the in vivo experiments in a diabetic rat model in the second phase. Instead of tissue explant experiments, it might be more informative to conduct normal skin experiments in a wounded normal rat model, varying the immunosuppression regimen. • The proposed aims are coherently linked and should provide new insights on wound healing. • The applicant addressed potential pitfalls for each of their aims.
No:	*none*

2	
GWG Votes	**Is the project feasible?**
Yes: 12	• This project is feasible to be achieved. It is very likely that the applicants will achieve their goals. • The proposed team is appropriate for this project. They have the expertise in iPSC-MSCs. However, they should show that they can differentiate iPSCs into ECs. • The aims are feasible. This is a fairly general approach. • The proposal is feasible. • The aims are likely to be achieved within the proposed timeline. However, the probability of success is compromised by the danger of allogeneic cell loss due to immune rejection.
No: 1	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**

Yes:
12
	• The project design addresses the influence of race, ethnicity, and gender.
	  • Specifically, the applicant considered sex as a biological variable by proposing in vivo studies in Aim 2 in both male and female ZDF rats.
	  • In the future, they will continue using cells from both male and female donors.
	  • Also, they will be using iPSCs from the CIRM repository that includes a racially and ethnically diverse diabetic patient population.
	• Good, specific plans to account for sex and age diversity.
	• Overall, yes, but there is not a clear plan for addressing race, ethnicity, sex, gender and age diversity among iPSC lines.
	• This project will be useful for underserved populations.
	• The assembled team is deeply committed to supporting and promoting diversity, equity, and inclusion in research. All investigators are actively involved in outreach efforts through their own laboratories.
	• Clear plans for outreach and educational activities.

No: 1	*none*

Application #	DISC0-14377
Title (as written by the applicant)	Understanding the mechanisms and developing therapeutics for the neurodegenerative Parkinson's disease using human iPSCs
Research Objective (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 disease-modifying therapeutics using human iPSCs models.
Impact (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 for Parkinson's disease (PD)
Major Proposed Activities (as written by the applicant)	• Develop a new scalable high content screening platform for mDA neurons derived from hiPSCs using a CRISPR/Cas9 engineered TH-reporter hiPSC lines suitable for live imaging. • Use the hiPSC derived human mDA neuron platform to validate the neuroprotective potential of RAAS inhibitors and other candidate drugs previously discovered from our in vivo zebrafish screen. • Develop a 3D midbrain organoid platform from TH-reporter hiPSC cell lines to evaluate the neuroprotective potential of candidate drugs in a complex 'in vivo like' environment. • Engineer multiple hiPSC lines from different gender and background with TH-reporter using CRISPR/Cas9 to discover therapeutics that will benefit all individuals. • Develop a hiPSC-based CRISPRi/a screen platform in mDA neurons to identify new mDA neuron specific survival genes and pathways. • Use CRISPRi/a screen results to identify and validate new genes and pathways that can synergize with RAAS inhibition for further neuroprotection.
Statement of Benefit to California (as written by the applicant)	Parkinson's disease (PD) is the most common movement disorder and the second most common neurodegenerative disorder. In 2020, California had the most death due to PD in the US (4147, NIH). PD not only deteriorates the health of patients in California and worldwide, but also poses severe burdens on their families and the society. The economic cost of PD is estimated to be at least $51.9 billion a year in the US (Yang et al. 2020). Apart from symptomatic managements, there are no disease modifying therapeutics for PD.
Funds Requested	$1,570,824
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." 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	77
Median	75
Standard Deviation	8
Highest	92
Lowest	70
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	5
(1-84): Not recommended for funding	10

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 proposal addresses the mechanisms of dopamine (DA) neuron loss in Parkinson's disease (PD), which is a critical knowledge gap. • The application tests the hypothesis that specific blood pressure lowering agents (RAAS inhibitors and other candidate drugs), identified as neuroprotective for dopaminergic neurons in zebrafish, are also neuroprotective for human iPSC derived dopaminergic neurons. • Since Parkinson's Disease (PD) is characterized by degeneration of dopaminergic neurons, the project has the potential to lead to a disease-modifying therapy. • Some doctors may already prescribe these agents off-label for patients with PD (or high risk for PD). Thus, research is needed to figure out if this is really a good idea. • Roughly one million people in the US have PD. There are no disease modifying agents. Symptomatic treatments have major limitations. • It is worth taking fairly long odds in PD research, because the potential payoff is very large. • Clearly this is an important area with clinical relevance. • This type of large-scale, iPSC-based screening assay can be employed for other subtypes of neurons and other diseases.
No: 0	*none*
GWG Votes	**Is the rationale sound?**

Yes: 11	• The rationale for the project is logical and based on interesting data obtained in zebrafish studies. Extending these findings to mammalian systems makes sense.
	• The preliminary data are strong. The applicant previously developed a model of DA neuron degeneration in larval zebrafish. This model was robust and suitable for high-throughput screening. Of >1400 bioactive small molecules screened, the applicant uncovered a series of RAAS inhibitors and other candidate drugs that alleviated the loss of DA neurons in the model fish.
	• Several electronic-health-record retrospective studies have shown that PD patients on RAS inhibitors have better outcomes than those that are on other antihypertensives.
	  ◦ In a retrospective review of hospital records published in 2021 (N = 308 white patients), significantly improved outcomes were recorded for hypertensive PD patients on RAAS inhibitors compared to hypertensive PD patients on other anti-hypertensive medications.
	  ◦ The above result was replicated in a South Korean cohort and published in 2022. However, no mention of the South Korean cohort was made in the proposal. The proposal should cite more review papers on the potential role of RAAS inhibitors in PD.
	• The project is significantly relevant to human biology and disease.
	• The project is for the most part associated with sound rationale. However, the rejuvenation associated with iPSC reprogramming is a relevant factor that needs to be better considered.
	• More background text on the genetics and interplay of environmental factors, including the importance of GBA1, might strengthen the application.
	• Not enough preliminary data are provided on cell differentiation outcomes.
	• Not enough preliminary data are provided showing degeneration / pathology when cells are treated with conduritol B-epoxide (CBE).

No: 3	• Making dopamine neurons from hiPSC is not novel, though the proposal incorporates improved markers. Another asset is that they have candidates in hand from their zebrafish studies.
GWG Votes	**Is the project well planned and designed?**

Yes: 12	• The applicant proposes two approaches to induce PD-like phenotypes in hiPSC-derived DA neurons. Both are well-represented in the field of PD modeling. One is to introduce mutations into the hiPSCs - leveraging a unique capability of iPCSs that cannot be obtained in any other system short of clinical trials. The other is to use an epoxide toxin (CBE). These alternative approaches are both worth testing. • Moving from zebrafish to human iPSCs is a reasonable step towards potential translation. • The project is well planned but a bit optimistic. • The timeframe and annual budget above $400,000 are appropriately justified. • The proposal is based on data published by the lab indicating that an inhibitor of RAAS is able to attenuate toxicity of CBE in zebrafish larvae. The data include findings in dopaminergic neurons. The neuroprotective effects appear partial, sometimes marginal. • Similar concerns relate to the clinical data suggesting that RAAS inhibitors slow down the progression of PD. The effect size seems to be marginal, though it reaches statistical significance. • There is no evidence confirming that the compounds' neuroprotective action is due to inhibition of RAAS rather than another property. • Inhibitors of RAAS are widely used drugs to control blood pressure. Are they reducing the incidence or progression of those patients affected by PD? It would be helpful to see a literature overview addressing this question. • CBE will be used as toxin for dopaminergic neurons. CBE is an inhibitor of glucosylceramidase (GCase) and is often used to establish a pharmacological model of Gaucher disease. It is not typically considered a model of PD - i.e., the effects of CBE on DA neurons are secondary. In mice, administration of CBE establishes a Gaucher phenotype and DA neurons are reportedly not affected (Rocha at el. Antioxidants & Redox Signaling 23:550, 2015). • One strategy might be to focus on genetic models, and reserve the CBE model as an alternative approach. Alternatively, the applicant might strengthen the proposal by providing a table of known PD model systems that could be used to screen for therapeutics, and compare and contrast the approaches. • Various methods are proposed to describe any effects of the test compounds, including live cell calcium imaging, lysosomal and mitochondria tracking, and quantitation of dopaminergic cell neurite number and complexity. These studies will generate nice-to-have data but do not contribute fundamental additional information. • The proposed activities intended to look for new targets are not needed yet. • Why not test in mammalian animal models? The CBE toxicity mouse model has been established by other investigators. • Potential pitfalls are identified but sufficient alternative approaches are not presented.
No: 2	*none*
GWG Votes	**Is the project feasible?**
Yes: 11	• The applicant plans to use a targeted CRISPRi/a screen to identify pathways involved in dopaminergic neuron degeneration. iPSC-derived DA neurons will be treated with CBE and compared with untreated control cells. In absence of preliminary data, it is difficult to predict the probability of success of the planned studies. • The PI brings adequate experience and expertise to the program, but does not seem to have been involved in the generation of the preliminary data that form the basis of the application. The PI is also not a co-author on the key papers. Why isn't the laboratory head the PI on this project? • The applicant should provide more preliminary data showing their ability to generate hiPSCs and derive DA neurons, as planned. • I am a bit unsure about the feasibility. For example, cell differentiation in 96-well plates is not easy, and not enough preliminary data are provided to show that the applicant can do this. The use of CBE to induce cell loss is not clearly validated and may stall the project. • 96-well plates may be too aspirational. • The team is qualified, but the percent effort of some team members may be too low. • The team has access to all the necessary resources to conduct the proposed activities. • The budget is appropriate for the research proposed.

No: 3	• This project is overly ambitious. The cell differentiation protocol is not yet developed, and the neurodegenerative effect in the model is insufficiently validated as a proxy for human disease.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• There are no concerns. • The proposal includes derivation of hiPSCs from a diverse cohort of donors including female, African, Admixed American, and Asian participants. • Yes. The applicant plans to use ethically diverse cell lines from the CIRM hiPSC repository (however, this is a lot of extra work without clear justification). • The project outcomes extend or validate the applicability of regenerative medicine discoveries to underserved populations, including underserved racial/ethnic communities. • The applicant has partially described prior efforts or proposed plans for outreach, partnership, or educational activities to inform the development of DEI within the research project.
No: 0	*none*

Application #	DISC0-14403
Title (as written by the applicant)	Plasticity and Endogenous Regeneration in Dental Injury and Repair
Research Objective (as written by the applicant)	We seek to understand how cellular plasticity, which is cells' ability to switch fates based on environmental cues, can support dental injury repair by studying injury repair in both mice and humans.
Impact (as written by the applicant)	Our findings will generate the knowledge necessary to tailor new regenerative dentistry avenues based on cell and molecular behavior, in turn facilitating the development of new dental treatments.
Major Proposed Activities (as written by the applicant)	• Investigate mouse incisor lineage reprogramming in response to physical and radiation injury • Characterize the extent and limitations of cellular plasticity in the finite-growth human and mouse molars • Integrate datasets and conduct cross-species tooth comparison to identify of key signaling pathways and cell populations that could trigger increased regenerative potential in human teeth
Statement of Benefit to California (as written by the applicant)	Stem and progenitor cell plasticity has long been described as the gateway to developing regenerative medicine and dentistry therapies. We believe that our work can provide economic benefits to the state by laying the groundwork for commercial efforts to alternative, more sustainable and more equitable treatments against dental decay, establishing the state as a leader in regenerative dentistry.
Funds Requested	$1,341,968
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." 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	77
Median	75
Standard Deviation	4
Highest	90
Lowest	74
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: 10	• This proposal aims to compare cellular plasticity during dental tissue regeneration between mouse incisor and molar, and then assess human dental tissue plasticity during injury using single cell RNA sequencing. If successful, this project will advance the understanding of endogenous dental cell plasticity during tissue repair. • The long-term goal of the proposed research is to inform our understanding of regeneration in both ever-growing and rooted teeth, which will provide a foundation to develop novel molecular therapies that stimulate regeneration in teeth. • The applicant uses mouse teeth as the model system and proposes that similar regenerative pathways exist in human teeth. • This proposal aims to understand lineage plasticity during endogenous regeneration in homeostasis and in response to injury in the mouse incisor, mouse molar and human molar. Although mechanisms of tooth regeneration remain insufficiently understood, it is hard to see how proposed studies will advance understanding of regeneration and stem cell trans-differentiation in human tooth.
No: 4	• It is not clear to this reviewer that the study of mouse incisor plasticity in response to injury addresses a major bottleneck to the use of stem cell or genetic therapy for human health. • Although this project has the potential to generate valuable omics data, it's unlikely to provide any new scientific insights or breakthroughs in the field. The data will be primarily descriptive, with no significant discoveries or revelations. For example, the project won't shed light on novel mechanisms by which dental stem cells differentiate. Therefore, while the project may be successful in achieving its goals, it's unlikely to have a major impact on the overall understanding of stem cells. • Will help understand dental cell plasticity, but the potential applications to regenerative medicine are moderate. • The link of this study to the application of stem cell or gene therapy in human health is not strong.
GWG Votes	**Is the rationale sound?**
Yes: 11	• The link between Aim 1 studying mouse incisor plasticity in response to injury, to Aim 2 studying human molar regeneration during repair, is weak. • The applicant provided scRNA-seq data from human molars showing pulp cell heterogeneity (Fig 5). These data demonstrated that the applicant is familiar with scRNA-seq techniques. • The link of this project to human biology and disease is still limited after revision. • While the mouse incisor is a continuously renewing organ, the comparison between incisor and rooted molar (which has limited regenerative capacity) is reasonable. It is not clear how the studies of mouse incisor will inform better understanding of mechanisms underpinning regeneration of the human molar.
No: 3	• The connection between mouse and human studies is unclear.
GWG Votes	**Is the project well planned and designed?**
Yes: 11	• The applicant proposes two aims for this project. While Aim 1 focuses on investigation of mouse incisor plasticity, Aim 2 will examine molar plasticity in mouse and human. A clear connection between Aim 1 and 2 is missing. Also, the relevance of Aim 1 to human health is missing. • Aim 1 will test use of single cell RNAseq to test the hypothesis that plasticity is triggered in the dental tissues in response to injury to allow healing. Aim 2 will examine the plasticity of dental mesenchyme in the human and mouse molar during repair. These proposed aims will generate meaningful results in the field of dental regeneration. • Strength: The proposal incorporates fundamental research in tooth development and regeneration. • The applicant addressed potential pitfalls for each of their aims. Discussion of the potential heterogeneity between mouse and human mesenchymal populations is lacking.

No: 3	• The aims are disconnected. Aim 1 is entirely focused on studies of incisors where regeneration is dependent on epithelial stem cells, whereas human tooth regeneration is mediated by mesenchymal cells. It is not clear how Aim 1 will be relevant to human biology.
GWG Votes	**Is the project feasible?**
Yes: 12	• The proposed team is appropriate for studying mouse incisor and molar plasticity. • The proposed aims are feasible to be achieved within the proposed timeline. This project is feasible.
No: 2	• The proposed aims and expected outcomes are vague.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• The applicant stated that sex and age balance of their sample will be incorporated into the experimental design. Thus, their approach will yield a comprehensive and informative analysis representative of diverse communities. • This project will be useful for underserved populations. • The applicant plans outreach to DEI students. • The applicants state they are lifetime DEI advocates. The revised proposal justified the choice of ethnicity and biological sexes of the human samples.
No: 1	• The applicant plans to account for sex and age differences, but there are no clear plans regarding race and ethnicity. Past and future outreach and educational activities are well described.

