APP #	TITLE	BUDGET REQ	FUND?	SCORE (MEDIAN)	Mean	SD	Low	High	Y	N	Resubmission	Previous CIRM Funding	Area of Impact
DISC0-13901	Drug Discovery for Dilated Cardiomyopathy using Patient-Derived Human iPSCs	**$1,350,000**	**Y**	**91**	92	2	90	95	14	0	N	N	Development of a model of dilated cardiomyopathy for future drug discovery
DISC0-13757	Drivers of trophoblast stem/progenitor cell identity in human placenta	**$993,881**	**Y**	**90**	92	3	85	99	15	0	N	N	Understand human placental development; formation of the maternal/fetal interface during pregnancy
DISC0-13801	Control of OCT4 abundance and function in human stem cells	**$1,415,301**	**Y**	**90**	89	1	86	90	15	0	N	N	Understand how stem cells maintain identity to more effectively generate iPSC
DISC0-13914	Developing a Human Model of Sporadic ALS Using Machine Learning and Robotic Microscopy	**$1,406,622**	**Y**	**90**	89	1	85	90	15	0	N	N	Create an iPSC model of ALS for future drug discovery
DISC0-13765	Engineering AAV capsids for transduction of neural and muscle stem cells	**$999,999**	**Y**	**90**	88	3	80	93	14	1	N	N	Identification of viral capsids that can better target neural and muscle cells for gene therapy
DISC0-13788	Modulation of human alveolar stem cells to promote lung regeneration and avoid pulmonary fibrosis	**$1,626,001**	**Y**	**89**	87	4	80	92	12	3	N	N	Understand how molecular regulators of lung stem cell function might promote lung regeneration
DISC0-13875	Developing a microglia replacement therapy	**$1,577,979**	**Y**	**87**	87	2	85	90	15	0	N	N	Develop an approach to restore microglia function in the brain
DISC0-13750	Generation of cortical organoids with tunable areal identities by spatial engineering of morphogens	**$1,497,032**	**Y**	**86**	86	1	85	88	15	0	N	N	Create organoids representing specific cortical regions to model disease for future therapy development
DISC0-13816	Towards a trophectoderm stem-cell model representing human blastocysts of the highest implantation potential	**$1,584,000**	**Y**	**85**	85	4	74	90	12	3	N	N	Understand the molecular determinants of successful human blastocyst implantation
DISC0-13806	Development of universal off-the-shelf iPSC derived dendritic cells for use as patient specific anti-tumor vaccine	**$1,625,998**	**Y**	**85**	85	3	80	90	7	7	N	Y	Proof of principle studies for developing a dendritic cell vaccine for cancer treatment
DISC0-13808	Development of a stem-cell based approach to interpret global effects of genetic variants contributing to neurodevelopmental disease risk	**$1,518,982**	**Y**	**85**	84	1	80	85	8	7	N	N	An approach to better understand the impact of genetic variants of key regulatory genes involved in neurodevelopment disorders
DISC0-13937	Plasticity and Endogenous Regeneration in Dental Injury and Repair	**$1,346,851**	**N**	**82**	81	2	76	85	3	12	N	N	
DISC0-13784	Establishment of a novel approach to systematically study the dynamic organization of protein complexes in stem cells	**$1,515,601**	**N**	**80**	83	3	80	91	5	10	N	N	
DISC0-13735	Decoding human embryo development in 3D with optogenetics	**$1,000,000**	**N**	**80**	82	4	76	87	4	11	N	N	
DISC0-13822	hPSC-derived enteric ganglioids for cell therapy in gastrointestinal motility disorders	**$1,589,307**	**N**	**80**	81	1	80	82	0	15	N	N	
DISC0-13810	Defining the source of dysfunction in monogenic Intellectual Disability Syndrome neurons	**$1,500,337**	**N**	**80**	80	3	70	86	1	14	N	N	
DISC0-13823	Using Human Neurons to Model Parkinson's Disease and Develop Therapeutics	**$1,578,001**	**N**	**80**	80	2	75	84	0	15	N	Y	
DISC0-13697	Targeting adipocyte progenitor cells to treat age-associated obesity	**$999,924**	**N**	**80**	79	2	75	82	0	15	N	N	

APP #	TITLE	BUDGET REQ	FUND?	SCORE (MEDIAN)	Mean	SD	Low	High	Y	N	Resubmission	Previous CIRM Funding	Area of Impact
DISC0-13706	Overcoming barriers for airway stem cell gene therapy for Cystic Fibrosis	**$1,472,857**	**N**	**80**	79	3	74	83	0	15	N	N	
DISC0-13748	Role of eIF4G2, a putative regulator in translation initiation, in pluripotent and somatic stem/progenitor cells	**$1,739,760**	**N**	**80**	79	4	70	84	0	14	N	N	
DISC0-13780	Neural Stem Cell Aging and Neurodegeneration	**$1,574,807**	**N**	**80**	79	1	75	80	0	15	N	N	
DISC0-13705	Functional genomics to study cellular convergence across ASD risk genes in neurodevelopment	**$1,577,425**	**N**	**80**	78	4	70	83	0	15	N	N	
DISC0-13726	Stem Cell-Based Bioengineered Therapies for Kidney Failure	**$1,280,388**	**N**	**79**	78	8	65	90	5	10	N	N	
DISC0-13804	Investigating the Role of Microglia in Autism Spectrum Disorder Using Patient-Derived hiPSCs in Culture and Cerebral Organoid Models	**$1,842,358**	**N**	**77**	77	4	70	80	0	15	N	N	
DISC0-13918	Decoding Firefox: A New Stem in Cancer Stem Cells	**$1,399,886**	**N**	**76**	75	6	65	83	0	15	N	N	
DISC0-13926	Establishing an organoid-based preclinical model of epilepsy and neuronal network dysfunction	**$1,224,585**	**N**	**75**	76	3	70	82	0	15	N	N	
DISC0-13795	Characterization and Evaluation of Gene-Editing Efficacy to Correct RAG2-Dependent Omenn Syndrome	**$1,578,000**	**N**	**75**	75	4	70	80	0	15	N	N	
DISC0-13707	Fundamental disease-driving features of human astrocytes	**$1,560,000**	**N**	**75**	74	3	70	80	0	15	N	N	
DISC0-13789	Single stem cell polymeric encapsulation to improve and elucidate mechanisms of stroke recovery	**$1,577,996**	**N**	**75**	74	3	65	75	0	15	N	N	
DISC0-13834	In vivo engineering of immune cells for cancer therapy	**$1,584,001**	**N**	**73**	72	3	65	75	0	14	N	N	
DISC0-13769	Expression of Extremophile Genes for Increased Stress Tolerance of Stem Cells	**$1,178,539**	**N**	**70**	72	6	60	85	1	14	N	N	
DISC0-13899	Elucidate the role of MSH3 in repeat instability and neurodegeneration in Huntington's disease.	**$1,651,428**	**N**	**70**	72	3	70	78	0	15	N	N	
DISC0-13763	Targeting pluripotency-related DPPA2/4 cancer functions	**$1,609,873**	**N**	**70**	69	3	65	75	0	15	N	Y	
DISC0-13689	ESPRESSO Phenotyping enables the study of neural stem cells phenotypic heterogeneity and its link to spinal cord injury transplantation efficacy	**$1,366,077**	**N**	**70**	68	4	60	75	0	15	N	Y	
DISC0-13869	Dissecting the molecular determinants of metastatic breast cancer stem cells	**$1,519,219**	**N**	**70**	68	4	60	75	0	15	N	N	
DISC0-13783	Cell Biology of Induced Pluripotent Stem Cells	**$1,574,808**	**N**	**65**	64	3	55	70	0	14	N	N	