Application #	DISC0-14457
Title (as written by the applicant)	Modeling and understanding alveolar hypoplasia in Down syndrome using iPSCs-derived alveolar type II cells
Research Objective (as written by the applicant)	Understanding alveolar progenitor cell defects in Trisomy 21 and the genes/pathways associated with them will allow for developing therapeutic approaches for individuals with DS.
Impact (as written by the applicant)	Although Trisomy 21 (T21) affects multiple organ system, respiratory complications are the major cause of death in kids and adults with Down Syndrome (DS). The causes of lung disease in DS remain poorly understood.
Major Proposed Activities (as written by the applicant)	• Create an in vitro model to study alveolar defects in Trisomy 21 using T21 and euploid iPSCs lines • Elucidate the defects in progenitor cell commitments to alveolar type II (AT2) and alveolar type I (AT1) cells in Trisomy 21 • 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 • Define the role of the mesenchymal niche in the alveolar hypoplasia phenotype observed in T21 • Generate unique resources (iPSCs) from diverse racial and ethnic background that will become available to the scientific community to study other comorbidities of DS (intestine, pancreas, liver) • 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 (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 costs are 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	$1,604,418
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.” 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	77
Median	75
Standard Deviation	2
Highest	80
Lowest	75
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: 12	• The research question posed in this proposal is very important. Down syndrome is associated with development of alveolar hypoplasia and authors show that this is independent of congenital heart disease. Authors have ethical approvals for use and access to lung tissue and have expertise in studies of lung development. • Individuals with T21 are at high risk of dying as a consequence of lung diseases. Lack of animal models has been a limitation on an understanding of the mechanisms driving the defect. The use of lung cells from iPS is novel. • I agree with the authors of the potential to 1) advance knowledge of disease mechanisms in T21 lung hypoplasia, 2) establish in vitro models to study T21 lung disease, 3) determine molecular mechanisms that drive defects in progenitor cell specification and function in T21 and 4) generate iPSC resources that will allow modeling of other DS-morbidities. • The government and private funding of research for trisomy 21 is probably below what it should be compared to the prevalence of the disease. This proposal would help reverse that deficit. • Patients with T21 have severe effects on lung development. • Use of primary fetal cells is interesting and a strength. • Funding research needing fetal tissue is one of the reasons CIRM exists. It is important in trisomy 21 to validate the model system against real human tissue; so a very appropriate use of fetal tissue. A successful model system would reduce the future need for fetal tissue.
No: 1	*none*
GWG Votes	**Is the rationale sound?**
Yes: 11	• The applicant's previous work demonstrated that lung anomalies in DS occur prenatally in about 70% of the samples examined, suggesting that the defects originate at the progenitor cell level. • A model system for T21 lung disorders would be useful. • The applicants state: "However, preliminary data from our group showed that lung defects can occur independently of congenital heart disease (Fig. 1)." Figure 1 may show this for one individual, assuming they don't have congenital heart disease. It is an N=1, whereas this should be a statistical claim. • The applicants state they will combine data to use a "personalized medicine" approach to identify risk factors for developing lung disease in individuals with T21. So little is understood about T21 and DS that it may be premature to personalize results before understanding them in an aggregate manner.
No: 2	• The preliminary data does not justify the main hypothesis and proposed experiments.
GWG Votes	**Is the project well planned and designed?**
Yes: 6	• Computational issues are a concern and should be done prior to experiments.
No: 7	• The characterization of the changes in AT2 and AT1 cell differentiation in fetal and postnatal T21 lung samples and age-matched controls using fluorescent imaging and gene expression could provide valuable information. • The use of tissue from newborns is an important aspect of this proposal. • Aim 2 is centered on the role of FGFR2 signaling, the experimental design for this aim is logical. • The preliminary data does not really support that they will be able to detect significant differences in lung cells from T21 vs. wild type samples.

	• Unfortunately, I can not appreciate the preliminary data provided. I do not see any differences between non-T21 and T21 samples in the Figures 3, 4, 5, so the hypothesis that T21 lungs have more mature AT2 cells or lower FGFR2 expression does not hold. • The lack of bioinformatics support is reflected in the inappropriate interpretation of preliminary results. • Power calculations and power analyses need to be done prior to a grant application, as we need to know if the project is adequately powered and if enough funds are allocated for enough samples/replicates to reach this power, and if the experimental design is sound. • The authors propose to use iPSC-AT2 derived from normal and T21 donors and compare expression of AT2 cell markers at different time points after differentiation completion. Based on the authors hypothesis, defects in AT2 differentiation in DS occur at the earlier stages of progenitor development (before cells become AT2), therefore it is not clear why such a study design was chosen. • Aim 1C proposes to select a time point with "the most changes", however it is unclear this will be informative. You may want the time point where you see the first changes. These are more likely to be informative and/causal. By the time many things are changing, many of these changes may be irrelevant and distracting. • In Aim 1C the hypothesis involves variation in epigenetic signals. If there is variation in these signals between individuals, an N=3 is way too small. • There is discrepancy in the title and text for Aim 1C. Clustering cell types is quite a long way from identifying changes in regulatory networks. There is some relevant text on regulatory networks but it is vague. • A time point is different from a timeline; this language should be clarified in the proposal.
GWG Votes	**Is the project feasible?**
Yes: 7	• Preliminary data are not strong. • Differentiation of iPSC into type 2 cells is questionable.
No: 6	• Better preliminary data is needed. Need to demonstrate lung differentiation from T21 iPS. • There is a concern about the ability of this group to differentiate iPS cells into AT2. Also, the ability of iPS-derived AT2 to transdifferentiate into other cell types is questionable. • It might be problematic as AT differentiation of iPSC takes more than 25 days and applicants aim to study them for a period of approximately 70 days. • It appears that the iPSC lines are not established yet. • Three listed key personnel at 5% FTE are probably skilled enough but no single person has enough allocated FTE to handle the proposed bioinformatics tasks. 5% is about enough time to attend a meeting or two a week and give advice, but not enough time to curate data, write scripts, analyze data, and craft results for publication. • Given the problems with bioinformatics, it is recommended to identify a data project manager which is currently listed as to be named. • The applicants make an erroneous statement about ToppGene. ToppGene does not do cell type association. This sentence is identical to an erroneous sentence from an abstract of a published paper which was also erroneous, suggesting that this text may have been pulled from an Internet search rather than personal expertise.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• T21 impacts a diverse population. • Yes, there is a good description. • Applicants state that they will generate iPSC from diverse donors. However, the applicants do not specify the numbers on the availability of donors from different ethnicities/races and how this reflects the ethnic diversity of California.
No: 0	*none*

Application #	DISC0-14385
Title (as written by the applicant)	A novel population of autologous neural precursor cells for the treatment of brain injury following germinal matrix hemorrhage in preterm infants
Research Objective (as written by the applicant)	Characterization and differentiation of autologous cerebrospinal fluid (CSF)-derived neural stem cells for transplantation to prevent cerebral palsy in preterm infants, without lifelong immunosuppression
Impact (as written by the applicant)	Preventing periventricular leukomalacia and cerebral palsy/developmental disability in premature infants following germinal matrix hemorrhage (GMH).
Major Proposed Activities (as written by the applicant)	• Collect, characterize and expand CSF derived stem cells from preterm infants following germinal matrix hemorrhage as part of the standard of care • Characterize the potential of CSF-derived neural stem cells to generate cells for transplant. • Test ability to genetically engineer CSF neural stem cells
Statement of Benefit to California (as written by the applicant)	Preterm birth is one of the leading causes of infant disability and death. The incidence of preterm births in California is 10%. Germinal matrix hemorrhage and resulting periventricular leukomalacia are the most common neurological complications of premature newborns and are strong predictors for cerebral palsy, seizures and mental retardation. Transplantation of these cells may dramatically reduce the incidence of these complications by repopulating the damaged brain area.
Funds Requested	$1,510,035
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." 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	75
Median	75
Standard Deviation	4
Highest	80
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: 11	• Periventricular leukomalacia (PVL), which is caused by injuries in the germinal matrix and white matter in premature surviving infants, represents a significant public health problem and is associated with high costs to society. • The proposal focuses on a disease with limited therapeutic options. The life-long complications for the infants are significant and novel or improved treatment options will be of great significance. The proposed experiments will provide insights into the biology of the utilized stem cells. • The studies will further the understanding of stem cell mechanisms and may identify new approaches for the treatment of brain deficits in prematurely born babies. • Development of strategies that promote oligodendrocyte progenitor cells (OPCs) and myelin repair after the injury could significantly improve the recovery of surviving infants. • If successful, the project could have an impact on newborns with GMH and could alleviate brain damage. • Approaches to induce neurogenesis and OPC proliferation have been tested but clinical trials thus far have failed. Stem cell therapies are promising but rely on paracrine effects rather than on providing appropriate cells directly. • The obtained data, especially from Aim 1, will provide biological insights for the cell types that are central to this proposal.
No: 3	• The significance is limited because the approach is only valid for preterm infants with already existing intracranial pathology.
GWG Votes	**Is the rationale sound?**
Yes: 12	• The proposed studies are well designed and based on the current knowledge in the field of stem cell biology. • The proposal is based on extensive existing and preliminary data. These data provide a high degree of confidence that the proposed studies can be executed and will yield meaningful results. • The generation and propagation of autologous CSP-derived NSCs that are engineered to directly differentiate to oligodendrocytes and interneurons to replenish neurons and promote myelination could provide a long term strategy to prevent/alleviate PVL. • The preliminary data for Aim 1 are well presented. It is likely that the goals of aim 1 can be accomplished. The preliminary data also demonstrates the investigators can conduct the proposed experiments. • Aim 1 will generate data which will characterize a distinct set of stem cells. This is likely to yield insights into potential cellular mechanisms. • OPCs and interneuron transplants together with additional trophic factors after glutamate excitotoxicity (GME) have been tested and show promising results, but the therapeutic potential is limited.
No: 2	• Ultimately, the proposal is build on the rationale that the differentiated cells have the potential to reverse or positively influence PVL in GMH. While Aim 1 is well described, Aim 2 lacks sufficient preliminary data or evidence that this approach in fact has some likelihood of success. Therefore there are some concerns related to the overall rationale of the project. • Aim 2 is underdeveloped. The proposed animal experiments are described in a cursory manner with limited information on the experimental design, data collection, outcomes, and interpretations. This is a major limitation of the proposal which reduces the overall enthusiasm. • This proposal is incremental to existing approaches.
GWG Votes	**Is the project well planned and designed?**
Yes: 8	• The timeline is appropriate. • Overall, the proposed studies are well planned, however, some of the methodological aspects are lacking details. For example, the applicants only provide a reference for the co-culture system that will be used to select differentiated cells for transplantation. • It is unclear whether the co-culture system will be used as a gating step to select cells tested in mice, or if the co-culture and in vivo systems are used in parallel.

	• If the project is completed successfully, the applicants plan to move the autologous transplantation approach to humans. Studies beyond the mouse transplantation studies, which are included in this application, may be necessary before going to human studies. • Aim 1 is likely to provide interesting insights. Aim 2 is underdeveloped and it is not clear whether important insights can be obtained. • Both aims discuss alternative approaches, however Aim 2 is underdeveloped.
No: 6	• Aim 1 is straightforward, and the rationale to characterize CSF-derived populations is clear. • Aim 2 (transplantation of naïve and engineered CSF populations and determination of differentiation potential) is speculative, due to the limited preliminary data. • The transplantation study in Aim 2 will only test myelination; interneuron transplantation is not addressed. • Potential preclinical models are not discussed. These models may be necessary to study the relevant of transplantation in addition to showing in vivo potential. • The major advantage of this proposed approach over already existing approaches is that the transplants would be autologous. The application would have been strengthened if the risks of immunosuppression needed with other approaches had been addressed. • The proposed study to test transplantation into immune-deficient mice does not address why an autologous transplant might be needed.
GWG Votes	**Is the project feasible?**
Yes: 12	• The studies are well designed, and the project is likely going to be successful. Timelines are aggressive but potentially achievable. • The location represents a unique strength of the proposal. The tight integration of research and clinical activities and the access to patient-derived cells will be an asset for success. • There is good integration of research and clinical activities and access to patient-derived cells. • The team appears to be qualified. • Resources are adequate. • The budget is appropriate.
No: 2	• The proposal is technically feasible but there is limited discussion of pitfalls. • Aim 1 seems feasible, but due to limited details it is challenging to address the feasibility of Aim 2.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• Past and ongoing efforts at the institution are documented and are impressive. • The project does adequately address the influence of race, ethnicity, sex, gender, and age. • There are clear disparities of incidence and outcomes in preterm infants as a result of race and disparities of income. The applicant's institution provides care for patients without any restrictions based on race or financial ability to pay for care. • The project outcome, if successful, would benefit underserved communities.
No: 1	• A clear, project-specific plan to address diversity is not provided.

Application #	DISC0-14470
Title (as written by the applicant)	Physiological and pathophysiological roles of eIF4G2, a putative regulator in translation initiation, in pluripotent and intestinal stem cells
Research Objective (as written by the applicant)	To reveal novel mechanisms of gene regulation important for intestinal stems in mouse and human
Impact (as written by the applicant)	Deeper understanding of stem/progenitor cell biology will result in better management of cells and facilitate their applications to cell therapies.
Major Proposed Activities (as written by the applicant)	• We will utilize human induced pluripotent stem cells to reveal novel mechanisms in gene regulation in multiple stem/progenitor cells. • We will analyze mouse models to fully understand roles of the revealed novel mechanisms in gene regulation in stem/progenitor cells. • We will elucidate molecular mechanisms of the novel pathways by using both mouse and human models.
Statement of Benefit to California (as written by the applicant)	Our project will reveal novel pathways essential for multiple stem/progenitor cell systems. Understanding of such pathways will lead to better management of cells and promote development regenerative medicine using stem/progenitor cells. We would like to overcome diseases by science and thus contribute to the State of California and its citizens.
Funds Requested	$1,739,760
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." 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	75
Median	75
Standard Deviation	1
Highest	78
Lowest	75
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	• Understanding fundamental pathways regulating stem cell biology is an important goal. • Advances knowledge of the role of translational control in the biology of stem cells. Mostly working with mouse cells. eIF4G2 was identified in 1997 as a gene important for pluripotent stem cells. Here the applicant will extend this to intestinal stem cells in mouse and human. • It has been shown in a number of studies that translational control mechanisms play roles in stem cell development and differentiation. See Saba et al (2021) Translational control of stem cell function, Nature Reviews Molecular Cell Biology 22, 671–690. Not clear that this particular component of the mRNA translational machinery is a major bottleneck. • Not clear that the proposed approach to examine eIF4G2 will significantly advance a gap in knowledge. • Results will provide new insights into translational mechanisms in stem cells with potential impact on their future expansion for therapeutic use • The proposal is highly biochemical and mechanistic, and use of iPSCs and organoids and in vivo knockout systems are well described. • Yes, any one of the aims would contribute to the field, and there are several aspects to the proposal.
No: 5	*none*
GWG Votes	**Is the rationale sound?**
Yes: 11	• Translation of mRNAs to proteins is a key component of cellular function and regulation of this process at different levels, including translational initiation, is likely important for normal stem cell function. The specific gene identified here was found in a screen for factors important for pluripotent stem cell function and thus there is good rationale that further investigation might reveal new insights into stem cell function. • The isolation of the novel initiation factor, eIF4G has been followed up with knockout studies in mice and some initial biochemical studies in cell lines to try to understand the molecular mechanism of action. There is certainly sufficient evidence that this gene may play interesting roles in stem cells to warrant further investigation. However, most of the work is in the mouse system and the extension to human iPS cells is still quite preliminary. It is not clear that loss of function in human has the same effect as in mice.
No: 3	• Understanding intestinal stem cells is relevant to human disease. • Rationale fell short. • The hypothesis that eIF4G2 plays a role in stem cells is sound, although the focused rationale as to why this is critical target for moving the field forward is less clear. • The evidence for the stem cell effect is based on decreased expression in conditional knockout (KO) mice. However it's not clear that this is due to stem cell-intrinsic regulation given the lack of specific effects for the model, although certainly plausible. • Many of the findings could be argued to be involved in non-stem cells. A set of experiments that show the targets are stem cell specific in type, species and in non-stem cell populations is not proposed. This does not rule out this target effect stem cells, but they equally could have been identified cheaper, easier and faster in less challenging non-stem cell system, eg., cell lines. • The rationale to use mouse vs. human systems throughout is unclear. • The basis of how differences in intestinal stem cells vs. PSC will be defined or even evaluated is unclear, along with the association of Crohn's disease. • In Aim 1, does age of the mice matter? Why in vitro first? How will in vitro results be compared to or guide in vivo results? Also stomach and intestine, how is this going to compared? • The iPSC knockout lines have not been generated. More information on the number of clones and somatic source is needed. How will data from in vivo and in vitro mouse work be deployed to understand iPSCs knockout cells? • In Aim 2, how will the protein analysis in Aim 2 be done? Which proteins, methods, and approach? How will the KO method be decided? What are the criteria for a candidate and validation? Why organoid in mouse, and human iPSCs? Are species differences expected?