APP #	TITLE	BUDGET REQ	FUND?	SCORE (MEDIAN)	Mean	SD	Low	High	Y	N	Resubmission	Previous CIRM Funding	Area of Impact
DISC0-13923	The influence of human neural stem cells on autoimmune and regenerative function in mouse models of multiple sclerosis	**$1,549,209**	**N**	**65**	64	3	60	70	0	15	N	Y	
DISC0-13805	Evaluating the role of ancestry in chronic liver disease using human induced pluripotent stem cells	**$1,521,473**	**N**	**60**	61	4	50	65	0	15	N	N	
DISC0-13852	Therapeutically exploring transplanted stem cell death	**$1,357,039**	**N**	**60**	61	6	50	70	0	15	N	N	
DISC0-13779	Immune Tolerization to AAV Capsid for Gene Therapy	**$1,578,000**	**N**	**60**	58	6	50	70	0	15	N	N	

Application #	DISC0-13901
Title (as written by the applicant)	Drug Discovery for Dilated Cardiomyopathy using Patient-Derived Human iPSCs
Research Objective (as written by the applicant)	Our team will use patient-derived stem cells to discover safe and effective drugs for cardiovascular disease.
Impact (as written by the applicant)	The use of patient-derived stem cells to discover novel drug targets for cardiovascular disease, identify drug candidates, and establish a clinical trial in a dish to evaluate drug safety and efficacy.
Major Proposed Activities (as written by the applicant)	• Use patient-derived induced pluripotent stem cells (iPSCs) to generate different cardiac cell types. • Identify drug targets that cause cardiovascular disease using iPSC-derived cardiac cell types. • Identify drug candidates from doing a virtual screening of chemicals. • Evaluate and modify drug candidates to improve safety and/or efficacy for humans. • Validate drug candidate for safety and efficacy in patient-derived human iPSC cell models. • Validate drug candidate for safety and efficacy in structurally complex 3D cardiac organoids.
Statement of Benefit to California (as written by the applicant)	Dilated cardiomyopathy affects ~1 in 2,500 people and often leads to heart failure. The discovery of novel drugs that are safe and effective will be life-saving for many worldwide, including California citizens. Our team is also based in California and has actively recruited patients from California to generate induced pluripotent stem cells. We also collaborate with a California university and California-based companies.
Funds Requested	$1,350,000
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: 91

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	92
Median	91
Standard Deviation	2
Highest	95
Lowest	90
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	14
(1-84): Not recommended for funding	0

KEY QUESTIONS AND COMMENTS

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

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 14	• LMNA-related dilated cardiomyopathy (LMNA-DCM) is among the most prevalent forms of inherited heart disease. This well-presented proposal uses the most innovative technologies including human patient iPSC lines, high throughput screening for druggable targets, and structure-based molecular docking for identification of candidate small molecule drugs. Subsequent studies include structural optimization and validation in iPSC-based models. • This proposal aims to identify new drugs for treating LMNA-DCM using induced pluripotent stem cell (iPSC)-derived cardiomyocytes (CMs). If successful, the project will address a major bottleneck in the field. • This project will elucidate the cardiac cell secretome and identify small molecules that target secreted proteins. Thus, it will have a big impact in the stem cell field. • Identifying mutation-specific pharmacotherapies is highly impactful and is a significant area of need. • If successful, this project will contribute to the advancement of world class science. • Not only does this project have the potential to find potential pharmacological therapies for LMNA-DCM, but it will also significantly advance the field of iPSC technology, accelerate drug discovery and development, and help de-risk severe adverse events in clinical trials. • No concerns.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 14	• Yes. Using patient iPSC-derived cardiac cells to study diseases and potentially find drugs for treating diseases has a sound scientific rationale. • The rationale is based on strong previous work from the investigators, demonstrating proof of concept. • The applicant's preliminary results indicate that normal and DCM iPSC-derived cells can be distinguished based solely on their secretory profiles, e.g. by the release of key signaling molecules such as IL6/8, TNFα, MCP1, LIF, and VEGFA. • Strong preliminary data support feasibility of each proposed aim and capability of the research team to accomplish the proposed studies. • The applicant has established ten patient-derived iPSC lines specifically for this project. • This project is significantly relevant to cardiac disease research and treatment. • DCM is a serious problem and this project has high potential to find a specific therapeutic. • The workflow proposed here will advance the field of drug discovery. • No concerns.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**

Yes: 14	• The overall design is excellent with stepwise logical interrogation of the secretome and exosome profile of patient-derived cells, followed by validation and small molecule design.
	• The planned Aims are appropriate and should give meaningful results.
	  ◦ Aim 1: Elucidate cardiac secretome and exosomal miRNAs in LMNA-DCM using iPSC- derived cardiac cells to identify druggable targets.
	  ◦ Aim 2: Identify small molecules that modulate targets using structure-based molecular docking to computationally screen compound libraries.
	  ◦ Aim 3: Validate compounds for safety and efficacy using iPSC-derived cardiac cells to determine drug candidates.
	• The project plan is logical and highly innovative; feasibility of each aim is supported by strong preliminary data.
	  ◦ The project starts with high throughput screening assays utilizing human patient iPSCs lines to find druggable targets;

	• Then the applicant will use a structure-based molecular docking in silico approach to identify candidate small molecule drugs; • Next the applicant will use chemical synthesis and structural optimization to improve the best candidate(s); • Finally, they will validate the efficacy of the resultant compounds in iPSCs models. • Yes, but the alternative approaches are weak. For example, the applicant claims that they do not see major differences between healthy donor and patient-derived iPSC-cardiac cell secretomes, this will be due to cell immaturity. I'm not convinced that cell immaturity would affect the differences. Also are there other possible explanations? • I don't see a clear strategy for choosing the top target candidates (from vast proteomic and transcriptomic datasets) for further development. • The strategy for target prioritization is not described. • The timeline is reasonable. • No concerns.
No: 0	*none*
GWG Votes	**Is the project feasible?**
Yes: 14	• It may not be feasible to complete differentiation of all ten patient-derived iPSC (and ten healthy donor-derived control iPSC) to all three cardiac cell types, and make tissues. • It is important to recognize that stem cell differentiation varies a lot between different iPSC lines. Some lines may have very low yield for certain cell/tissue lineages. • This will require a lot of time for re-optimization. • The team is qualified for this type of work. • The team has access to the necessary resources for this research. • The aims and expected outcomes are logical. Based on the track record and experience of the team, this project can be achieved in a timely manner. • Yes, based on strong preliminary data and the expertise of the team. • The proposed budget is too high. $600,000 total - i.e., $200,000 per year for three years - is more reasonable. • No concerns.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• They will plan to schedule monthly outreach drives, to be held in neighborhood community centers and places of worship in the greater area to identify a broad representation of potential participants. • The applicants described their approach to diversity in great detail, in particular providing information on the numbers of iPSC donors from various ethnic, racial, gender backgrounds. • Yes; excellent emphasis on diversity in cell lines from diverse patient backgrounds. • Strong DEI section.
No: 0	*none*

Application #	DISC0-13757
Title (as written by the applicant)	Drivers of trophoblast stem/progenitor cell identity in human placenta
Research Objective (as written by the applicant)	This proposal will elucidate the mechanisms that identify a stem/progenitor cell population in the human placenta.
Impact (as written by the applicant)	This proposal addresses our current knowledge gap on human placental development related to formation of the maternal/fetal interface during pregnancy, and placenta-associated pregnancy complications.
Major Proposed Activities (as written by the applicant)	• Identification of DNA regions, called enhancers, regulating placenta stem/progenitor cell-specific identity • Identification of novel proteins regulating placental stem/progenitor cell-specific identity • Construction of a reference for human placenta stem/progenitor cells in uncomplicated pregnancies • Identification of abnormal mechanisms in placental stem/progenitor cells affected by Trisomy 21
Statement of Benefit to California (as written by the applicant)	California has a significant racial/ethnic diversity, with 14.5% Asian and 5.8% Black or African American. Racial/ethnic minority groups are at higher risk of pregnancy complications due to placental defects. This proposal will result in the development of strategies for prevention, detection, and intervention for various placental disorders, decrease pregnancy loss, and improve pregnancy outcomes, with impact on the physical/mental well-being as well as economy across Californian citizens.
Funds Requested	$993,881
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	92
Median	90
Standard Deviation	3
Highest	99
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 the epigenetic mechanisms driving the transcriptional program of trophoblast stem/progenitor population in early human placenta, thus addressing a major bottle neck in our current knowledge. • If successful, this project will have at least two major impacts: (1) predict epigenetic mis-regulation driving abnormal trophoblast stem/progenitors in various placental defects and (2) validate and optimize in vitro models. • This will strongly contribute to world class science and represents a major step forward. • Defects in placental development may cause serious disorders of pregnancy and these are not evident in animal models, probably because of species-specific differences in placental development. Therefore filling this gap in our knowledge is essential for understanding mechanisms underlying preeclampsia and other pregnancy disorders. • The project focuses on characterizing mechanisms of placenta formation in natural human pregnancy and using this information as a baseline for comparison with in vitro models of placental development. • The study clearly defines and addresses this significant gap in our knowledge and the potential application of stem cell models in the future. • Appropriate use of precious materials and the knowledge gained from them could significantly contribute to the field. • CIRM is in a special position to support this critical research on human embryonic and fetal tissue, which is under threat in the current legal/political climate.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 15	• This rationale is sound and a good case is made for the work that has been proposed. • Very strong rationale, including the fact that animal models display species-specific attributes in placentation and do not display similar pregnancy complications. Thus, a human signature is required for both clinical applications as well model validation. • The project is directly relevant to human biology and disease. • Strong preliminary data on trophoblast stem cells from in vitro models. • Preliminary data is minimalistic given the nature of the project but taken together with the expertise of the PI in placental development sufficient to support the feasibility of the proposed project. • Project aims to discover molecular regulation of human trophoblast stem cell identity. It is clear that these cells exist, but they have not been well characterized at the epigenetic level in vivo. Epigenetic abnormalities can affect placental development. • There are well-described human-relevant in vitro models including those derived from pluripotent stem cells. However the authors rightly insist that the in vitro data are impossible to interpret without detailed knowledge of the corresponding processes in vivo to use as a gold standard reference point. • The authors therefore plan to study some specific mechanisms which they hypothesize to be important with several cell lines.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 15	• Acquiring basic knowledge about our own development is urgent. • The project is straight forward and well designed with 3 specific aims. The first two aims are basic biology and the last targets the clinical situation of trisomy 21. • The budget rationale is appropriately justified. • Some pitfalls and alternative approaches have been identified, although the major aspect is the abundance of the biological material. • Access to earlier material would be valuable. • Potential difficulties with CHIP-seq acknowledged. • Plans for dealing with large numbers of candidate distal regulatory elements not well developed.
No: 0	*none*
GWG Votes	**Is the project feasible?**