	• In Aim 2, it is unclear how microarray vs. RNA-seq will be used, and why microarray is required if RNA-seq is to be performed. The scRNA maps origin and rationale is unclear, as the depth of these techniques is very different, unless it is being used for validation. • Unclear how aim 2a is different or overlapping with 2b global efforts? The rationale for the validation studies performed for 2a candidates and 2b candidates is unclear. Why is there then a discovery CRISPR screen despite the effort for candidate identification? • Why are some elements of Aim 3 profiling for true targets not done before functional validation experiments that are mentioned throughout steps of Aims 1 and 2? • Some evidence for role of eIF4G2 in species specific or somatic vs. PSC stem cell vs. non-stem cells would have strengthened the proposal.
GWG Votes	**Is the project well planned and designed?**
Yes: 7	• The project focuses on the role of eIF4G2 in pluripotent and intestinal stem cells. It will combine phenotypic analysis of loss of function in mice and in human stem-cell derived intestinal organoids, with biochemical analysis of the molecular pathway of action of the protein. This is a logical progression but the work on the human iPS cells is less well described and designed at this time. • Generating functional intestinal organoids from iPS cells to study the phenotypic effects of loss of function will not be simple and is not described extensively here. There is still some concern that this particular pathway needs to be integrated into a broader view of the fundamental aspects of mRNA translational control in stem cells. • The potential benefit of mouse vs. human and intestinal vs. PSC stem cell biology being carried throughout the grant is unclear. • Candidate targets, global candidate targets, and direct targets can be streamlined. The design for this order, and how these will be compared and focused is unclear and there are several areas of redundancy especially in the functional validation studies at each of these stages. • Is the applicant expecting differences in regulation targets or mechanism in PSCs vs. intestinal SCs? • The knockout causes a block in neuro-ecto lineages, yet neural biology is not being investigated. This seems to be in misalignment with foundational findings. • A plan is provided, but timelines are needed for generation of the reagents used eg., iPSC KO lines. This may provide a major pitfall in expected goals given the short duration of the grant. • Timelines and the need to generate cellular and molecular reagents is a concern eg. iPSC lines, construct tags, etc. • Redundancies of validation and use of multiple methods for similar RNA and protein candidates is unlikely to be useful and absorb time and funds.
No: 7	• The approach is not stem cell specific. • The rationale for exclusively using the described model is not clear if the goal is to specifically target stem cells.
GWG Votes	**Is the project feasible?**
Yes: 12	• Inducible KO in all tissues in adult shows particular hematopoietic and intestinal problems. They propose this to be stem cell-specific role but it could be that these are sites of active translation. Need to do stem-cell specific deletion to determine whether defects are truly stem cell specific. Aim 1 will only use the inducible KOs and look at different cell types in mouse and intestinal organoids. • Aim 1b gives minimal details on how they will translate the phenotypic analysis into the human iPS system. • Aim 2 seeks to find specific target mRNAs regulated by eIF4G2. They will use candidate approach and they will attempt to determine targets by comparing RNA levels and protein levels in human inducible knockdown cell lines +/- DOX. They have some preliminary data identifying mitochondrial proteins as targets. Will pursue further. This aim is appropriate next step. • Aim 3 proposes an extensive set of experiments using current technologies to explore biochemical function of the protein. There are appropriate local partners with expertise in these approaches. These experiments are appropriately focused on pluripotent stem cells in both mouse and human for the in-depth biochemical functional analysis and target identification.

	• A big problem would seem to be still the lack of clear insight into the role of the two factors. They describe one set of experiments directly comparing results of knockdown of the two factors (Aim 3b). This is an important control and should be carried through into other experiments as it may give the best insight into the different roles of the two factors. • Timelines and need to generate cellular and molecular reagents is a concern, eg., iPSC lines, construct tags, etc... • Team is appropriately qualified and staffed and has access to all the necessary resources to conduct the proposed activities. • Likely requires more funding given the proposed experiments.
No: 2	• The extent of global analyses and validation proposed does not seem feasible. This concern was raised before and does not seem to be addressed.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• Race inclusion is not clearly indicated. • Minimal discussion but the institution has an extensive DEI program.
No: 0	• The diversity and source of cell lines generated or gender of mice are mentioned, but are not detailed in the proposal. For example, the source of iPSCs, etc.

Application #	DISC0-14518
Title (as written by the applicant)	Investigating pediatric hematopoiesis in situ during steady state
Research Objective (as written by the applicant)	Human pediatric hematopoiesis has not been thoroughly investigated. This makes interpreting corollary studies of diseased children difficult. Here we aim to bridge this gap in knowledge.
Impact (as written by the applicant)	An understanding of native pediatric hematopoiesis during healthy steady state development to compare samples from diseased settings and post therapeutic interventions with in the future.
Major Proposed Activities (as written by the applicant)	• Characterization of hematopoietic stem cells (HSCs) and their frequencies throughout development (flow cytometry) • Thorough characterization of phenotype of pediatric HSCs (CyTOF) • Transcriptomic analysis of pediatric HSCs (scRNAseq) • Transcriptomic analysis of pediatric bone marrow stroma (scRNAseq) • Bone marrow cues that regular changes in pediatric hematopoiesis (proteomics)
Statement of Benefit to California (as written by the applicant)	Our institution is currently a leading hospital in stem cell transplantation therapies and pediatric diseases. However, during ongoing clinical trials at our institution, we have realized there is a large gap in knowledge in healthy human pediatric hematopoiesis compared with disease settings, making interpretations and clinical approaches subpar. Here, we aim to fill this knowledge gap to help with clinical treatments and interpretations for patients in California, and worldwide.
Funds Requested	$1,513,522
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." 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	75
Median	75
Standard Deviation	4
Highest	81
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: 12	• This proposal aims to gather information regarding pediatric hematopoiesis based on a hypothesis that hematopoiesis, and specifically the interaction between hematopoietic stem cells (HSCs) and stromal cells the stem cell niche, differs between children and adults. • The impact would be (i) the discovery of methods or molecules to manipulate hematopoiesis in children and (2) the provision of baseline, expected numbers of HSCs in healthy children. • Yes. Baseline profiling of HSCs using human samples is needed. • Preliminary data demonstrate that are fewer HSCs in early life stages of non-human primates with an expansion of HSCs thereafter. • There is a key knowledge gap in the understanding of hematopoiesis in children. Most studies use adult or cord blood. In particular, the number and properties of hematopoietic stem cells (HSCs) during the pediatric period is not known. This proposal will use bone marrow (BM) samples from healthy, sibling donors to address this issue. • The project is quite closely defined in terms of generating scRNAseq and proteomic data from HSCs and bone marrow stroma in children versus adults. The data will be a resource for the community. • There are other studies on pediatric hematopoiesis that could be complementary - e.g. https://chanzuckerberg.com/science/programs-resources/single-cell-biology/pediatric-networks/the-childhood-hematopoiesis-and-immune-development-atlas/
No: 3	*none*
GWG Votes	**Is the rationale sound?**
Yes: 12	• There is a need to better understand hematopoiesis in children, as the applicant properly describes. • The project is clearly defined in its goals and approaches. It is not overly ambitious. • This is a good team with solid capabilities. • The applicant provides some preliminary data from HSCs from pediatric BM samples as compared to HSCs from adult BM samples. The data suggest that children have lower numbers of HSCs, but not lower differentiation capacity. This is based on a very small cohort. Scaling up these studies will be a challenge.
No: 3	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 11	• The project design is quite simple and straightforward. The applicant will use current approaches to identify surface protein markers, and scRNAseq to characterize cell phenotypes, of both HSCs and bone marrow stromal cells from both pediatric and adult healthy BM. • The major pitfalls are (i) accessing sufficient healthy BM samples of defined ages, sex, ethnicity to provide a significant dataset and (ii) accumulating sufficient numbers of cells representing rare populations of HSCs. These difficulties are acknowledged and mitigated to some degree by enriching for CD34+ cells. However, overall this will be a challenging project. • The timeline and order of experiments should be adjusted. The applicant proposes to study HSCs first and then turn to the stromal cell analysis. Given that the BM samples will be hard to come by, these studies should be designed to run on the same samples at the same time. • Could the applicant identify examples where differences in hematopoiesis contribute to medical needs? This would increase the potential impact of the application.
No: 4	• Threshold differences and the number of samples needed are not well specified.
GWG Votes	**Is the project feasible?**
Yes: 10	• The applicants have access to samples from pediatric and adult bone marrow donors and will use these to continue the characterization of HSCs and stromal cells using next generation sequencing.

	• The applicant will apply flow cytometry, cyTOF and single cell RNAseq to HSCs and stromal cells from pediatric and adult BM samples. The approaches are relatively straightforward and collaborators are in place to help move the project forward. • The applicant's access to sufficient samples is not guaranteed. • The feasibility of getting significant sample sizes may make implementation difficult. • The PI has extensive research and clinical expertise in BM transplantation, with special emphasis on development of non-genotoxic conditioning for transplantation and running clinical trials. The PI does not have a strong background in single cell analyses like those proposed here. The PI has post-docs with some experience, and has access to collaborators at the institution's stem cell center. • It's not clear that the PI has a dedicated data manager to manage the single cell data to be produced.
No: 5	• There are serious concerns about getting enough cells to have the statistical power to resolve differences with age.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• This project could improve outcomes of BM transplants and gene therapy for kids in many different groups. • The applicant will access a diverse population of siblings coming forward as donors for sick family members. • The applicant is very much involved in community outreach. • This will provide a baseline resource for a wide community. • The proposal shows good attention to diversity.
No: 0	*none*

Application #	DISC0-14581
Title (as written by the applicant)	Zika virus pathogenesis at single cell resolution: uncovering cellular mechanisms and therapeutic targets
Research Objective (as written by the applicant)	We will detail the genetic effects of Zika virus on specific neural stem cell (NSC) types in the developing human brain, define the role of innate immunity, and develop a tailored platform for screening therapies.
Impact (as written by the applicant)	The proposed experiments will aid in creating targeted therapies by providing a detailed cellular map, that does not yet exist, of how Zika induces microcephaly in the developing human brain.
Major Proposed Activities (as written by the applicant)	• Standardize the protocol for infecting tissue samples with Zika virus. • Identify the genetic changes induced by Zika virus in diverse cell populations of the developing human brain. • Create an anatomical map of the innate immune system response to Zika virus in developing brain cells. • Generate 3D brain organoids ("mini-brains") that include the cell types found to be involved in the response to Zika virus, validate the system, and utilize for large scale drug screening.
Statement of Benefit to California (as written by the applicant)	Zika caused recent outbreaks of microcephaly in unborn children in South America and Asia and travel-associated cases have appeared in California in 2022. Climate change is predicted to spread the mosquito vector and Zika in California, particularly in the central valley where West Nile virus is already endemic. This study leading to better understanding of disease pathogenesis and the prospect of effective prevention will benefit citizens in vulnerable population centers throughout California.
Funds Requested	$1,605,342
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." 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	75
Median	75
Standard Deviation	4
Highest	80
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	• The potential role of innate immunity in mediating central nervous system (CNS) sequelae to virus infection represents a gap in our understanding. The proposed project addresses this gap by studying the innate immune response to Zika virus infection. • The project could contribute to a better understanding of the innate immune response to viral infections, and its effects on the brain. • The findings may have implications for the development of treatments or preventive strategies. • Emerging mosquito-borne viruses have a decent chance of becoming the next pandemic, and in the meantime cause considerable worldwide morbidity. • It is worthwhile to explore the basic biology of Zika BEFORE the next pandemic. • A deeper understanding pathogenesis of zoonotic viruses is needed. • Yes, though it is not clear how relevant Zika virus infection will be to the CNS effects of other viral infections.
No: 1	*none*
GWG Votes	**Is the rationale sound?**
Yes: 9	*none*
No: 5	• Understanding exactly which CNS cells Zika infects may be needed before this more specific research on the innate immune response can be done. • The gap in knowledge needs to be clarified in the proposal, with reference to prior Zika virus studies.
GWG Votes	**Is the project well planned and designed?**
Yes: 8	*none*
No: 6	• The titration study proposed in Aim 1 may be insufficient to fully capture the effects of different viral loads on the innate immune response. • Only four single-cell RNA-seq experiments are proposed. These may not provide a sufficiently comprehensive understanding of the expression profile. Also, does the study include controls? • The rationales for Aim 1 and Aim 2 are not well-established, particularly in the case of the spatial analysis proposed in Aim 2. • Is there a simpler method to determine what cell types are affected? E.g., a cell-death assay? Perhaps even microscopy? Transcriptomics may not be needed for this project. • Aim 3 appears to be underdeveloped and may not be feasible with the technology available. It is unclear whether the imaging techniques proposed will allow for high enough resolution to distinguish subcellular compartments. • It's not clear how much new data will be generated that will impact the field. • Precious fetal material requires a more rigorous project plan. • No pitfalls are presented.
GWG Votes	**Is the project feasible?**
Yes: 12	• The proposed experiments appear to be feasible, although they are ambitious in scope. • While there may be some technical challenges associated with the spatial analysis proposed in Aim 2, the team appears to have the necessary skills and experience to overcome these challenges. • The team has access to the necessary resources and equipment to conduct the experiments outlined in the proposal. • The budget for the project appears to be appropriate given the scope of the work proposed. • The plan is straightforward and should be feasible.
No: 2	• More preliminary data are needed to demonstrate that organoids and slices can be infected. • More attention to potential variability from slice to slice and organoid to organoid is needed.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**

Yes: 14	• The project outcomes would extend the applicability of regenerative medicine research to underserved populations because Zika disproportionately affects underserved populations. • The lines and tissues to be used will be derived from a wide variety of genetic backgrounds. • Viral borne diseases disproportionately affect the economically disadvantaged. • Prior efforts or proposed plans for enhancement of DEI are missing.
No: 0	*none*

Application #	DISC0-14587
Title (as written by the applicant)	Implementing a coupled system of integrative ML modeling and data validation in Alzheimer's disease
Research Objective (as written by the applicant)	We will build a pipeline to identify & validate drug targets for Alzheimer's disease using machine learning and a stem cell-derived microglia/neuron co-culture system.
Impact (as written by the applicant)	Alzheimer's disease (AD) has no effective treatments available. This work will suggest new microglia-related drug targets and uncover mechanisms underlying the role of microglia in AD.
Major Proposed Activities (as written by the applicant)	• Extend an ALS focused machine learning model to AD • Make global predictions of cellular states downstream of perturbations and determine causal genes relevant for neuroprotective microglial states for use as therapeutic targets. • Iterate model to integrate data generated by our co-culture iPSC system. • Validate putative targets using antisense oligos and scRNAseq in co-culture system • Employ imaging pipeline for functional characterization of putative targets in co-culture system
Statement of Benefit to California (as written by the applicant)	California has one of the largest Alzheimer's disease (AD) populations in the US, with approximately 690K patients. Our machine learning model and patient stem-cell derived brain cell co-culture system suggests new drug targets for AD. In a state as diverse as CA, it is crucial to consider that drugs can have variable efficacy in different patient populations. Our model can take into account patient background and other features, suggesting drug targets for CA's diverse patient population.
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." 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	75
Median	75
Standard Deviation	1
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: 11	• There is a great need to develop new therapies, treatments and cures for Alzheimer's Disease. • AD represents a major health burden. The treatment options are limited and there are not many potential new drug treatments or drug targets available. This proposal will use iPSC derived cells for disease modeling in a multi-cellular disease model. This has the potential to provide important and relevant insights into the disease process. • The aim of the project is to build a pipeline to identify & validate microglia drug targets in AD. There is a need for new drug targets, and microglia represent a reasonable area of focus. • Using stem cell-derived cells for disease modeling is a well established approach for diseases with limited availability of disease models. The proposed identification of novel disease networks addresses a clear bottleneck in drug development. • If successful the identified networks and mechanism are likely to provide valuable insights into the disease process. • Machine learning may help distinguish the roles of microglia. • A similar approach have already been applied by the team to ALS, suggesting the potential applicability to other diseases.
No: 3	• The studies may further the understanding of stem cell mechanisms and may identify new approaches to Alzheimer's disease and other neurodegenerative diseases. They are not directly addressing an identified bottleneck for therapeutic development.
GWG Votes	**Is the rationale sound?**
Yes: 11	• The overall rationale is well established. The proposed co-culture of relevant disease cell types is appropriate given our current understanding of the overall disease process. • The project is based on sound rational. The idea is to identify new drug targets in AD based on data from databases, public data, and internally generated data. The internal data will be collected from a co-culture system of microglia, neurons and astrocytes from patient iPSC lines. This will be used to test targets via in vitro perturbations, and to generate molecular and imaging data, which is in turn will be used to refine and expand the computational model. • The proposed analyses are highly innovative and in the context of this proposal are relevant. • The applicants provide extensive and excellent preliminary data supporting all aspects of their proposal. • The preliminary data related to the data analysis are highly innovative and well presented. • If successful, the experiments and the data analysis will provide important and novel insights. • The applicants provide good preliminary data supporting the computational model and pipeline, but less data on the cell system. • The proposal is based on the assumption that microglial cells exist in two functional states, neuroprotective and neurotoxic. This is an oversimplification, as pointed out in a recent expert review, which concludes that "this dualistic classification of good or bad microglia is inconsistent with the wide repertoire of microglial states…." (Paolicelli et al., Neuron, 110:3458, 2022). • The applicants base their classification of microglia under an assumption that the neuroprotective state is determined by four factors. This is not sufficiently justified in the application, and details regarding how this state was characterized are lacking.
No: 3	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 7	• The applicants provide interesting data on using their approach for ALS, where they identified a critical gene regulating the status of microglial cells. This allowed them to identify secondary genes, and to demonstrate that manipulation of these genes by siRNA affected the status of microglial cells. • The overall study design is mainly supported by the preliminary data.