Yes: 15	• Well designed project that will yield valuable information on human trophoblast development. • The aims and expected project outcome will be achieved in the proposed timeline. • The work is certainly feasible within the timeframe of the project. • PI is an early career stage investigator with expertise in trophoblast biology, has a strong publication record in this field, and is experienced in molecular biology. A collaborator is a computational biologist and head of a computational biology center. • The proposed team is extremely well qualified and well staffed. • The institution's excellent research facilities and its proximity to two other institutions uniquely qualifies them to accomplish the goals of this project. • The budget is appropriate. • More dedicated bioinformatician time might be helpful.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• This proposal goes to great lengths to adequately address and account for the influence of race, ethnicity, sex, gender and age diversity. • DEI addresses the patient population; underrepresented minorities have a susceptibility to high risk pregnancy including placental pathologies. • This project's outcomes validate the applicability of regenerative medicine discoveries to underserved populations. • Study population comes from a clinic with high Hispanic representation. • PI has demonstrated various types of prior and on going outreach in the form of monthly discussions with their team on topics related to DEI and the team participates in other forms of educational outreach in the Hispanic/Chicano community. PI also serves as a mentor in an institutional mentor program to serve underrepresented minority students. • PI has a good record of DEI mentorship.
No: 0	*none*

Application #	DISC0-13801
Title (as written by the applicant)	Control of OCT4 abundance and function in human stem cells
Research Objective (as written by the applicant)	Our work will reveal an essential pathway that establishes precise levels of the OCT4 transcription factor and protects stem cell identity despite genetic or environmental stress.
Impact (as written by the applicant)	Our work will enhance our ability to generate and expand high-quality induced pluripotent stem cells from older patients or patients of neurodegenerative disease for use in regenerative medicine.
Major Proposed Activities (as written by the applicant)	• Aim 1: By identifying partners of the OCT4 species that is degraded through quality control, we will determine how OCT4 stabilization disrupts transcription factor complexes in stem cells. • Aim 1: By following OCT4 in cells lacking quality control, we will determine how aberrant OCT4 complex formation impedes pluripotency gene expression and stem cell self-renewal. • Aim 2: We will determine how the pluripotency transcription factor OCT4 drives expression of quality control components in stem cells to ensure its own activity. • Aim 2: With help of CRISPR/Cas9-mediated editing, we will determine the importance of OCT4 activity for the expression and activity of its own quality control system in stem cells. • Aim 3: Exploiting the Huntingtin protein, we will determine how aggregation-prone and neurotoxic proteins interfere with OCT4 function in stem cells. • Aim 3: We will determine whether overexpression of quality control components enables more efficient generation and expansion of iPSCs from Huntington's Disease patients.
Statement of Benefit to California (as written by the applicant)	Regenerative medicine is a promising approach to treat neurodegenerative diseases affecting thousands of Californians. However, disease mutations, older patients, or environmental stresses compromise the quality of transplanted cells and thus limit the benefits of regenerative medicine. By dissecting how stem cells maintain their identity despite mutations causing neurodegenerative disease, this work will spur the development of new therapeutic options for currently untreatable pathologies.
Funds Requested	$1,415,301
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	1
Highest	90
Lowest	86
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 targets improvement of iPSC expansion and function, addressing a major bottleneck in regenerative medicine. • The proposed work will investigate and explore the conserved Gene expression Quality Control (GQC) pathway that safeguards pluripotency by targeting its transcription factors. Improving the stability of iPSCs would have high impact in regenerative medicine. • The importance of OCT4 in PSC biology cannot be understated. Thus, efforts to better understand how OCT4 is regulated are at the heart of stem cell biology. The PI provides exciting preliminary evidence in support of the main approach. • The knowledge to be gained from the proposed work may impact how induced PSC are generated, in particular in cases where GQC mechanisms may need to be invoked. • The successful execution of the grant will have worldwide impact. • A fundamental question in pluripotency is being explored here. • The technology is novel and can be transformative. • Likely major impact, but specific to PSC expansion. • Unclear that the number of PSCs is an issue, vs. the inability to control and specify differentiation. Few, if any use of iPSCs is due to inability to generate sufficient numbers of iPSC clones, and no references are provided to suggest otherwise in the proposal.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 15	• The preliminary data presents the identification of a protein by an unbiased genetic screen as an essential regulator of pluripotency and as an essential component of GQC pathway involved in OCT4 degradation, connecting activity to protein aggregation provides a very strong basis to support the 3 specific aims. • The main rationale for the work is solidly based on the PI's expertise and preliminary evidence. • The preliminary data are well presented and provide compelling support for the proposed work. • Previous paper to establish the pathway to pluripotent transcription factors forms a strong basis of this proposed work. • The proposed work directly addresses a key aspect of human PSC biology, and may impact the generation of regenerative approaches that make use of PSCs. • The project is highly relevant to human biology and disease. • The rationale behind the study is very strong. • Straight forward and highly mechanistic proposal. • The proposal spans PSCs and OCT4, as well as Huntington's disease and breast cancer, and uses gastrointestinal cancer in mouse development as a basis for exploration. Aim 3 is disconnected from Aims 1-2 and unclear why Oct-4 and huntingtin is tested in a PSC state. • Use of Huntington iPSC lines would be more focused and provide genome context for this exploration. • Given the heterogeneity of human PSCs, multiple lines should be tested and examined, certainly more than one line. • Fig 1 suggests dichotomy of gene expression for several markers. This also indicates incredible heterogeneity. Given the number of cells that are clonally pluripotent is less than 1 in 10K, this data is difficult to interpret, and may not support the stochiometric precision suggested by the applicant. This is also not quantitative, e.g., RNA-seq, etc... • There is good rationale for project but little preliminary data.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**

Yes: 15	• The proposed work is outlined as three main Aims. The first aim is focused on addressing the mechanism of GQC regulation of OCT4 protein stability. • Aim 2 will address the role of OCT4 and partners in regulating protein expression. A careful dissection of the UBR5 promoter are proposed, including in the context of cellular stress, which is an important strength. • Aim 3 will examine how huntingtin mutations that increase protein aggregates are sensed by the protein and how this mechanism may impact its role in GQC for OCT4 function. A key aspect the experimental approach in this aim is to over-express the protein in fibroblasts to determine whether this would impact reprogramming into PSCs, even in the presence of stress, which is an important test of their hypothesis. • Well-designed and thorough set of experiments and constructs in place. • Outcome analysis and pitfalls are appropriately addressed. • The project and timelines are in line with CIRM priorities. • The budget rationale is appropriately justified. • It would have been a good idea to also propose whether OCT4 mutants are able to function to induce PSC reprogramming of fibroblasts, and whether this would have been affected in the absence of protein expression. This might have more directly tested the main hypothesis about the role of the protein in PSC generation and maintenance. • While specific Aim 3 seems a little bit out of place and the connection to huntingtin protein remains a little vague, overall the project is planned appropriately. • Other human PSC lines should be tested and compared, including Huntington's iPSC lines in Aim3. • If reporters for the protein do not work, endogenous loci will be targeted as this may be due to chromatin modifications that are required.
No: 0	*none*
GWG Votes	**Is the project feasible?**
Yes: 15	• Very well constructed proposal and very doable. • The project is high risk and also high gain • The project is feasible within the timeframe of the proposal. • Yes, the proposed aims are well structured and feasible within the outlined timeframe. The proposed team is superbly and extremely well qualified to direct and carry out the work. • The team is perfectly suited for the execution of the specific aims. • The PI team has access to the world class scientific resources at the institution. • Highly unique world renowned experts. • Tools in place, experts in the field. • The research environment is well equipped and supported to carry out the work. • The budget proposed is appropriate for the research proposal. • Everything in place.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• The proposal has adequately addressed and accounted for the influences of race, ethnicity, sex, gender and age diversity. • The project outcomes extend the reach of this work to undeserved communities. • PI has sufficiently outlined previous efforts for outreach and educational activities. • The PI points out and addresses these factors in the proposal. • Not relevant for this project that focuses on technology development.
No: 0	*none*

Application #	DISC0-13914
Title (as written by the applicant)	Developing a Human Model of Sporadic ALS Using Machine Learning and Robotic Microscopy
Research Objective (as written by the applicant)	We will develop the first human stem cell model of sporadic ALS (sALS) to identify disease mechanisms in the most common form of ALS and to discover drugs to treat the vast majority of ALS patients.
Impact (as written by the applicant)	Failure of drugs to treat sALS may be due to the use of models of familial ALS to establish preclinical efficacy. Our model of sALS may increase discovery of drugs to treat a wider range of patients.
Major Proposed Activities (as written by the applicant)	• Establish that the larger population of iPSC derived motor neurons (i-MN) from 20 sALS patients have significantly increased risk of death compared to controls. • Establish that the larger population of i-MNs from 20 sALS patients have increased morphological changes indicative of early degenerative phenotypes compared to controls using machine learning. • Establish that i-MNs from sALS patients express impaired protein clearance, TDP43 turnover and/or mitophagy compared to controls. • Determine the utility of our sALS platform for drug discovery by testing if small molecule autophagy inducers and mitoxantrone slows degeneration of neurons from patients with sALS.
Statement of Benefit to California (as written by the applicant)	Neurodegenerative diseases are a major health problem in California, especially since there are no disease modifying therapeutics for any of these diseases. Our studies focus on ALS. While this is a rare neurodegenerative disease, our development of the first model of sporadic ALS could lead to the discovery and eventual development of the first therapeutic to slow progression of this disease and provide an example for developing similar models for more prevalent neurodegenerative diseases.
Funds Requested	$1,406,622
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	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	• Yes, this project develops one of the first human motor neuron models of sporadic Amyotrophic lateral sclerosis (ALS) using induced pluripotent stem cell-derived from patients. • Harnessing both unique high-throughput imaging and analysis, the project applies single cell high content screens to characterize time-varying properties of motor neurons from sporadic ALS patients. • The i-MN models used in this study are a tool for increasing understanding of neurodegenerative mechanisms and potential treatment targets in ALS. While it is not focused on a stem cell-based therapy, these models have potential to address a key knowledge gap in the ALS field regarding mechanisms associated with sporadic forms of disease. • Models based on familial ALS mutations only represent a small portion of ALS cases. The i-MNs in this study are a highly valuable resource to study mechanisms underlying more common sporadic ALS. Coupled with automated longitudinal imaging technology, the biosensor, and novel machine learning methodologies, these models represent an important tool for interrogating early disease mechanisms and treatment strategies, and as such have potential to make a major impact in the ALS field. • Yes, these important models and sophisticated techniques offer incredible potential to contribute much-needed insight to the ALS field. • The findings will enable a first-in-kind look at mechanisms at play in a human model of this complex, sporadic disease. They will also support testing of targeted therapies in a model of sporadic disease, and assessments of early disease indicators. Importantly, models of familial disease have yet to lead to development and approval of an effective ALS therapy; better treatments are critically needed. • The techniques and knowledge gained from the proposed studies could also be applied to stem cell-based models from other complex neurodegenerative diseases, extending potential impact of the strategies and tools once established. Mechanisms and therapeutic targets may also translate to other disorders. • ALS is need of new treatments. • ALS has currently no cure and the focus in this proposal is on sporadic ALS.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 15	• Given that there are no current models of sporadic ALS, which accounts for the majority of cases, this project is incredibly relevant to human biology and disease. It provides, for the first time, a platform to model and assess various aspects of disease pathogenesis in sporadic forms of ALS to study neurodegenerative processes and discover novel therapies. • All commonly used models of ALS are based on familial mutations, which impact only 10% of cases. The use of i-MN to model sporadic ALS is innovative and enticing and offers the means to look at cellular events associated with sporadic disease. • A key strength of this proposal is that it develops a phenotypic screening tool and novel cell lines to study sporadic ALS, an area where there are large gaps in scientific and translational knowledge. • Another strength includes the resources made can be used broadly and shared to augment the impact of this work. • Using robotic microscopy (RM) along with the sensor can identify cells committed to death while they are still alive in order to support investigations into disease mechanisms and therapeutic screening. • Applying machine learning methods to assess morphologic cell features in an unbiased manner has potential to provide a means to identify and quantify early signs of degeneration in i-MNs. • The applicant's affiliation with the Answer ALS consortium has facilitated access to all necessary iPSC lines for the proposed study, and their ability to generate the i-MN model is supported by the preliminary data.