	• Pitfalls are identified and some alternative approaches are presented. • Potential pitfalls are discussed, but concerns about the design of the study remain. • The project is well planned but there are unaddressed caveats with the cell model. • The proposal is consistent with the urgency of CIRM's mission. • Having the project shorter than 3 years is not well justified.
No: 7	• The proposal is premature. • Alzheimers cell lines may not be fully appropriate for this approach, and the approach may be too preliminary. • The proposal lacks important detail on the overall study design specific for AD. For example, how many experiments will be performed, what cell lines will be used, and what are the race, sex, and phenotypic characteristic of the cell line donors? What quality control measures will be employed to determine adequate differentiation of the iPSCs? • The study design is heavy on data analysis and lacks details on the experimental design.
GWG Votes	**Is the project feasible?**
Yes: 10	• The experiments are likely to be accomplished in the proposed timeframe. • The team is highly qualified. • All necessary resources are available. • The budget appears appropriate. • The team is well composed and represents expertise in computational modeling as well as stem cell biology.
No: 4	• The proposal is written in a style heavy in conceptual statements and does not provide much experimental detail. The preliminary data provide some confidence that the proposed goals can be achieved. • The project seems overly ambitious for the proposed 2 year duration. Plans described under Aim 2 include co-culture systems and antisense oligo treatment. Such studies will require significant effort and time. • The computational parts of the project are achievable, but the cell models are more risky.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 12	• DEI aspects are adequately discussed in the application. • The proposal might validate the applicability of regenerative medicine discoveries to underserved populations. • Prior efforts or proposed plans for outreach, partnership, or educational activities appear appropriate. • The applicants partly address DEI principles in that the need for diversity in iPSC lines is recognized but not incorporated in the project plans.
No: 2	• Principles of DEI cannot be evaluated with certainty, because it is not clear what cell lines will be used.

Application #	DISC0-14531
Title (as written by the applicant)	Stimulating a cartilage-resident progenitor population for regeneration in aging and OA
Research Objective (as written by the applicant)	Proposed studies will provide new knowledge about cartilage-resident stem cells
Impact (as written by the applicant)	A successful outcome can lead to a stem cell based treatment for cartilage injuries and osteoarthritis (OA), a painful, debilitating disease that limits mobility and adversely affects quality of life
Major Proposed Activities (as written by the applicant)	• Identify a novel cartilage-resident stem cell population and its dysfunction with aging • Investigate prostaglandin signaling as a regulatory pathway for the function of these progenitor cells • Utilize mouse models of OA as well as patient samples to evaluate the effect of modulating prostaglandin signaling for a beneficial effect in OA pathogenesis
Statement of Benefit to California (as written by the applicant)	Our proposed research can identify a stem cell based therapeutic strategy that can directly benefit cartilage regeneration in age-associated osteoarthritis. California being the most populous state has an ever-increasing elderly population that will greatly benefit from a successful outcome of these studies.
Funds Requested	$1,500,261
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." 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	3
Highest	78
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: 11	• This proposal aims to activate endogenous chondroprogenitor cells to regenerate cartilage tissues in OA and aging mice, as cartilage has low regenerative potential in adults. This project, if successful, will lead to a novel therapeutic approach for cartilage tissue regeneration by activating endogenous stem/progenitor cells. • Articular cartilage repair is an important target. The applicants propose to rejuvenate cells by modulating the prostaglandin pathway, which has been shown to be important in muscle rejuvenation. • Successful completion of these studies will identify a novel cartilage progenitor population and a candidate drug, a small molecule modulator of prostaglandin signaling, that can 'rejuvenate' and restore the function of dysfunctional chrondroprogenitors. This would meet a major unmet need in cartilage repair and amelioration of OA. • The applicants' prior findings demonstrating rejuvenation of sarcopenic muscle via modulation of the prostaglandin pathway in mice is compelling, as are the preliminary data showing that a similar intervention benefits cartilage in mice and in human OA explants. • Elegant computational tools have been optimized by the team for analysis of murine joints, which raises the technological contributions and innovation of the proposal. • The causal role of cartilage stem cells in mediating the cartilage rejuvenation in the mice results is a bit speculative. Moreover, the translation to human cartilage is not strong. The hypothesis that prostaglandin levels would be important mechanistically could be supported by in vitro studies where the relevant molecules are directly added to explants. This study would be important to address both anabolic and catabolic activities of prostaglandins, and this additional preliminary data would help to lower the risk of the proposal.
No: 3	*None*
GWG Votes	**Is the rationale sound?**
Yes: 11	• This project is motivated by solid prior work from the investigator showing regeneration of muscle and by the preliminary data in mice and OA human explants provided. The data support that prostaglandin modulation is doing something beneficial to the cartilage tissue and the work is potentially very exciting. • Activation of endogenous chondroprogenitor cells to regenerate cartilage tissues in OA and aging is novel and exciting. • The role of prostaglandins in joint degeneration and OA is controversial, but in many ways this proposal represents a paradigm shift where a strategy to increase local levels of certain prostaglandins leads to cartilage growth/regeneration rather than break down. In this context, the method of action/dosing of the small molecule needs to be studied carefully with regard to the local downstream prostaglandin levels. • The weakness of the proposal is the tie of prostaglandin to a stem cell population within cartilage, which is circumstantial at this point. The translatability to human cartilage needs additional preliminary data to provide confidence of relevancy. Moreover, the local concentrations of downstream prostaglandins which are hypothesized to underly the beneficial impact of the small molecule are unknown. The rationale for the applicants' proposed strategy versus direct augmentation or agonism of downstream prostaglandins should be articulated. • The data the applicants collect may not be consistent between animal and human models, which may undermine the importance of studying the detailed spatial orientation of stem cells through and across the cartilage tissue or the overall underlying stem cell mechanism. The latter raises the general concern of how translational the proposed mice studies will be to the human condition. • This project mainly focuses on mouse models with limited human related studies. • It is unclear how the data generated in Aim 2 will be used to improve the small molecule treatment regiment.
No: 3	• The proposal does not address the putative cellular targets of the small molecule, and whether they are present in cartilage.
GWG Votes	**Is the project well planned and designed?**

Yes: 8	• The focus on modulating prostaglandin signaling in situ is based on exciting studies of muscle cell rejuvenation. • The proposed studies can be highly impactful as they aim to define a cartilage stem cell population and its spatial context as well as a molecule to stimulate these progenitor cells for a beneficial effect for cartilage regeneration. The advantage here is the incorporation of newly developed cutting-edge single cell techniques coupled with detailed functional outcomes to study this chondroprogenitor population. • The preliminary data demonstrates the ability to carry out Aim 1,2, and 3 of the proposal. Without stronger evidence that (1) the small molecule's actions are solely due to modulation of downstream prostaglandin levels levels and (2) that the data is translatable to human, the meticulous analysis to be performed on murine tissues derived from in vivo studies may not be justified. • Mechanistic questions regarding the small molecule may be better addressed in vitro, where tissue explants (including the human OA explants of Aim 3) or isolated cells could be subjected to well-defined doses, and changes in stem cell populations determined. • Yes, pitfalls regarding the method of processing tissues for molecular profiling are explained, as are optimizations in the bioinformatics analyses of the samples. • The applicant did not discuss potential pitfalls extensively.
No: 6	• Preliminary data indicate that the small molecule is effective, but experimental design could be improved. • A certain part of Aim 1 is an incremental aim, which only confirms the applicants' preliminary findings. • Aim 3 is limited to performing some in vitro human related studies. In addition, experimental details and feasibility of Aim 3 are not established. • A limitation of the proposal is that there is no real discussion of dosing (concentration or duration) of treatment with the small molecule. The concentration of downstream prostaglandins that will be modulated in response to the small molecule is critical as literature studies describe anabolic and catabolic impacts of prostaglandins on cartilage. • The applicants' prior work used intraperitoneal administration, whereas the current project will use also use intra-articular routes. How will efficacious concentrations of the small molecule be determined? • It is not clear that human cartilage has the same stem cell population as reported for mice, and it is not clear if the chondrocyte markers that will be used will be sufficient. • In the mouse model, it is possible that mechanism of action of the small molecule may be attributed to stem cells from other joint tissues (e.g., fat pad, synovium) recruited to cartilage, or through modification of the surrounding joint tissue secretome that impacts chemical crosstalk with cartilage, as opposed to direct action within cartilage.
GWG Votes	**Is the project feasible?**
Yes: 10	• Based on previous work and preliminary data, there is no concern regarding the ability of the team to carry out the proposed studies. • The proposed aims are feasible to be achieved within the proposed timeline, and the team has access to the necessary resources to conduct the project. • The preliminary data and expertise of the investigators provide confidence that the proposed research can be carried out as outlined. • The team has access to the necessary resources to conduct the project. • The proposed budget is reasonable.
No: 4	• It is not clear that stem cells are involved in the proposed mechanism.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• Yes, as cartilage degeneration and OA is more prevalent in underserved individuals whom are laborers and rely on physical activity for their occupations, this proposal is applicable to these populations. • The applicants propose that their work will improve quality of life for older persons. • The applicant has a track record of promoting diversity, inclusion, belonging, equity, and justice at their institution and in research. • In the mouse models, age is considered as a variable and both young and aged mice are employed as OA models. Similarly, both male and female mice will be utilized for these studies. However, it is known that the kinetics of OA progression upon injury are widely

	different in male and female mice; hence these cohorts will be analyzed separately. For human patient samples, the applicants will ensure that the samples reflect diversity of race, ethnicity, sex, gender and age. • The applicant described that they will ensure that the samples reflect diversity of race, ethnicity, sex, gender and age in both mouse and human studies; however, the details were not provided in human patient sample collection.
No: 1	*None*

Application #	DISC0-14567
Title (as written by the applicant)	The influence of human neural stem cells on autoimmune and regenerative function in mouse models of multiple sclerosis
Research Objective (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 (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 (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. • We will characterize a novel molecule expressed in Tregs that may be involved in maturation of oligodendrocyte precursor cells (OPCs) into functional oligodendrocytes. • 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. • 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. • We will establish the influence of Tregs on microglial pro-inflammatory vs. remyelination gene expression in response to hNPC administration to mouse MS models. • 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 (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	$1,549,209
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.” 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	3
Highest	75
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	• The proposed studies will improve the understanding of stem cell mechanisms involved in demyelination and remyelination in MS. • This revised grant maintains its thematic focus on advancing the use of stem cell therapy for multiple sclerosis (MS). • The gap in knowledge addressed by the project is the understanding the pathologic role and therapeutic targeting of inflammation in MS. Here, the PI proposes to examine the role of T regulatory cells or Tregs in dampening demyelination and conferring remyelination. • The bottlenecks identified in this project are profiling Tregs (i.e., antigen specific) and understanding the mechanism of action. • If the project shows that inflammation is reduced by hNPC-mediated recruitment of Tregs, Treg-derived peptide antigens, or adoptive transfer of Tregs then this opens a new avenue of treatment for MS. • Significance is not clear.
No: 2	• Current MS therapies are capable of blocking autoimmune T cell infiltration into the central nervous system. However, these are largely ineffective in the context of progressive stages of the disease. Regenerative approaches to improve myelin restoration or remyelination are being actively studied as a means to prevent axonal injury and neurodegeneration in regions of chronic demyelination. • Previous approaches have utilized the transplantation of human stem cell-derived neural precursors as agents capable of influencing the inflammatory environment in animal models of MS and thereby altering the disease process. These have been implicated in the promotion of remyelination though this remains an outstanding question. • The lack of effect of NPC transplantation on the clinical score reduces the likelihood that the effects described have a meaningful impact on disease outcome.
GWG Votes	**Is the rationale sound?**
Yes: 8	• The rationale to focus on Tregs for the modulation of MS inflammation is sound. • It appears that compelling data accompany hNPC-Treg-mediated reduced demyelination and increased remyelination • The use of hNPCs and the testing of the cells in clinically relevant animal models demonstrate the significant relevance of the project to human biology and disease. • There is a concern about effect sizes in the earlier data providing the basis of the application. Demyelination and remyelination in both the mouse models, while statistically significant, are not robust and show major overlap of treated and untreated populations.
No: 6	• The scientific rationale is reasonable. • Preliminary data are not compelling. • There was no effect on clinical score in one model following NPC transplant. • Some key data are not shown or refer to unpublished work. • There are major problems with the preliminary data and their interpretation. This substantially reduces enthusiasm for this project. • Figure 1 is unreadable. • Figure 2 shows reduced lesion volume. The significance of this is unclear as NPC transplant did not differ significantly from dermal fibroblasts. • Figure 3 does not include quantification of the imaging data. • In Figure 4, remyelination vs. normal myelination cannot be reliably distinguished using the described label-free approach.
GWG Votes	**Is the project well planned and designed?**

Yes: 8	• The experiments are logical. Aim 1 will examine the role of CNS antigen presentation by hNPCs on Tregs. Aim 2 will examine the mechanisms by which NPC-altered Tregs may enhance remyelination. • Controls are discussed. Preliminary data in vitro is compelling. • While technically advanced, it is unclear what the hypothesis to be tested is, and the specific rationale for including the proposed approach above the existing methodology. • The applicants address an earlier critique: "....focus should be placed on understanding the mechanism for the unique immune rejection response mice have to hNPCs" by showing new data that human dermal fibroblasts do not promote remyelination. However, the new data in Fig. 2 is not convincing.
No: 6	• The revised experimental design seems to incorporate many of the previous concerns, however there remain outstanding weaknesses. In particular, the PI argues that human dermal fibroblasts do not elicit significant therapeutic effects and referred us to Figure 2. A careful examination of Figure 2 panel B reveals a statistically significant reduction in demyelination, contrary to the PI's argument. • For the new microglia assessment, the experimental design is not well developed - when and where will the tissue sampling be conducted? • In response to delineating the direct contribution of the implanted cells to functional recovery, the PI deflected their response by noting that the focus of the project is on hNPC-mediated immunomodulatory mechanism. The PI proposed the use of non-viable hNPCs which is responsive to this previous concern.
GWG Votes	**Is the project feasible?**
Yes: 11	• The project is feasible. • Team is qualified, well-staffed, and their roles are specifically outlined. • All necessary resources for this project are in place. • The project is somewhat ambitious but the investigator and team are excellent. • Aim 1 will test whether specific populations of Tregs generated in vitro are able to affect myelination in the mouse models. The experimental approach is well-designed and a positive demonstration of such effects would be a significant achievement. • Aim 2 will attempt to identify molecular and cellular mechanisms and specifically test the hypothesis that reduced neuroinflammation and increased proliferation of OPC are responsible. The experiments are well-designed. Results can be expected to answer the specific questions posed, however, it is not clear to what further studies or questions they would lead.
No: 3	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• There is a section detailing all these factors in experimental design, team make-up, and envisioned MS patient enrollment. • Project outcomes are catered to the underserved communities. • The PI has been active in this area.
No: 0	*none*

Application #	DISC0-14346
Title (as written by the applicant)	Nodal Organoids to Dissect the Molecular and Cellular Architecture of Cardiac Pacing
Research Objective (as written by the applicant)	We propose to use human induced pluripotent stem cell (hiPSC)-derived pacemaker cardiomyocytes and fibroblasts to generate sinoatrial node (SAN) organoids that will permit exploration of the tissue architectural determinants of cardiac pacing.
Impact (as written by the applicant)	There is currently no way to model the human cardiac pacemaker due to the complexity of its cellular constituents and tissue architecture, creating a barrier to using stem cells for biological pacing.
Major Proposed Activities (as written by the applicant)	• We will aggregate hiPSC-derived pacemaker cardiomyocytes and cardiac fibroblasts in engineered substrates to generate human sinoatrial node (SAN) organoids. • SAN organoids will be characterized at the physiological, structural/architectural, and transcriptomic levels using pacing, physiological recording, microscopy, and sequencing. • SAN dysfunction will be modeled in SAN organoids by activating fibroblasts and assessing SAN organoid structure and function using pacing, physiological recording, and imaging. • The role of the teneurin family of cell adhesion molecules in regulating SAN tissue architecture will be defined in SAN organoids created with TENM4 and TENM3 loss of function hiPSCs.
Statement of Benefit to California (as written by the applicant)	The proposed research will address disorders of cardiac pacing, among the most common types of heart disease. These diseases affect hundreds of thousands of Californians, who must live with implantable cardiac devices. By overcoming barriers in this area, our work will create a new platform for discovery in arrhythmia biology, and will be a first step towards developing a new generation of treatments that will obviate the need for implantable devices for Californians suffering from arrhythmias.
Funds Requested	$1,598,398
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." 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	3
Highest	82
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: 11	• This projects aims to develop an organoid model of the sinoatrial node (SAN), to use this model to study architectural features of the SAN that underlie its pacemaking ability, and to define the roles of specific intercellular adhesion pathways in creating and maintaining SAN architecture. • The sinoatrial node (SAN) is a small but critical structure that initiates the heartbeat. SAN tissue cannot regenerate, so pacemaker cardiomyocyte (PC) malfunction, loss of PCs, or fibrosis in the SAN results in SAN dysfunction, a common disease that cannot be prevented or reversed with current treatments. • There are a number sinus node diseases for which the underlying disease mechanisms are not well understood. • The proposal aims to develop an iPSC derived model of the SAN which can build the basis for disease modeling. Aim 3 proposes to study a potentially disease relevant pathway. • The proposed organoid model has potential as a new experimental platform and disease model. • If successful the proposal can elucidate disease mechanisms. • A biological model to study SAN will be important. • If successful, this project will have a major impact on modeling SAN and its related cardiac diseases. • This kind of study is important to achieve pacemaker activity for transplants.
No: 3	*none*
GWG Votes	**Is the rationale sound?**