	• Presented and published data demonstrate that the platform can reliably provide precise, longitudinal monitoring of single cells. Additionally, the data from 4 ALS and 4 control i-MN lines supports their ability to track the increased degenerative phenotype of the ALS i-MNs relative to control i-MNs using this platform. • The probe is innovative and has been published, with data supporting its ability to identify neurons prior to death. The included figure, however, includes conflicting definitions of the red/green colors in panel A versus the legend, which complicates interpretation. It would also be helpful to know how long before cell death the signal is typically detectable. • The use of optical pulse labeling is an established technique and can be used to accomplish the proposed objectives focused on protein and organelle turnover. • The preliminary data support significant differences in i-MN morphology and neurite complexity and length that will facilitate the proposed machine learning methods proposed to evaluate early signs of degeneration. • Built on strong preliminary data. • The rationale is good in general but the heterogeneity of sporadic ALS is not sufficiently taken into account.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 15	• The project clearly outlines how to make a near-term impact through the development of quantitative methods to characterize iPSCs from sALS. • Aim 1 is designed to extend the preliminary work to a larger cohort of sALS and control lines to establish consistent, statistically significant disease phenotypes in sALS i-MNs. This will follow a similar paradigm to the preliminary studies, and while the number of lines is relatively low given the heterogeneous nature of sALS, the unbiased high throughput study design should support validation of the phenotype and could support future extended efforts using additional Answer ALS lines. • Aim 1 will also include validation of the platform via testing of therapeutics that slow degeneration in familial ALS models. Drugs will be assessed using dose response curves in both control and sALS i-MNs, and results will be analyzed in a comprehensive and blinded manner to support meaningful outcomes. • Aim 2 will use established assays to look at protein and organelle turnover in the 4 original sALS and control models, focusing on mechanisms of neurodegeneration that have been previously linked to ALS in other studies. This includes plans to assess commonly linked mechanisms in a human sALS model. • Aim 3 will further develop machine learning methods to quantify subtle morphological changes in human sALS i-MNs to establish a means to reliably detect early signs of neurodegeneration and pathomechanisms in living cells. This will be completed on the lines from Aim 1, and use machine learning methods applied in the preliminary and published studies. • For each of the proposed aims, controls and optimization is briefly discussed to support reliable results. The data sharing plan also includes additional details on experimental parameters, anticipated data, and experimental and analysis standards that further support the ability of the project to glean meaningful results. • The timeline is reasonable and supportive of achieving the proposed objectives during the study period, in line with CIRM's mission to address knowledge gaps to support future translational advances. • Minor weaknesses include no electrophysical assessments and multiomics or metabolic analysis in parallel with the phenotypic neuronal single cell screens. • Parallel multiomics would help. • There is no discussion of potential pitfalls or alternative approaches in any aim. • Lack of alternative approaches is a weakness. • The project is well planned and designed and makes use of front line technology. The neurocentric view is a bit limiting.
No: 0	*none*
GWG Votes	**Is the project feasible?**

Yes: 14	• Yes, the PIs have outlined a clear plan and assays to accomplish isolating and characterizing the iPSCs for sporadic ALS cases, and using this template for future studies including drug screens. • Based on the provided preliminary data and the fact that the iPSC lines required for these efforts are already established by the Answer ALS consortium and available in the laboratory, the proposed timeline seems logical and feasible. • The key personnel have extensive experience with the technical aspects of the proposed work as well as the assessment of neurodegenerative disease mechanisms. While the applicant has not served as PI on a previous grant, their lead role on other groundbreaking work in the field and their role in optimizing and applying many of the proposed tools support the ability to succeed in the proposed efforts with the support of the project team. • The team is well composed and experienced and well suited to carry out project. • While the data sharing plan indicated a lower number of in-hand ALS patient lines, all resources and equipment appear to be in hand to support the ability to quickly make progress on the proposed work if funded. • Yes, the budget seems appropriate for the proposed studies. • No pitfalls or alternative approaches.
No: 1	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• While the sALS samples are limited, the PIs make an effort to disseminate their technologies across demographics and researchers. • Yes, the applicant describes the extensive outreach and educational activities led by the Answer ALS consortium. They also mention a program at the institution focused on increasing representation of underrepresented groups in the sciences. • Makes use of well-characterized patient cells. • As the PI indicates, ALS is rare but affects people of both sexes and all ethnicities, with onset primarily occurring in midlife. The participant samples for this work are part of the Answer ALS cohort and represent both sexes and a range of ages, but are primarily Caucasian. However, there are plans to extend any identified disease signatures to subject samples from other races, such as African and east Asian. • This is not directly applicable at the current stage of the project, but the outcomes and any subsequent advances based on those outcomes have potential to impact all individuals affected by ALS.
No: 0	*none*