Yes: 10	• Yes, the rationale is clear. There are no representative in vitro models of SAN.
	• The underlying rationale is well developed. The need for a disease model is justified.
	• The proposed use of iPSC and approach to modify the underlying matrix composition are innovative.
	• There are preliminary data supporting the overall approach. The selection of a candidate cell signaling pathways in Aim 3 is logical and appropriate.
	• There are limited data to establish that the proposed micro-tissues will mimic the architecture of SA node. These tissues look and probably behave more like tissues already widely utilized for contractility studies.
	• If successful this proposal could shed light on general concepts of SAN function and pathology.
	• This project is based on sound scientific rationale.
	• Using iPSC-derived pacemaker cardiomyocytes (PCs) to reconstitute and engineer SAN tissue in vitro is a good idea.
	• Overall, yes, but I have two concerns.
	  ◦ The SAN has a distinct composition, size, shape and location. These all likely contribute to the SAN electrical activity, properties, and function. The applicants propose to model the SAN using organoids - in this case, iPSC cultured with certain micro-tissues (Fig 5) - and study the origin of SAN pacemaking activity in these organoids. However, the proposed micro-tissues are similar to micro-tissues used to model ventricular or atrial heart muscles and may not recapitulate SAN architecture. This substantially reduces the strength of the proposal.
	  ◦ The applicant states that the current cell differentiation protocol may be hard to scale, or may scale with low efficiency. They state that they have a new, improved protocol but do not share it (or describe key improvements), making

	this difficult to review. They also do not provide data showing differentiation efficiency with the new protocol.
No: 4	• It's not clear that this is a truly novel approach to study node structures. Other 3D models exist.
GWG Votes	**Is the project well planned and designed?**
Yes: 6	• Aim 1 is well developed. The proposed experiments to vary composition of the organoid components is appropriate. • The proposal lacks general innovation in the way the SAN is modeled. It is not clear what the difference is to a regular ventricular type micro-tissue. • Pitfalls and limitations are discussed. While the applicant discusses in great detail the structure and composition of an SAN, the proposal lacks some discussion on whether the organoids behave like an SAN. • The timeline is appropriate. • The three aims are appropriately planned, but are not particularly novel. For example, Aim 1 plans to optimize the ratio of pacemaker cardiomyocytes (PCs) and fibroblasts in SAN organoids. Are there other cell types in SAN tissue? • For Aim 2, the applicant did not propose an innovative way to promote maturation of PCs in SAN. • The applicant addressed some potential pitfalls, but not how to functionally test their engineered SAN tissues.
No: 8	• I think this is a great idea but I would like to see more proof-of concept data demonstrating that applicants can make SAN organoids that function as pacemakers. What if nodal fibroblasts are crucial for PC maturation? What if PCs will not mature in the organoids? • The preliminary data not very convincing. Is this really better than published protocol.
GWG Votes	**Is the project feasible?**
Yes: 10	• The applicant has established a protocol for iPSC differentiation into SAN PC and tissue engineering technology for assembling cardiomyocytes and fibroblasts into a 3D matrix using a micro device. However, they do not demonstrate that these are functional SAN organoids - this is essential to ensure the feasibility of the proposed work. • All aims can be accomplished during the proposed timeline. • This team is qualified to do the proposed research. • The team is highly qualified. • All resources are available. • The budget appears appropriate. • The budget is appropriate. • The project is feasible.
No: 4	• Feasibility is unclear at this point. Will the system actually function as a pacemaker?
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• The applicant's response is very general. Specific details in the experimental plans or outreach activities regarding influence of race, ethnicity, age or sex are lacking. • The proposal addresses the influence of race, ethnicity, sex, and age. • The proposal is applicable and relevant to underserved populations. • The applicants describe outreach, partnership, or educational activities. • The applicant plans to use iPSC lines from donors representing different races, ethnicities, and sexes. • This project will be useful for underserved populations. • The applicant describes outreach activities to DEI students.
No: 1	*none*

Application #	DISC0-14533
Title (as written by the applicant)	Investigating the Role of Microglia in Autism Spectrum Disorder Using Patient-Derived hiPSCs in Culture and Cerebral Organoid Models
Research Objective (as written by the applicant)	Through the use of patient stem cells we will model MEF2C haploinsufficiency syndrome, a debilitating form of autism, and identify at a molecular level the role of the immune cells in this disorder.
Impact (as written by the applicant)	This research sets the stage to identify potential targets or therapeutics for MEF2C haploinsufficiency syndrome, a debilitating form of autism that currently has no FDA-approved treatment.
Major Proposed Activities (as written by the applicant)	• Perform RNA-sequencing on MEF2C patient and healthy microglia • Analyze RNA-sequencing data from MEF2C patients and healthy microglia • Develop human stem cell-derived co-culture model containing microglia, neurons and astrocytes and probe for differences in patients with MEF2C haploinsufficiency syndrome • Develop and characterize 3D organoid model with human stem cell-derived microglia component • Perform single cell RNA-sequencing on MEF2C mutant and healthy organoids with microglia component • Identify and test potential drug candidates identified in screening platform to reverse abnormalities associated with MEF2C haploinsufficiency syndrome in organoids
Statement of Benefit to California (as written by the applicant)	Recent studies show that MEF2C activity not only affects myocerebrohepatopathy spectrum (MCHS) but also other forms of autism spectrum disorder (ASD) because MEF2C drives the activity of other ASD-related genes. Thus, while we are developing a model to target the MCHS form of ASD, identified compounds in our model may also prove effective for a much large group of ASD patients. As ASD is now reported to occur in 1 in every 44 births in the USA, the benefit to the ASD community in California is potentially immense.
Funds Requested	$1,837,714
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.” 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	1
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: 11	• The project is focused on a severe form of ASD (MEF2C haploinsufficiency syndrome, MHS) for which there is no cure. Previous work by this group identified defects in neuronal and astrocytic cells. They now propose to extend the analysis to microglia, which might be dysregulated in models of ASD with disease-causing mutation in MEF2C. • The applicants propose to study autism spectrum disorder (ASD) using hiPSC-derived microglia (or isogenic controls) from patients with MEF2C haploinsufficiency syndrome (MHS). They also propose to investigate aberrant inflammation and potential therapeutic targets. • Phenotypic and molecular analysis will be coupled with a drug repurposing approach to identify drugs that are suitable to correct aberrant phenotypes observed in human microglia. • While the current study is focused on an extremely rare disease, the approach has the potential to be useful for many applications where microglia may play a role. • The use of complex human organoids will address the knowledge gap of the potential role of microglia defects in this disease. • Developing an in vitro model system entirely derived from human hiPSCs with more human-like microglia characteristics holds promise to enable improved quality and human-relevant studies. • The proposed project and the use of the proposed 3D system incorporating microglia has a potential to significantly impact the field. However, the biological advancements of this proposal are likely to be incremental. It has recently been shown that MEF2C-deficient microglia were sufficient to produce autism-related behaviors in mice, so the next step of testing this in humans is logical. • Understanding autism disorders using cerebral organoids is attractive but this approach may only apply to clear genetic forms of the syndrome. The wider applicability is unclear. • The applicants frequently use "wild-type" in a confusing manner, as this term typically applies in animal experiments only.
No: 3	• The application describes a new approach to understanding the pathobiology of a rare neurodevelopmental syndrome resulting in autism and intellectual disability. • The project considers the potential role for microglia in this ASD disorder, an area that has been previously overlooked. • The applicants do not provide a convincing case for how the drug screening proposed in Aim 2 will yield results. • In their description of the relationship between MHS and ASD, the applicants should refer to a published systematic review (https://doi.org/10.1002/ajmg.a.62412) suggesting that not all MSH patients have ASD. In that respect, it is important to clarify how ASD status will be ascertained in the context of this proposal. • The applicants' statement that ASD is characterized by "decreased cognitive abilities" (among other behavioral symptoms) is not correct, as many individuals with ASD do not show decreased cognitive ability, nor is this a factor in ASD diagnosis.
GWG Votes	**Is the rationale sound?**
Yes: 11	• The scientific rationale is clear and sound. • Microglia are already known to affect synaptic pruning during development and are thus an attractive target for disorders that show disruption of synaptogenesis. • Studies in mice suggest that targeting loss of MEF2C to microglia is sufficient to affect pyramidal neurons and elicit autism-related behaviors. • The applicants have identified a relevant syndrome and have developed robust microglia differentiation protocols. Appropriate isogenic control and variant lines are available to perform these studies. • The proposed approach is consistent with published mouse data.

No: 3	• Though it is reasonable to speculate that microglia are important in MEF2C haploinsuffciency disorder, the applicants and others have noted multiple problems in neurogenesis, neural development and maturation in this condition. It might be difficult to tease apart the roles for microglia in a co-culture system since neuronal deficits seem to be cell-autonomous. • The rationale rests heavily on data from a microglia specific conditional knockout mouse published earlier by another group. Preliminary data from the applicant in control and variant cell lines support some changes in microglia phenotype, but could just relate to differences between clones rather than gene-dependent differences. Data on additional patient lines would strengthen the rationale behind the study. • The preliminary data on effects of MEF2C deficiency on microglia show a minor increase in activation state, minor decrease in particle uptake, and failure to produce cytokines in response to stimulus. The applicants should summarize the potential functional implications of these data. For example, do they describe a coherent cell fate, and how do they relate to previous studies of the effect of this gene on the myeloid lineage?
GWG Votes	**Is the project well planned and designed?**
Yes: 5	• Overall the project is well designed. • The applicants do not provide preliminary data addressing the extent to which microglia integrate into organoids.
No: 9	• The establishment of human brain microglia from normal yolk sac is interesting. The applicant provides data showing that the transcriptional signature of induced microglia is consistent with primary human microglia transcriptomes, which is a strong indication that the model the applicant is using to generate microglia is suitable. • The phagocytosis assay of microglia support the hypothesis that induced microglia have impairments compared to an isogenic wild-type control cell line. • The applicants justify their use of organoids by stating that mouse models have issues and limitations. The applicants should also address the limitations of the organoid system. • Pitfalls are mainly limited to technical feasibility issues and do not clearly describe how the applicants will address ambiguous data. • Power calculations should be clearly presented and explained. • The major strength of this project is the use of the cerebral organoids containing yolk-sac derived microglia cells, and the applicants extensively discuss why this approach is better than classical 2D cultures. In that respect, reverting to 2D cultures because of the low throughput of organoids to more rapidly screen for compounds is not a good alternative strategy. • It is not clear how outcomes of Aim 1 are informing Aim 2. • Aim 1 relies on the purity of induced microglia to determine whether any observed defects are endogenously associated with MEF2C deficiency versus exogenous factors. However, the purity of these cultures is not well described, so this aim is redundant with Aim 2. • Project Aim 1 should reveal whether there is a significant and consistent microglia phenotype in hemizygous cells. If this is not observed, the rationale for Aim 2 disappears. Without data on patient microglia, it will be difficult to know if observations in vitro are relevant in vivo. The proposed drug screen for effects on neuronal hyperexcitability do not seem to rely on the microglial model, and could be a separate project. • The ELISA data presented in Fig 8 are intriguing but the relevance of the stimulation studied in this figure to ASD is not clear. Is the applicant focused on the early synapse pruning function of microglia or the late immunfunction of these cells in a more mature brain? The time line of pathology and the various roles of microglia during development make this challenging to evaluate. • It is not clear if the concentrations and penetration of inflammation-inducing factors in Aim 2 will be adequately controlled. If these treatments do not produce an effect, could that be due to lack of exposure? It is also not described at what stage "inflammation" will be induced and what relevant event will be modeled (for example, pruning in early synapse development or inflammation in more mature brains). • The organoid example that is shown in Figure 9 suggests an absence of certain cell populations in the mutant organoids. Is this a reproducible phenotype and does this have

	functional relevance? The application would have been improved if there was discussion on the representation of various cell populations in mutant and wild-type organoids. • It is not clear why co-culture experiments are not repeated in the context of inflammatory stressors. If that is an important aspect of the microglial pathology, it is perhaps even more relevant in the context of other cells.
GWG Votes	**Is the project feasible?**
Yes: 9	• The proposal is feasible. • The investigators have outstanding track records and have the required expertise to conduct this work. • Concerns about the interdependency of the aims may present challenges to feasibility.
No: 5	• The proposal may be feasible. The applicants provide data indicating that microglia integrate into the organoids, although the yield is very low. Their approach will likely require the addition of IL-34, which could mask neuronal defects in the organoid. • The applicant suggests that they will overcome the low yield of microglia by seeding experiments. However, the yield of microglia after seeding seems equally minimal (according to Figure 4), raising the question of whether the number of cells recovered for the single cell RNA analysis will be sufficient to make useful comparisons. The applicants do not discuss this consideration. • It is not clear if organoid model has sufficient throughput to be applied to screening approaches with complex readouts. • The timeline is "staggered," but experiments could be initiated in parallel rather than sequentially. • A failure to detect significant and reproducible microglia phenotypes in mutant cells will mean that the outcomes of this proposal are low impact.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• Yes, this proposal addresses DEI adequately. • The plans for outreach/educational activities are very basic and general. • The applicants will generate cellular models representative of diverse genetic ancestries. The patient community served by the team is diverse.
No: 0	*none*

Application #	DISC0-14456
Title (as written by the applicant)	Development of a synthetic cellular model of human embryonic Implantation
Research Objective (as written by the applicant)	The primary objective is to develop a reproducible and controllable 3D model of human embryogenesis and embryo implantation using CRISPR-based epigenome editing in human embryonic stem cells.
Impact (as written by the applicant)	We lack a cellular model that can recapitulate the biology of embryo implantation in the dish. This project will address this limitation by establishing a novel model to reproduce the biology of implantation.
Major Proposed Activities (as written by the applicant)	• Generation of major cell types of a human preimplantation embryo • Formation of the human embryo-like models induced by CRISPR technology • Establishing a cellular assembly to mimic interaction between human embryo and uterus wall • Single cell genomics and microscopy to systematically characterize human embryo-like models
Statement of Benefit to California (as written by the applicant)	This proposal puts California at the forefront of the rapidly emerging field of synthetic human embryology. Given the entrepreneurial spirit of California and Californians, we anticipate transnational applications of our human embryo model to address clinically relevant problems in the field of assisted reproductive technology in the near future.
Funds Requested	$1,510,160
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." 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	7
Highest	80
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: 11	• There is a recognized bottle neck in the understanding of human implantation. This process has a high failure rate and better systems are needed to study implantation in humans. The applicant intends to make better 3D models of the early human embryo and combine these with 3D endometrial models to explore interactions in vitro. Development of a robust model of human implantation would have an impact on assisted reproductive technologies and interventions to promote improved early pregnancy survival. • The project has clear relevance to human health and disease, making it an important area of research with the potential for significant impact. • If robust and reproducible, these in vitro model systems could be used for drug screens and other methodologies to improve human development at this critical time period.
No: 3	• The project addresses a key knowledge gap in our understanding of stem cells and their application to human biology and disease. Specifically, the project focuses on human implantation, where there is a major bottleneck in discovery due to the lack of good models. • Successful completion of the project has the potential to yield new insights into this critical period of development and impact the fertility field. • Implantation of the human embryo is a crucial feature of a successful pregnancy and major differences in this process exist between mouse and human. No good models of implantation exist as of yet, but this proposal will not resolve the lack of good models.
GWG Votes	**Is the rationale sound?**
Yes: 11	• By using human stem cells, the project has the potential to yield insights that are directly applicable to human biology and disease, making it an important and promising area of study. • Preliminary data from the mouse system is very compelling, indicating that this approach has the potential to generate blastoids that more closely resemble the natural implantation process. • The project has a sound rationale, as it builds upon previous work that has successfully generated blastoid structures and demonstrates the feasibility of using the CRISPR system in this context. • Preliminary data in mouse shows that inducible CRISPRa of lineage-specific transcription factors can generate cells with desired lineage properties, and that mixed cultures of these cells form 'preimplantation cylinder structures." This is important preliminary data but it is not clear what these structures represent. • The overall concept of generating an in vitro stem cell-derived blastocyst model (blastoid) and an in vitro endometrial organoid and combining them to study implantation is sound and is being pursued in multiple labs around the world. The claim is that current blastoid models are inefficient and not robust. However, the applicants do not reference several key papers on blastoids (Liu et al 2021, Yu et al, 2021, Yanagida et al 2021), some of which also demonstrate robust systems. • The use of transcription factors to drive cell fate towards extraembryonic lineages has been reported by others and was used to generate models of early postimplantation development (Tarazi et al Sept 2022 Cell 185: 3290; Amadei et al 2022 Nature 610: 143; Lau et al 2022. Cell Stem Cell 29: 1445). These papers were published before submission of this grant and should be acknowledged.
No: 3	• The applicants currently do not have a demonstrated ability to create the required human blastoids, and other groups have already created endometrial organoids. • The CRISPRa strategy proposed in this application is not trivial and levels of transcription factors may not be sufficient to achieve the necessary outcomes. • The design of the co-culture seems too simplistic given the complexity of implantation.
GWG Votes	**Is the project well planned and designed?**
Yes: 8	• The project is well-designed and appropriately planned, with a clear and logical sequence of steps. • The first step involves assembling the blastoids, which is followed by a detailed analysis of their structure and function.