Application #	DISC0-13765
Title (as written by the applicant)	Engineering AAV capsids for transduction of neural and muscle stem cells
Research Objective (as written by the applicant)	The studies will identify and characterize new gene therapy vectors able to deliver gene editing components to stem cells. to enable treatment of diseases involving both muscle and brain.
Impact (as written by the applicant)	New AAV capsids that target muscle and neural stem cells will enhance the number of neurological diseases able to be treated with AAV-based approaches
Major Proposed Activities (as written by the applicant)	• Screen an AAV library to identify AAV capsids that transduce both muscle and neural stem cells. • Test the best capsids in human cells and tissues.
Statement of Benefit to California (as written by the applicant)	The project has the potential to create a valuable resource that could be used in research or to treat patients with devastating neurological disorders. The project will employ four individuals to carry out the project, which will create jobs within the state.
Funds Requested	$999,999
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	88
Median	90
Standard Deviation	3
Highest	93
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: 15	• Gene therapy for muscle stem cells is a very worthy goal. • Successful completion of the proposed project would definitely yield a significant incremental step forward for gene therapy. If the goals are achieved to the maximal degree possible, the impact will be major.

	• Capsids that target specific tissues while de-targeting the liver are critically needed. • Significant area of need to target stem cells and de-target the liver. • However, the applicants do not make a strong case for the need to target muscle and nerve stem cells at the same time, which weakened the application. For example, while some patients affected by muscular dystrophy do experience cognitive impairment, stark differences in the tissue themselves and in the expressed dystrophin protein could mean that separate strategies are more appropriate. Dual targeting of muscle and neural stem cells seems novel but premature.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 15	• Strong rationale based on previous work by the PIs and strong preliminary data. • Producing and screening of recombinant viral capsid libraries is a generally accepted approach to identify modified capsids with improved targeting attributes. AAV is the most widely used viral vector for gene therapy. Most ongoing clinical trials use native AAV vectors. Several new biotechnology companies use such approaches to generate capsids that target specific organs. The proposal is within the main stream of these important efforts. • The screening for tropism is scientifically sound. However there are nuances in how disease affects these very different tissues that could have been better considered.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 15	• The proposed studies are outlined with clarity and are likely to produce meaningful results. • It is designed to give results that could inform and address the listed aims and some alternate approaches are presented. • Very well written and well organized with logical flow. • The choice of the animal model could be refined.
No: 0	*none*
GWG Votes	**Is the project feasible?**
Yes: 15	• Project plans and timelines are appropriately ambitious. • The project seems achievable within the proposed timeline, the team is appropriately qualified and the needed resources seem readily available. • Project well within the area of expertise of the PIs who have already demonstrated feasibility of the approach.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• The application identifies sources and resources that are diverse, and outlines an active and continued involvement in DEI-focused activities. • Adequate.
No: 0	*none*

Application #	DISC0-13788
Title (as written by the applicant)	Modulation of human alveolar stem cells to promote lung regeneration and avoid pulmonary fibrosis
Research Objective (as written by the applicant)	Understanding regulators of human alveolar lung stem cell function will promote more normal lung regeneration after injury and avoid the nearly untreatable problem of advanced pulmonary fibrosis.
Impact (as written by the applicant)	Idiopathic Pulmonary Fibrosis (IPF), Adult Respiratory Distress Syndrome (ARDS), and other chronic fibrotic lung disorders.
Major Proposed Activities (as written by the applicant)	• Modify human fibroblasts to create a supporting niche promoting normal alveolar stem cell differentiation in ex vivo organoids, a model of the stem cell/fibroblast crosstalk prominent in injured lungs. • Develop an in vivo co-transplantation model of human lung stem cells plus stem cell promoting fibroblasts for better engraftments and expansion in injured lungs of immunocompromised mice. • Elucidate signaling pathways necessary to reverse or prevent the pro-fibrotic effects of abnormal human lung stem cell function prominent in the pathobiology of fibrotic lung diseases.
Statement of Benefit to California (as written by the applicant)	This application is focused on a set of lung diseases that are difficult to treat and associated with significant morbidity and mortality for citizens of California. The risk factors include advancing age, smoking, and exposure to inhalants that damage the lung. They all ultimately produce chronic scarring that impairs lung function. The goal of this research is to improve lung stem function which we believe will promote better lung regeneration after injury and attenuate scarring.
Funds Requested	$1,626,001
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: 89

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	89
Standard Deviation	4
Highest	92
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: 15	• Reduced lung regeneration capacity is a key contributing factor to inefficient recovery after lung injury, and a hallmark of several incurable lung diseases. • Reduced lung regeneration capacity associated with aging severely limits the quality of life of the elderly. • Therefore, it is fundamentally important to understand the cellular and molecular mechanisms driving the process of lung repair and regeneration in the human lung. • If successful, this project should advance the use of transplantation as therapeutic strategy in the lung, provide proof of concept that we can direct a desired stem cell fate in vivo in the context of a hostile environment, and provide insights into therapeutic modulation of declined lung regeneration in chronic disease and aging. • This project is highly significant; it has the potential to develop an effective strategy to alleviate fibrotic lung injury through cellular reprogramming. • No concerns.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 15	• Yes. The rationale is based on recent published findings from the group showing important distinctions between mouse and human alveolar type II (AT2) stem cells in their plasticity. Their key finding was that during fibrosis or/and aging, human (but not mouse) AT2 cells undergo transdifferentiation into the KRT5 basal cells incapable of gas exchange function. This transdifferentiation is driven by the aberrant signaling from surrounding mesenchymal cells. • The applicant has demonstrated feasibility of AT2 transplantation with subsequent engraftment and improvement of lung oxygenation function. • In the current proposal they want to extend these findings to develop a co-transplantation method to regulate human AT2 stem cell fate following engraftment into injured lungs and explore if it is possible to reverse KRT5 basal cells to an AT2 state. • The scientific rationale is sound and is based on previously published work and promising preliminary results. • No concerns.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 14	• The research plan is thoughtful, logical, and supported by strong preliminary data. The experimental design has good balance between in vitro studies in primary human lung cell organoids and data from in vivo model. It also incorporates innovative high throughput CRISPRi screening analysis and multi-omics. • The project is well-planned and designed. • Overall, yes, but the CRISPR experiment is risky and needs to be modified.
No: 1	*none*
GWG Votes	**Is the project feasible?**
Yes: 10	• The proposed aims are logical. Based on the track record and expertise of the research team in executing similar studies on the past, this is feasible in 3 years' time. • The project is feasible. The specific aims are realistic and an extension of previously published work. • The personnel and commitment by the staff need to be adjusted to execute the work.
No: 5	• Staffing is an issue. Major screens are quite risky, and have unknown outcomes. • Effort and staffing need to be addressed. • The screen may not work out as described. • The project is under-staffed.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**