	• The final step involves simulating the uterine environment, which is an important and necessary step in understanding the implantation process and the formation of blastocysts. • The approach is highly systematic and methodical, which is critical for achieving meaningful and reproducible results. • The project employs a range of cutting-edge techniques and technologies, including CRISPR gene editing, that are ideally suited for investigating the complex biology of blastoids. • The strengths of this proposal lie in the CRISPRa screens for novel transcription factor combinations that will drive human ES cells towards trophoblast and hypoblast lineages without complex culture conditions. Preliminary data in the mouse is supportive of this approach. The applicants have established a collaboration to bring endometrial organoids to the project and the combination of the two will be the desired outcome. • The CRISPRa screen of multiple transcription factors to find the best combination to drive trophoblast and hypoblast development is a rational approach. This is also the applicant's core expertise and should generate helpful data not only for this study but for other approaches to understanding early human development. However, there ultimately may not be sufficient expression level modulation to obtain the desired fate changes. • Pitfalls and alternatives are discussed in detail. • There are many challenges along the way for success in this project. The applicant recognizes this and proposes possible alternate models for both blastoid and endometrial components. If the endometrial organoids are not successful in combination with blastoids, the applicant will try trophoblast alone in trophospheres. This approach is appropriately cautious, given that it is still early days for these model systems. • The overall issue of how closely these stem cell models mimic normal development and implantation remains a challenge. Careful comparison with existing and emerging data from human embryo material will be needed.
No: 6	• There is a lack of preliminary data to support Aim 1 (the immunofluorescence results from mouse are not sufficiently convincing). Others in the field have published key aspects of Aim 1 and the proposed co-culture approach. • Aim 2 is not described in sufficient detail and lacks preliminary data supporting how this aim will aid in the understanding of human embryo implantation, which is the stated goal. • The applicant does not address considerations for bridging their proposed model and the true in vivo process. Co-culturing blastoids and endometrial organoids does not adequately account for human-specific aspects of implantation.
GWG Votes	**Is the project feasible?**
Yes: 9	• The project is highly feasible, with experiments that can be completed within the proposed timeline. • The team has the necessary expertise in key areas, including in vitro culture, CRISPR gene editing, and data analysis, which are all critical for the success of the project. • The research lab has the necessary resources to carry out the experiments, including access to state-of-the-art equipment and facilities. • The team has a strong track record of accomplishment in related areas of research, which provides confidence in their ability to successfully carry out the proposed work. • The applicant has core expertise required for this project, and the team includes a dedicated data manager. The applicant has also established critical collaborations for CRISPR screening and the endometrial model. • The project leverages existing expertise and resources, which helps to minimize the risk of delays or unexpected obstacles. • The budget for the project appears appropriate, given the scope and complexity of the work to be undertaken. • Aim 1 claims current approaches to making blastoids are inefficient and variable. As such, the applicant proposes to use induced transcription factor expression to force lineage decisions and then recombine cell types. This is very similar to a recently published study which used Cdx2 and Gata4 to prime embryonic stem cells to extraembryonic lineages. The “synthetic embryos’ produced resembled early postimplantation embryos but presumably the culture conditions could be modified to promote blastoid formation.

	• Aim 2 will build a combined blastoid/endometrial model of implantation. This is important and also under study in other groups, which have reported using implantation of blastoids with endometrial cells (Khoei et al 2023, Nature Protocols).
No: 5	• The proposal is impacted by a lack of preliminary data, and gives the impression that the applicant is starting at the beginning in a field that has progressed beyond the studies included in this application.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• The plan for diversity, equity, and inclusion is discussed in detail. • This project extends the applicability of stem cell research to underserved communities because access to assistive reproductive technology is problematic in underserved populations. • The objective addresses the racial inequity in access to assistive reproductive technology, but it is not clear how this study will address this. The applicants may be able to make embryo models from racially diverse groups in the future, but ultimately this proposal does not address socioeconomic issues.
No: 1	• Studies proposed in Aim 1 and 2 do not specifically addresses DEI.

Application #	DISC0-14559
Title (as written by the applicant)	Machine Learning to Guide Design of CRISPR Engineered T Cell Therapies for Cancer
Research Objective (as written by the applicant)	We will build the methods and experimental approaches to CAR T cell engineering to allow characterization of diverse CAR T cell CRISPR modifications for cancer treatment efficacy
Impact (as written by the applicant)	Our project will improve CAR T cell cancer therapies; we will characterize novel CRISPR edits to engineer behaviors in CAR T cells; we will develop analysis pipelines for live-cell imaging data.
Major Proposed Activities (as written by the applicant)	• Build public database of live-cell imaging experiments. This database will include live-cell imaging experiments and other publicly-available data from other labs (database). • Build open-source software platform for the analysis of live-cell imaging experiments. This software package will process, compress, and phenotype imaging data (open-source software). • Publication on the machine learning approaches to phenotyping the live-cell imaging data (paper). • Publication on the machine-learning assisted selection and experimental validation of CAR T cell modifications to produce a specific, desirable behavior (paper). • Paper on machine learning selection of multiple CRISPR modifications that, in combination, produce specific, desirable behaviors (paper). • Database that describes the phenotypic effects of all tested CRISPR CAR T cell edits (database).
Statement of Benefit to California (as written by the applicant)	The proposed research will benefit the State of California by dramatically expanding access to CAR T cell therapies for cancer patients. The project has the potential to create therapies for diseases other than cancer as well. The computational methods and CRISPR editing will accelerate the development of these therapies for individual patients to provide timely, life-saving solutions. We will provide research opportunities to students and researchers in the area.
Funds Requested	$1,710,858
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." 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	8
Highest	84
Lowest	50
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: 11	• The value of a standardized and freely available database for live cell imaging would be widespread. • Being able to do pooled optical screens would help T cell therapy development. • Causal inference is difficult and it is rare to find in research proposals. The invocation of time dependencies to infer causality is appreciated, innovative, and welcome. • There is no need for a proposal to only have relevance to stem cells, although at least one other reviewer thought a technology specific to stem cells would be more appropriate. The applicants may wish to emphasize and call attention to the particular value of this approach to stem cell research. • Relation to stem cell biology is unclear but could impact many groups in general cell biology. • Unclear if there would be a major impact on scientific knowledge in the stem cell/regenerative medicine field, but this is a novel application and creative.
No: 4	• The project aims to understand how genetic changes alter cell-cell interactions, cell killing, and cell motility. • If successful, the project could help identify characteristics of cell dynamics impacted by novel genetic manipulations that may influence the design of next-generation genetically engineered immune cell therapies. • While this proposal does not directly apply to stem cells, it does propose to study the cellular dynamics of genetically engineered immune cell therapies as regenerative medicine. The complexity of cellular dynamics is not completely understood and the impact of genetic manipulations on cell motility and function is also not well understood. This proposal aims to utilize live cell imaging to elucidate some details related to cellular dynamics. • The proposed imaging tools are not clearly of high impact to the wider community.
GWG Votes	**Is the rationale sound?**
Yes: 10	*none*
No: 5	• The proposal suggests that live-cell imaging of cellular dynamics throughout CRISPR screens of CAR T cells will improve understandings of how specific genetic manipulations contribute towards cell-cell interactions, cell motility, and cell killing. However, studies of genetic perturbations that influence cell activity can already be performed and there is no indication that live-cell imaging in this proposal can enhance that knowledge. • The proposal does not identify a biological problem with CAR T cell cellular dynamics or provide a hypothesis that can be tested and is therefore not significantly relevant to human biology and disease. • Proposal does not identify a biological problem with CAR T cells that cellular dynamics would solve. • The application and relation to stem cell biology is difficult to pinpoint. The rationale behind the need is unclear. Who will deposit this data and how will this be deployed? Academic and private sector? • Examples of how dynamics of CRISPR screens have missed important biology would strengthen the position and foundation of the PI's proposal. • There are no preliminary data included, only descriptions of data. In addition, the rationale document is not written cohesively; there are two distinct styles of writing, the references are not listed sequentially and they do not align with the references document. • Pooled screening approach should be demonstrated. • Unclear if large databases are annotated for racial background, sex, or age that would allow deployment of their proposed strategies. • Figures are not found. Difficult to access.

GWG Votes	Is the project well planned and designed?
Yes: 6	*none*
No: 9	• Strong integration and use of Aim 1 to Aim 2 CRISPR screens using T-cell modeling systems for CAR-Ts. • The pitfalls of too much data and too many cellular phenotypes is identified and alternative approaches of integrating additional cell phenotypes for study is proposed. • The application provided pitfalls, and given the expertise, these are very likely to successfully mitigate concerns. • The proposal lacks an integration between the genetic manipulations and screens and live-cell imaging. Specifically, there is no plan to spatially isolate the genomes or transcriptomes of cells in order to correlate cell signaling, genetic manipulations, and characteristics of cell dynamics. While the proposal suggests that fixed-cell imaging has limitations on studying cellular heterogeneity, there is no clarity on how the proposed study will provide more specific information of single cells. • A demonstration of how the approach is better than state-of-the-art approaches should be provided (e.g., over imaging studies of serial CAR T cell killing). • The approach needs to deconvolute a pooled set of perturbations. It is not clear how this would be done. • Preliminary data are not strong. • The annual budget appears to be inflated - less than $30K in supply costs for annual budgets of over 10x that amount.
GWG Votes	**Is the project feasible?**
Yes: 8	*none*
No: 7	• Very qualified staffed and very well supported. Pioneers in their fields. • The facilities and resources available are sufficient to conduct the proposed activities. • The project is very likely feasible, but highly dependent on number of CRISPR screens and reproducibility. • One investigator is an expert in machine learning for biological systems and one investigator is an expert in systems approaches for genetic manipulations in cell therapies. The integration of expertise to form a more cohesive proposal is recommended. • The proposal has flaws that limit the feasibility to integrate live-cell imaging studies of cellular dynamics with genetic perturbations and changes in cell signaling. Additionally, temporal changes in cell signaling is necessary to understanding the complexity of cellular dynamics - these are not proposed studies. • The budget is not appropriate for the limited studies proposed.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 12	• The methods of addressing these principles in their technical work should be described.
No: 3	• Use of diverse samples, and integrating sex and background as parameters of their machine learning approaches. • Prior educational activities to inform the development of DEI within the research project is described. • The proposal does not include any mention, except in the statement on DEI, of race, sex, or gender as biological variables that may influence cellular dynamics. Inclusion of training opportunities for underrepresented minorities is mentioned, but representation of the diversity of cancer patients is not considered. • There is no evidence that the proposed studies would extend the applicability of regenerative medicine discoveries to underserved populations. • Minimal description.

Application #	DISC0-14530
Title (as written by the applicant)	Engineered injectable pre-vascularized microporous implants for neural stem cell transplantation after stroke
Research Objective (as written by the applicant)	We propose to develop an injectable soft material that can encapsulate brain cells and promote their long-term survival through blood perfusion to repair the brain after stroke
Impact (as written by the applicant)	If the proposed study is successfully achieved, we will have developed a nanotechnology-based stem cell therapy that enhances cell survival and integration in the brain after transplantation
Major Proposed Activities (as written by the applicant)	• Fabrication of the engineered material • Generation of the neural stem cells • Encapsulation of neural stem cells in the material and brain injection in the stroke lesion • Evaluation of brain inflammation and cell survival • Evaluation of vessel formation • Evaluation of tissue repair and recovery of neurological deficit
Statement of Benefit to California (as written by the applicant)	The proposed research will benefit the State of California and its large population of diverse ethnicity, gender, age, and socioeconomic status.
Funds Requested	$1,605,001
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." 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	65
Median	65
Standard Deviation	5
Highest	70
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: 9	• The applicants propose to investigate the development of an innovative hydrogel-based therapeutic nanotechnology specifically designed to promote brain tissue regeneration and functional recovery though vascular formation in the injured brain, and blood perfusion of the transplanted neural stem cells. • The PI hypothesizes that their biomaterial system will enhance long-term survival and integration of the transplanted neural progenitor cells (NPCs) with the host brain and will promote post stroke recovery. The project is relevant to the application of stem cells to the treatment of stroke, and it addresses a key knowledge gap in this field. • This project holds promise to deliver a major break through in the stroke field. • The project identifies that graft survival remains poor or non-existent within the ischemic core after stroke. Augmenting this will likely enhance not only graft survival but functional recovery. • The project defines the development of hydrogels/nanoparticles that may enhance NPC graft survival and function. • If the project shows the product leads to enhanced graft survival and function following transplants into the ischemic core then this will have an impact on the scientific knowledge and clinical application of cell therapy for stroke, which remains an experimental treatment that produces mediocre and variable clinical outcomes.
No: 5	• Neural cell transplants for stroke treatment is a tough area. If successful, this approach would have impact. However, it is not clear that this approach is innovative enough to drive success.
GWG Votes	**Is the rationale sound?**
Yes: 10	• The rationale for the proposal is based on the hypotheses that co-culture of cells in the gels prior to transplantation will result in a self-assembly of the vascular constructs with the NPCs. These pre-vascularized cell assemblies would then provide immunoprotection of the graft and will stimulate vascular anastomosis of the graft with the host vasculature to achieve blood perfusion and functional integration of the transplanted NPCs. • Preliminary data and relevant publications are solid. • The scientific rationale is sound and the project is well thought through and well designed (with the exception of inclusion of male and female mice). Alternative approaches for potential experimental issues are well addressed. • Clarification is needed on what was accomplished in the previous 5-year CIRM grant with another PI that appears to set up the present grant.
No: 4	• Incremental advances over previous study
GWG Votes	**Is the project well planned and designed?**
Yes: 7	• Overall, the present experimental design is a small incremental advance from previous grant. There are many technical aspects proposed for hydrogel development that should have been answered already on the previous grant and appears to be merely replicated on Aim 1. Aims 2 and 3 need to be more thematically coalesced using clinically relevant in vitro and in vivo disease models to truly probe angiogenesis, axogenesis, anti-inflammation, and functional recovery across all treatment conditions of the product. • Aim 1 seems like a repeat of previous grant and publications. Also, the in vitro setting without oxygen glucose deprivation modeling does not replicate the in vivo setting thus the optimization is likely not tested in the best disease model. • Aim 2 should be Aim 1 as this is the innovation of this grant as opposed to the previous CIRM grant of hydrogel development. Again, in vitro studies do not incorporate the disease model of oxygen glucose deprivation, thus not the best cell condition to replicate a clinically relevant in vivo setting. • Aim 3 is the most pivotal set of experiments testing the novel hypothesis of whether the hydrogel improves NPC survival, differentiation, and other cell fate behaviors, yet underdeveloped. Angiogenesis and axogenesis are mentioned briefly as outcome parameters, but whether these outcomes relate to the transplanted cells or host tissue is not clear. Furthermore, the main thesis of anti-inflammation is not mentioned as a parameter. • What is the stroke animal model that will be employed here to appreciate the locus of the infarct, the targeted stereotaxic transplantation, and the cylinder and grid-walk tests? This

	animal modeling is critical because the size/severity/location of the infarct will guide the target location and dose of the gel and NPC to be transplanted to afford functional effects. • For example, if middle cerebral artery occlusion model is used then the striatal lesion cavity more prominent to the transplants would be intrastriatal, but if there is a cortical lesion cavity transplants will be intracortical. Such variation in stroke anatomical location raises the question whether "lesion cavity" is influenced by the brain anatomy and whether the product can similarly sequester inflammation and induce angiogenesis and axogenesis leading to functional outcomes regardless of stroke anatomical location. • Functional/behavioral recovery is introduced as outcome parameters in Aim 3. However, without introducing these parameters in Aim 2, we will never know whether hydrogel alone or hydrogel plus NPC is the main source of therapeutic functional effects in these conditions. • Pitfalls and alternative approaches will need to be spelled out. • Timelines can be more aggressive since Aim 1 appears to be a repetition of the previous grant.
No: 7	• The success of the aims is in question because supportive preliminary results are not provided in the application. Thus, it is not clear whether the proposed experimental plan will generate meaningful results. • The design of the project is not optimal. Functional assays to address the clinical significance of the proposed approach are only proposed for Aim 3, which will commence in year three of the project. • Lack of behavioral assays for measuring improvement in stroke models is a concern.
GWG Votes	**Is the project feasible?**
Yes: 8	• Team is qualified and well-staffed. • All resources required for the successful conduct of the project are in place. • It seems that less than ten animals per group will be used. If those animals are mixed males and females, then it will not be possible to reach any meaningful conclusions regarding male vs female effects. Therefore, either the number of animals should be doubled or one sex be used. • The most important goal is to get meaningful results, and if that means using only males or only females, that is fine. Using both sexes in numbers which will not allow for any meaningful statistical analyses is not justified and is not the meaning of DEI. • Project aims and outcomes are feasible but as noted above should be consistent across all aims.
No: 6	• Not clear that hydrogels will be the big breakthrough, they have been used in previous studies by the PI and others. • This is a highly risky project, and it is unlikely that the goals of the specific aims will be achieved within the proposed timeline. • The qualifications of the team to conduct the proposed work are in question. The publication record of the PI in the field of the study is modest, especially in the last several years. The co-investigator, at 5% effort, will be responsible for cell culture and induction of dopaminergic cortical neural cells. Presumably, these cells will be generated from iPSCs; this is not explicitly stated in the application.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 12	• This is actually not really applicable here as this is a study done on mice. Although the applicants have stated they will use both males and females I have concerns regarding the number of animals used to actually get meaningful results. • To account for the influence of gender, the project will use equal numbers of male and female mice of the same age in each group at every time point. Data will be analyzed as average between values obtained in male and female mice, or as separate groups to assess any differential therapeutic efficacy between male and female. Future clinical studies will include populations of diverse race/ethnicity, gender, age, and socioeconomic status. • There is a section addressing race, ethnicity, sex, gender, and age diversity, including the experimental design (male and female mice), targeted envisioned stroke patient population (all iterations incorporated), and the research team's engagement with the California community.

No: 2	*none*

Application #	DISC0-14543
Title (as written by the applicant)	Effects of antipsychotic drugs on early brain development and cellular function in schizophrenia compared to controls
Research Objective (as written by the applicant)	Do antipsychotic drugs help or hinder brain development? Is susceptibility to schizophrenia affected by antipsychotic drugs during pregnancy? How do antipsychotic drugs help in schizophrenia?
Impact (as written by the applicant)	Antipsychotics (APDs) in pregnancy statistically increase risk of psychiatric illness in vulnerable children at high risk of psychosis. The impact of APDs on the developing brain will be studied.
Major Proposed Activities (as written by the applicant)	• Continue development of induced pluripotent stem cell (iPSC) lines from lymphoblast cell lines collected from controls, and a multiplex pedigree with schizophrenia in Costa Rica. Characterize iPSCs for karyotype and differentiation. • Develop directed cortical organoids from iPSCs in the presence of aripiprazole and olanzapine. Preliminary dose-response curves have shown high dosages of APDs impair organoid development. • Measure impact of APDs on spine and synapse development, mitochondria respiration, mitochondria copy number, and Complex I activity, in directed cortical organoids. • Cell-specific RNA-Seq to analyze alterations in organoids due to APDs. Do APDs increase transcriptomic alterations in family members with schizophrenia compared to unaffected or healthy controls? • Integrative cross-validity analysis of GWAS, postmortem transcriptomics of schizophrenia, and prior published cortical organoid studies. • Compare admixed controls to family members with high genetic risk for schizophrenia on all outcome measures to determine the organoid model variability and effect sizes in this study.
Statement of Benefit to California (as written by the applicant)	Antipsychotic drugs prescribed during pregnancy increase the risk of developing psychiatric disorders in childhood. Further studies of these risks are required. We will study an underserved segment of the population in stem cell research, predominantly admixed Hispanics, to understand the risks of prenatal antipsychotic drugs on brain growth and to ultimately provide better information to pregnant mothers of potential risks of antipsychotic drugs to their child's brain development.
Funds Requested	$1,571,057
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.” 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	64
Median	65
Standard Deviation	9
Highest	75
Lowest	40

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: 10	• The application is motivated by the findings of large meta-analytic studies which indicate that anti-psychotic drugs taken during pregnancy either increase the risk for neurodevelopmental disorders in the offspring or that no data are available. Direct tests in pregnant people have not been conducted. • The applicant addresses an important knowledge gap that potentially affects a growing number of individuals considering that the incidence of mental heath problems in general is rising. • The lack of insight of the impacts of anti-psychotics taken during pregnancy leaves parents without guidance and exposes their offspring to drugs that have not been tested in pregnancy. • Anti-psychotic drugs are widely prescribed to pregnant people without data from clinical trails. Epidemiological studies show differing result on potential effects on offspring, depending on the power of the study. Small studies tend to find that drugs are safe, while large studies describe associations with neurodevelopment disorders in offspring. • The application has the potential for high impact via the establishment of a fast screening platform for adverse effects. • Organoids are a model of choice to study human fetal development. • A classic problem in psychiatric research is the lack of adequate human model systems. If organoids could be deployed as a human model system, this could have benefits for drug development, selection, and basic science of stem cell response to various compounds. • If successful, use of brain organoids in screening for drug effects, or for characterizing their actions in the brain, would encourage other studies to use that model system. Given organoids are an early stage brain model, their use would also cover the action of stem cells. • The hypothesis of this study is not generally supported by prior human research, and is overly broad (covering all antipsychotic drugs) when there are generally negative findings for the complete class with regards to association with neurological disorders. Thus there's likely a risk that if this project does have a major impact, the impact could be misleading because it may be assumed to be representative of an entire class of drugs, when in reality the proposal is only testing two examples in this class with improper controls.
No: 4	• The aim of this study is to use stem cells to assess developmental toxicology of two widely used anti-psychotic drugs. • The background epidemiological data are not sufficient to support an urgent need for the study. • There is no evidence from epidemiology to support neurodevelopmental actions of these drugs. It is unclear how the applications expect to detect meaningful results from a small sample of patients and organoids.
GWG Votes	**Is the rationale sound?**
Yes: 8	• There is compelling evidence of the harmful effects of APDs during pregnancy. • Epidemiological studies cannot address causation, so more direct testing models are needed.

	• A set of appropriate readouts (i.e transcriptomic, bioenergetic, and cellular approaches) are used to test the effects of APDs on healthy and schizophrenia-risk iPSC derived organoids, which represent a major traget population for APDs prescriptions. • The two drugs that will be used in this application have already been associated with gestational maternal diabetes and neurodevelopmental disorders, but their specific impact on the fetal brain is not known.

No:
6
	• The rationale that organoids could offer a more refined screening mechanism for psychiatric drugs is sound.
	• The data on families with schizophrenia are interesting but suggest complex underlying genetics. It is not clear how this study will add to genetic understandings of schizophrenia.
	• Siblings have much less in common in terms of genetic effects on phenotypes than is often thought.
	• Preliminary data on pharmacologically relevant doses of compounds in the model would strengthen the rationale.
	• One cannot predict from clinical epidemiology what the expected organoid phenotype would be, or indeed whether the cortex is the likely anatomical substrate of potential neural developmental issues.
	• There is an absence of clinical data strongly indicative of developmental neurotoxicity for the compounds to be studied. One large study reported a hazard ratio of 1.36 for a range of behavioral and functional endpoints in a large population, for only one of the drugs the applicants will look at. Based on this evidence, the applicants would need to show that their study is powered sufficiently to detect an effect given a hazard ratio of 1.36.
	• The overall hypothesis that brain neurodevelopment will be negatively impacted by the administration of antipsychotic drugs is not supported by published data, which is consistently reported in a misleading fashion and summarized incorrectly in the application. The majority of studies of congenital malformation find no risk for APD's:
	  ◦ On congenital malformation risk, a recent systematic review and meta-analysis ..."suggests that there is no strong evidence to demonstrate an association between prenatal exposure to any antipsychotics or, in particular [small gestational age], and congenital malformations in children." This publication indicates that there is some support for high-risk antipsychotics leading to high birth weight, but no evidence for other APD's in doing so, and no evidence for the more concerning small gestational age (Zixuan Wang British Journal of Clinical Pharmacology 2021).
	  ◦ The largest neurodevelopmental study finds no effect for all but one drug. Notably, that study is explicit that they do not control for multiple comparisons, and the results would not be significant if they did. The conclusions section of the abstract states, "The findings of this birth cohort study suggest that the increased risk of [neurodevelopmental disorder, NDD] seen in children born to women who took antipsychotic drugs late in pregnancy seems to be explained by maternal characteristics and is not causally related with prenatal antipsychotic exposure" (Straub 2022 Jama Internal (https://pubmed.ncbi.nlm.nih.gov/35343998/). One can conclude from this study that there is no evidence that APDs as a class are influencing NDD. This is consistent with the other largest studies that did not break down drugs individually. In the current application, these studies are ignored or are incorrectly summarized as supporting evidence for the whole class of drugs.
	  ◦ According to another recent study (Liu, Jama Internal Medicine 2022): "In this register-based cohort study, maternal prescription fill for antipsychotics during pregnancy did not appear to be associated with standardized test scores in the offspring. The findings provide further reassuring data on offspring neurodevelopmental outcomes associated with antipsychotic treatment during pregnancy." This is a notable study in that it utilizes cognitive testing as opposed to mere diagnosis. This finding does not support the main hypothesis of the current application, and it's findings are not addressed by the applicant.
	  ◦ One hypothesis possibly supported by the data is that aripiprazole specifically has some effect on development. However, even this conclusion is suspect, as that study didn't correct for multiple comparisons.

	• Therefore, the applicant's hypothesis concerning antipsychotics as a class is repeatedly rejected by the literature and thus cannot support the proposed research. One line of investigation supported by the literature would be a study of aripiprazole vs other antipsychotics used as controls and which would be expected to show no effect.
GWG Votes	**Is the project well planned and designed?**
Yes: 7	• The study is well powered, and materials to enable research are available. • General benchmarking (inclusion of an appropriate control of a known neurotoxic drug) is missing, making it difficult to interpret the preliminary data. • The endpoints that will be analyzed seem appropriate but no preliminary data are provided regarding whether these endpoints will be informative. For example, how many spines will be counted per organoid, and from what neurons? The application is missing details to lend confidence that the analysis will show robust effects. • Measurement of oxygen consumption rate and extracellular acidification rate in organoids have been done by others. The applicant only shows oxygen consumption in lymphoblastoid cell lines (LCLs) and not organoids. A brief description of how this will be done would have helped lend confidence on feasibility. • The staining in Figure 6 is confusing, as the panel for one of the three markers seems to show a different organoid than the panel for other markers. In addition, the low dose of one of the tested drugs seems to be associated with a massive growth in the panel labeled with two of the markers compared to controls, but not in the panel for the third marker, and no explanation is provided. The organoids in Fig 6 show no clear ventricles, and the applicants do not describe how ventricle size will be quantified.
No: 7	• Use of single cell RNAseq, differentiation markers, and dendritic and mitochondrial assays are all logical ways to assess the state of an organoid. • It is not clear what the Costa Rica cohort adds to the study. The applicants do not present data to suggest that anti-psychotic medication on top of a disease genotype would add synergistically to risk of developmental abnormalities. • The application does not provide evidence that any postulated effects on neurodevelopment relate to the cortex. • In this revised application the investigators increased the number of non-related individuals in the control group. However, the details of the power analysis supporting this number of individuals are not spelled out. Because unrelated individuals are not going to be isogenic controls, more details related to this power analysis are needed to ensure that it is correct. • The experiments have no meaningful control, because human studies indicate non-effect of the majority of antipsychotic drugs. The applicants could select the most commonly prescribed antipsychotic drug and test it in comparison to another drug such as an SSRI that lacks evidence of affecting fetal brain development. However, the applicants state that they have insufficient funding for this comparison. The applicants should modify their proposal so that this critical negative control can be included. Alternatively, the applicants can include a positive control from a class of drugs that does have robust evidence of impacting neurodevelopment. • In the absence of proper controls, the proposed studies are biased towards declaring all antipsychotics a risk due to potential results with a small number of drugs in this class. • It is not clear that this study will lead to any definitive outcome regarding recommendations around the use of these drugs in pregnancy.
GWG Votes	**Is the project feasible?**
Yes: 10	• Resubmission resulted in an improved application. The applicant is now including 20 iPSC lines (10 male and 10 female) from unrelated controls and 8 age matched lines from high risk schizophrenia individuals. • In Aim 1, organoids will be exposed to drugs, and dose range will be defined by mimicking fetal-maternal circulation levels. The first level analysis will include ventricle formation, size and shape, neurogenesis, and apoptosis. • Aim 2 will identify changes via single cell RNA-Seq cell-specific analysis, dendritic spine morphology, and mitochondrial function.

	• There is no discussion about expected outcomes and challenges in the interpretation of results. For example, what outcomes would be consistent with the drugs being "dangerous," and how many parameters would have to be affected at each dose to place a drug in this category? • The techniques are overall feasible but a lack of preliminary data make it difficult to judge whether the applicant will be able to generate the data. • Investigators are qualified to carry out the work, but would benefit from expertise on developmental toxicology in the team. The application has many issues with positive controls, dosage and timing of drug administration, and proper endpoints.
No: 4	• Anticipating false positives is a key point in organoid research. It is doubtful that this has been accounted for in the proposed study because the investigators have already decided on the conclusion of the study by ignoring the large body of evidence that APD's as a class have no substantial risk of impacting neurodevelopment.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• The project encompasses a particular genetic isolate of Hispanic ancestry. The results will be applicable to patients of Hispanic genetic ancestry. • The study is mainly focused on a multiplex Hispanic family and an additional twenty healthy control volunteers from the same admixed population. While the study addresses under-representation of Hispanics in genetic and stem cell studies, the exclusion of other ethnicities still affects diversity. The applicants could propose to include samples from other ethnicities in future studies. • The applicants have access to diverse collaborators through their participation in a consortium. • The applicant is part of a genomics consortium that is focused on bringing the country-of-origin researchers into collaboration with the applicant's institution. While the investigators are actively working with country-of-origin collaborators in Costa Rica, the description of other efforts to increase diversity are generic.
No: 1	• The connection to the genomics consortium is useful and reasonable. • Although the Hispanic origin samples are commendable, it is possible that this study includes only male participants. • If this study is completed, it may discourage individuals from using necessary antipsychotic drugs, so this may be damaging to underserved populations.

Application #	DISC0-14591
Title (as written by the applicant)	Phenotypic characterization of ALS and control iPSC-derived motor neuron and microglia differentiation via an automated stem cell culturing platform
Research Objective (as written by the applicant)	We seek to improve reproducibility and scalability of stem cell modeling and therapies by understanding the factors that control differentiation of neural cells and disease mechanisms
Impact (as written by the applicant)	Gain knowledge of what factors affect differentiation outcomes, address major bottlenecks in reproducibility and scalability of stem cells, and possibly identify novel therapeutics for amyotrophic lateral sclerosis (ALS)
Major Proposed Activities (as written by the applicant)	• Use an automated workcell to grow and differentiate stem cells into motor neurons and microglia • Collect longitudinal imaging data throughout the differentiation process • Generate single cell RNA sequencing data, identify developmental and phenotypic/functional cell states, and determine regulators of these states. • Determine biological and technical features that regulate stem cell differentiation and functional cell states using correlation and gene perturbation • Use deep machine learning to distinguish undifferentiated cells from differentiated, and classify cells based on morphological states associated with phenotypic/functional state • Validate morphological states associated with phenotypic/functional state using staining and functional assay readouts.
Statement of Benefit to California (as written by the applicant)	California has one of the largest ALS populations. Since California has a unique ethnic diversity as compared to the U.S. and the genetic implications of ALS differ between ethnic groups, it is important to generate induced pluripotent stem cell (iPSC)-derived neural cell types from a variety of patients. Indeed, ALS cases with different etiologies may present different phenotypes. We seek to increase the representation of patients which will increase the chances of developing a therapy for Californian ALS patients.
Funds Requested	$1,181,250
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." 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	64
Median	65
Standard Deviation	6
Highest	75
Lowest	55
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	• iPSC and stem cells are a critical resource for understanding human diseases, yet aspects of high variability and diverse approaches to culture conditions makes it not only difficult to compare data across laboratories but limits power to identify subtle changes that might underlie the development of more dramatic defects over time. Thus a full understanding of the underlying reasons for variability in iPSC derived cells is a current bottleneck. • Promoting the inclusion of iPSCs from donors of different races and sexes is currently based on assumption of differences rather than on scientific reproducible data describing differences. Data generated in this proposal might lead to a better rational for studying cells across races and sexes. • The applicant proposes to identify new methods to improve in vitro culturing, and endpoint analysis that could impact scalability. These are necessary features for developing therapeutics but these tools are not discussed in detail.
No: 6	• The project aims to address a knowledge gap in stem cell research and regenerative medicine. • Specifically, it focuses on the automation of tissue culture and the application of artificial intelligence (AI) and imaging in data analysis. • The project has the potential to impact the field by optimizing current approaches to stem cell research and regenerative medicine. However, it may not necessarily lead to a revolutionary shift in the field, but rather a significant improvement in efficiency and accuracy of existing methods. • Further evaluation of the project's potential impact would depend on the specifics of the methods and results, as well as their relevance to current research needs and challenges in the field. • Scalability and reproducibility in disease modeling are important. However, many laboratories are addressing these issues and many groups are using AI for image analysis, so it is not clear why the applicants' approach has particular advantages. For example, a recent study in Neuron assessed many variables in modeling ALS across a large cell line panel. • Widespread application of the findings would require access to expensive specialist equipment and proprietary software, and it is not clear if the applicants' software will be open source. • It is not clear that this system will be made widely available and applicable to different research questions.
GWG Votes	**Is the rationale sound?**
Yes: 7	• The focus on motor neurons and microglia in ALS is reasonable considering the availability of many different cells lines and the observation that both cell types display intrinsic phenotypic differences. • The applicants have identified a specific area of interest within the field, namely microglia and motor neurons for ALS studies, and have discussed the potential significance of their project in addressing relevant questions and gaps in this area. • The project's rationale is based on a sound understanding of the current challenges and limitations in stem cell culture (reproducibility). • The proposed automation and data analysis methods are well-founded and have the potential to significantly improve the efficiency and accuracy. • The project's long-term approach is also justified, given the complex nature of in vitro culture and differentiation system.
No: 7	• We already know that iPSC-derived cells are variable and the expectation would be that the proposed approach could identify specific factors and patterns that could underlie

	variability in respect to gender or race. However, this is not addressed, and it remains unclear to what extent the collection of differences the applicants will observe would be informative of novel approaches to achieve homogeneous cultures. • The proposal lacks performance data for the automated cell culture system to be employed. Rather, the data provided documents successful implementation of established protocols in the applicant's lab. Preliminary data are not provided on AI morphological analysis. • The preliminary data show mainly diagrams of aspirational outcomes, not actual results.
GWG Votes	**Is the project well planned and designed?**
Yes: 5	• The experiments are well-described and there is a clear logical order to them, suggesting a carefully thought-out plan. • The design includes a clear definition of the research question and hypotheses, which will help guide the experimental design and analysis • The project team has chosen appropriate methods and tools to carry out the experiments, suggesting a strong understanding of the research topic and available resources. • The project team appears to have thoroughly considered alternatives and potential pitfalls, indicating a well-rounded approach. • The timeline for the project suggests a sense of urgency, which could be due to the importance of the research topic or external factors such as funding constraints.
No: 9	• This is an open-ended analyses of many cell lines using established parameters, and the impact is not clear. • The development of the automated system is not clearly described. The applicants also do not describe how it differs from the available Monomer system. • The assumption that spontaneous versus directed differentiation into induced microglia (MG) and induced motor neurons (iMN) can be easily distinguished by their classifiers is not supported by any preliminary data. • Details are missing and no examples of stains or images are provided. For example, the image in Figure 2 is not informative, as it is not clear whether these are clumps of cells or mixed organoids. • The choice and rational of time points in Aim 2 are not described, nor are the resolution of images and quantification of morphological features using generic dyes. • Aim 2 is very broad. Previous work has characterized these differentiation pathways, so it is not clear what this proposal is adding in this regard. • For Aim 3.2, negative controls need to be included, such as iPSC that do not show changes in phenotypic state. • The correlation of staining, scRNA-seq, and morphological data might be powerful, but it is not clear how these data will actually be integrated. It is also not clear how motor neuron or microglia function will be assessed, and this is critical to interpret the results. • The fallback plans mostly comprise reverting to existing methodologies/protocols. • The planned use of only 10 cell lines will not provide any confidence in the scalability of this system.
GWG Votes	**Is the project feasible?**
Yes: 7	• The project appears to be feasible, as the design is well-thought-out and the methods chosen are appropriate for the research question. • The project team is fully qualified to carry out the work, with expertise in the relevant areas of research and experience with the experimental techniques and methods used. • The institution has the necessary infrastructure to support the project, such as laboratory space, equipment, and computing resources, which suggests that the project can be carried out with minimal additional investment. • The chosen research topic is within the scope of the applicants' expertise and resources, further supporting the feasibility of the project.
No: 7	• While the applicant will be able to identify changes across cell lines that are cultured in identical conditions, the variability that might be due to changes in protocol that can in turn affect purity of cells, differentiation efficiency, viability, and similar parameters are not discussed. This approach will only address intrinsic variability but not variability due to extrinsic factors as originally stated. • Pre-defined cut-offs for determining what constitutes changes observed in the system are not clear.

	• Automated systems can be challenging to establish, and this proposal includes little in the way of preliminary data to suggest the platform can do what the applicants hope it can. • The applicant has built the automated platform that has an incubator, liquid handling operations, and daily imaging capabilities but other features (remote manual decision making, and collection of phase-contrast and immunofluorescence data using this platform against manually differentiated cells) have not been established. Therefore the project seems to be in the very early stages and feasibility is not clear. • The timeframe is unrealistic and the request for the full budget in year 1 is not justified. • The timeline is not feasible.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 11	• The project has the potential to address the impact of sex, ancestry and ethnicity on ALS relevant cell types. • The project might extend or validate the applicability of regenerative medicine discoveries to underserved populations, including underserved racial/ethnic communities, given that this method will allow investigation of very nuanced phenotypes.
No: 3	• The proposal does account for the influence of race, ethnicity, sex, gender, and age diversity, but this could be expanded. • Prior efforts or proposed plans for outreach, partnership, or educational activities to inform the development of DEI within the research project were not addressed. • DEI efforts are not addressed. • This project only employs 10 cell lines, and their distribution across male/female donors or diversity in genetic ancestry are not provided. Plans for outreach are vague.

Application #	DISC0-14510
Title (as written by the applicant)	Engineered nanotechnology for neural progenitor cell transplantation in Sanfilippo syndrome
Research Objective (as written by the applicant)	To develop a stem cell therapy for Sanfilippo B syndrome.
Impact (as written by the applicant)	There is no treatment for Sanfilippo syndrome, and other therapeutic approaches have failed in clinics. This proposal will develop a stem cell based therapy for Sanfilippo syndrome.
Major Proposed Activities (as written by the applicant)	• Generation of universal donor Embryonic Stem Cells (ESC) using state of the art genome editing technique. • Increase the level of the missing enzyme in universal donor ESC using state of the art genome editing technique. • Differentiate ESC into brain stem cells in vitro capable of secreting NAGLU (NAGLU-NPC). • Transplantation of NAGLU-NPC to evaluate if the cells can survive in the mouse brain and can repair brain tissue provide NAGLU enzyme. • Transplantation of NAGLU-NPC to evaluate if cells differentiate into functional neuron and integrate in the neuronal networks. • Transplantation of NAGLU-NPC to evaluate if cells can repair brain tissue and correct abnormal mouse behavior associated with Sanfilippo syndrome.
Statement of Benefit to California (as written by the applicant)	This application will help develop a stem cell therapy for Sanfilippo B disorder, a pediatric genetic disorder that currently has no treatment. If successful, this approach could be extended to several other lysosomal storage diseases, bringing a therapy for these catastrophic disorders.
Funds Requested	$1,605,000
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." 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	63
Median	60
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	• Sanfilippo syndrome is a severe disease with enzyme deficiency. Gene therapy has been attempted. This project tests the hypothesis: can neural progenitor cells (NPCs) bring the enzyme to the brain? • The project develops and utilizes NPCs that secrete NAGLU (the missing enzyme in Sanfilippo) to correct brain pathology. The therapeutic is intended as a complimentary treatment to gene therapy. • The hypothesis is that successful treatment for Sanfilippo syndrome requires both local delivery of NAGLU to the brain and gene therapy. • There is today a lack of treatment for cognitive decline in Sanfilippo syndrome. • The development and use of fabrication and cell engineering steps may extend the impact of this project.
No: 5	• The proposal addresses cell therapy for a rare lysosomal storage disorder for which there is no existing treatment. Gene therapy, hematopoietic cell transplants and enzyme therapy have been unsuccessful; use of neural stem cells has failed for multiple reasons that this project aims to address. • The study has flaws that will likely limit its impact on this disorder.
GWG Votes	**Is the rationale sound?**
Yes: 5	• Yes, the project is based on a solid scientific rationale. • There is a sound rationale behind the hypothesis that successful treatment for Sanfilippo syndromes requires both local brain delivery and gene therapy. • However, the applicant does not provide a clear rationale attempting to promote cell survival via use of a hydrogel. • Preliminary data for the methodology are presented.
No: 8	• The concept behind the proposal is the use of a hydrogel to promote survival, migration and immune protection for transplanted NPCs. It is not clear how this approach would lead to wider biodistribution of the NPCs in the host brain, which appears to be a key limiting factor of current therapies. • The evidence provided to demonstrate the hydrogel's modulation of immune response and neurogenesis comes from a stroke model and does not look at effects of engrafted cells. The hydrogel's effects are in most cases modest. • How will the transplanted cells survive and migrate once the hydrogel degrades? • What will heparin nanoparticles achieve in this context? • The rationale for facilitation of migration using hydrogels is not well developed. • Encapsulation may not help migration in the brain.
GWG Votes	**Is the project well planned and designed?**
Yes: 5	• Overall, yes, but the hydrogel may not work in vivo in humans. How does/will the applicant assess the fate(s) of transplanted NPCs in vivo? • Potential pitfalls and contingency plans are presented for the main strategy. The use of other cells as delivery vehicles is not considered. • Project plan and timeline demonstrate an urgency that is commensurate with CIRM's mission. • Pitfalls are not adequately acknowledged. • The project is well designed.
No: 8	• The project is very interesting and important. However, I have serious concerns about its design. It seems that many different experiments have been proposed to cover more ground. It would be better to streamline the project better, concentrating on the most important steps and doing them properly. • The investigators will look at many variables concerning hydrogel composition, but these will only be assessed in vitro. It is not clear how they will impact cell distribution or fate in vivo. • How will fate of NPC be assessed in vivo? GFP marker, differentiation markers? Do NPC continue to secrete enzyme at high levels following differentiation? Should NPCs alone be used as a control?

	• The applicants plans for assessing the fate of transplanted NPCs are not clear. • It will be difficult to understand the origin of the problem (i.e., what is wrong with hydrogel design) if poor results are obtained in the animal tests in Aim 2 and 3. • The applicant has not considered the limitations of the scale of small animal models in their approach to improving biodistribution. • Limitations of scale are not adequately considered. • Only some of the potential pitfalls are addressed and those, not adequately so. • Pitfalls are not adequately addressed.
GWG Votes	**Is the project feasible?**
Yes: 6	• Yes, but the project is very ambitious for the proposed 3 years. I have concerns about its potential to be achieved within the proposed timelines. • The project is feasible. • The team has the necessary expertise. • The team has access to all the necessary resources to conduct the proposed activities. • The budget appropriate for the research proposed.
No: 7	• Preliminary data show that NPCs can be engineered to secrete enzyme and that the group has the capacity to tune hydrogel properties. Some preliminary data indicate better survival of NPCs in hydrogel in vivo. • This is an interdisciplinary team including two well-qualified early career researchers with expertise in stem cell therapy and bioengineering. • Many experiments are proposed without prioritization. • The project outcome is likely to be incremental. • There are a number of aspects that require better strategy. • The team is good.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 7	• The applicant describes prior efforts or proposed plans for outreach, partnership, or educational activities to inform the development of DEI within the research project. • However, the selection of cells for this project does not account for the potential importance of diversity. • The applicant's response has limited details.
No: 6	• The DEI statement is poorly considered. Although patients with rare disorders are sometimes neglected, this is not what is captured in CIRM's DEI policy.

Application #	DISC0-14504
Title (as written by the applicant)	Promoting differentiation of adult stem cells for the treatment of Multiple Sclerosis and other demyelinating diseases
Research Objective (as written by the applicant)	Our approach using repurposed drugs to selectively drive differentiation of oligodendrocyte progenitor cells (OPCs) at sites of demyelinated lesions to enhance remyelination may have a significant impact on new Multiple Sclerosis (MS) treatments.
Impact (as written by the applicant)	A major issue in Multiple Sclerosis is the inability of human adult stem cells at the oligodendrocyte progenitor stage to further differentiate into terminal mature oligodendrocytes.
Major Proposed Activities (as written by the applicant)	• Determine common gene pathways leading to oligodendrocyte progenitor cell maturation. • Determine whether reactivated T cells or other immune cells provide the ligand which blocks oligodendrocyte maturation. • Evaluate potential combination therapies with repurposed drugs and current first line MS drugs.
Statement of Benefit to California (as written by the applicant)	MS is a devastating autoimmune disease that affects over 250,000 Californians and nearly a million Americans. Though immunosuppressive therapies can significantly delay the severity of the disease, a cure remains elusive and immunosuppressive drugs need to be administered for life. Current drugs have been associated with significant toxicity and eventually usually fail. If the studies proposed here are effective in animal models that translate to humans, a cure is envisioned.
Funds Requested	$1,920,000
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." 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	59
Median	60
Standard Deviation	7
Highest	65
Lowest	45
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	• The proposal address key gaps of knowledge in how OPSc mature into myelinating oligodendrocytes. • The results could lead to new therapeutics for MS. • Can be applied more broadly in demyelinating disorders. • The proposed project is based on a hypothesis that it is not the failure of numbers or migratory ability of OPCs, but rather inhibition of OPC differentiation at sites of demyelinating lesions that is associated with disease progression. • In its current form, the proposed project does not address a major bottleneck in the field. The design of this project is not likely to offer a transformative effect but rather a stepwise improvement.
No: 5	• The project aims to identify small molecules, their doses and combinations that will promote myelin regeneration in animal models of MS. • However, the experimental approach will be unlikely to address this bottleneck and is instead likely to provide only an incremental increase in our knowledge. Similar molecules have already been studied in the context of animal models of MS and have already made the translation into human clinical trials. • Clinical data are not discussed; findings in the literature are not integrated. • Differentiation of oligodendrocyte progenitor cells is reasonably well understood and the new insight is not clear.
GWG Votes	**Is the rationale sound?**
Yes: 8	• The project sets out to gain understanding of the signals that drive OPC maturation into myelinating oligodendrocytes and test repurposed drugs as a potential therapeutic. • The project is based on the hypothesis that it is the inhibition of OPC differentiation at sites of injury rather than migration that is the cause of demyelinating lesions. • Very extensive preliminary data on in vitro parts but not on the xenograft model. • Largely yes, however most of the study is based on a 10 years old study with little evidence of innovation.
No: 6	• The rationale is somewhat dated. There have been many studies that have identified small molecules capable of enhancing oligodendrocyte differentiation. The approaches described are not particularly novel and likely do not model the inhibitory environment present in chronic MS lesions. • Based on outdated models and information. • There is an incomplete/selective literature review that omit key papers that have elucidated the mechanisms of action of similar small molecules. • Human iPSC data is weak. The rigor of the human iPSC cultures is poor. O4 staining does not appear reliable and appears to label cells with non-oligodendrocyte lineage morphology. No other markers are provided, no quantification. • No preliminary data support the rationale for Aim 1.3. • There are no preliminary data provided pertaining to the studies that will examine the mechanism of action of drugs on human OPCs. What is the purity of these cells? What proportion undergo differentiation spontaneously, following small molecule application?
GWG Votes	**Is the project well planned and designed?**
Yes: 5	• The project is well designed. Mechanistic studies will be carried out in vitro and lead candidates will be tested in animal xenograft model of MS. • The project plan and timeline demonstrate an urgency that is commensurate with CIRM's mission. • Very extensive preliminary data are provided on in vitro parts but not on the xenograft model which is the difficult, but also most rewarding part. Alternative mouse models are discussed but preliminary data would be recommended. • This project proposal is quite difficult to follow. While the rationale is clear, it seems that the entire project is based on a 10 year old study - one wonders why that study has not been appropriately followed up since then. • This project seems to have two parts that are not well aligned; characterization and drug testing. While this is not a problem per se and is often done, here it is not clear how they are separated. Furthermore, this is not a very novel approach and a successful outcome, although important, is not likely to be transformative for the field.

No: 9	• The profile of differentiating OPCs and oligodendrocytes has been studied many times. The work proposed in Aim 1.1 and 1.2 is unlikely to yield a scientific impact. • Preliminary data is poor and methods are somewhat outdated. • Assessment of remyelination is not sophisticated. The approach using staining analysis of mature oligodendrocyte numbers cannot reliably distinguish generation of new oligodendrocytes vs. promoting survival of existing oligodendrocytes. • Description of individual experiments in the approach is vague. It is often hard to define what the experiments will be. What is the hypothesis, and how will this be tested? • The study of drug treatment as a prophylactic in the proposed animal model is not relevant for studies of myelin regeneration. In the literature, most effects have been attributed to alterations in the immune response. • Limitations are not well discussed.
GWG Votes	**Is the project feasible?**
Yes: 7	• The proposed team is appropriately qualified and staffed. • The team has access to necessary resources. • The budget is appropriate for the research proposed. • The project is feasible to carry out. The biggest risk is the in vivo model. • The proposed aims and experimental set up are not easy to follow. Furthermore, a project of this scale is hardly achievable within the proposed time frame.
No: 7	• No preliminary data provided that show the feasibility of transplant studies proposed in Aim 2.3. How will rejection of xenografted cells be prevented?
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 10	• Project outcomes extend or validate the applicability of regenerative medicine discoveries to underserved populations, including underserved racial/ethnic communities. • Partially so; addresses and accounts for the influence of race, ethnicity, sex, gender, and age diversity.
No: 4	• Unclear. Will any of the iPSC lines to be used be sourced from underserved populations? If not, what is the rationale for their selection? • Very generic, not appropriately addressed.