Yes: 15	• Although the nature of the study precludes the investigation of differences between race, ethnicity or gender, the applicant has acknowledged this and has incorporated plans to investigate differences between young and older lung donors. • Fibrotic lung injury has an increased prevalence in underserved lower income groups. Also, it affects the aging population. This project has a potential to address these health disparities. • No concerns.
No: 0	*none*

Application #	DISC0-13875
Title (as written by the applicant)	Developing a microglia replacement therapy
Research Objective (as written by the applicant)	To develop a new cell therapy for the brain by transplanting the brain's immune cells
Impact (as written by the applicant)	The brain cell therapy we envision could be used to treat rare genetic metabolic diseases, Multiple Sclerosis, brain tumors, as well as common neurodegenerative diseases including Alzheimer's disease.
Major Proposed Activities (as written by the applicant)	• Finding the best source of human cells that can be grafted into the brain • Understanding the necessary steps to prepare the recipient animal before transplantation that enable the integration of grafted cells into the brain • To develop a method that allows immune cell integration exclusively into the brain but not other organs
Statement of Benefit to California (as written by the applicant)	The goal of this research is to solve several key bottlenecks for the development of a cell therapy for the brain. As California's population becomes older, neurodegenerative diseases increase steadily and are predicted to represent a large socio-economic burden on California's society. This project has the chance to prevent or improve age-associated brain diseases like Alzheimer's disease which would not only benefit those Californians afflicted with disease but also the society as a whole.
Funds Requested	$1,577,979
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: 87

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

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

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 15	• Restoring healthy microglia function could lead to novel disease-modifying therapies, and for a number of neurodegenerative diseases. • The objective of this proposal is to develop a microglia transplantation therapy. • In addition to providing insights into how/if microglia replacement can provide therapeutic benefit in neurodegenerative disorders, this project also offers a possibility to filling some critical gaps in understanding the role of microglia in pathophysiology of a range of central nervous system (CNS) disorders. • If successful, this project could have a very high impact. This project has a potential to impact various CNS disorders from neurodevelopmental to neurodegenerative ones, but also those related to regeneration of CNS following injury. • Having cell therapies for CNS disorders is a very attractive goal.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 15	• Overall, the preliminary data supports the proposed project. • Using hiPSCs to generate an embryonically derived therapeutic cell is a sound proposition. • The rationale is sound and very clear. Preliminary data provide a necessary proof-of concept, however, high risk experiments are now warranted. • The team has developed and published a protocol that enables homogenous and efficient CNS incorporation of circulating myeloid cells following bone-marrow or hematopoietic stem cell transplantation. • This method is highly efficient at replacing microglia in the brain. This protocol is an improvement compared to conventional bone-marrow transplantation methods because circulating myeloid cells do not typically cross the blood-brain barrier efficiently. • This approach works well in mice, but the translatability to human cells remains to be demonstrated. In addition, drug toxicity prevents its application to chronic or slowly progressing neurological diseases.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 14	• While the experiments are presented in a logical order, some important experimental details are lacking, e.g., power calculations. Have any behavioral experiments been planned to assess possible functional consequences of proposed experiments? • What will happen if the Aim 1 fails? The alternatives proposed are not really convincing and the failure at this step would mean the end of the project. • Additional functional experiments could be done for the human cells within the brain. • Aim 1: The proposal states that mice will be lethally pre-conditioned, and this is a procedure established by collaborators for human cell transplantation. However, would a lethal dose of a chemo drug not affect the biological functions of the animal and therefore introduce a bias? This model is far from physiological. • The other experiments detailed in Aim 1 seem feasible, and the design takes into account several variables that may affect the results (e.g. test of various host conditioning protocols, various cell sources etc.). This is a strength of the proposal. • Another strength is the large panel of experiments proposed to characterize the function and transcriptomic profile of human circulation derived microglia-like cells. However, the preliminary data does not show images of the human cell populations that will be used in Aim 1. • A caveat of using the approach in Aim 2 is whether this technique triggers glia/immune reactivity that would confound the results. • Aim 3: The investigators propose to identify ways to restrict the cell replacement procedure to microglia, without affecting the entire hematopoietic system. The procedure is well explained but it is unclear whether they are looking for a procedure that is translatable to the clinic. I feel lukewarm towards the methods proposed in the third aim. • The pitfalls are well detailed and take into consideration biochemical and genetic alternatives.
No: 1	*none*

GWG Votes	Is the project feasible?
Yes: 15	• The team is highly qualified to carry out the proposed project. • The timeline proposed by the applicant is very well thought out and justified. I believe that with the collected preliminary data and the expertise of this group, the work will undoubtedly be brought to completion. • This application is led by a PI who is a clinician and highly productive scientist with the goal to bring new cell replacement therapy to the clinic for disorders affecting the brain. PI has repeatedly published in high impact journals, spearheaded novel fields of research and made very good use of prior CIRM grants. • The team is composed of several collaborator-experts in complementary aspects of the proposal. This is a major strength, as some of the techniques utilized required specialized knowledge.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• No concerns.
No: 2	• Unless I am mistaken, I have not seen any explicit mentions of this in the proposal/experimental plan, not even the sex of the animals to be used nor the cell lines. • A rather vague account is provided. • This has been addressed by the PI although it is rather limited to his own lab activities with a focus on the composition of the current research group and how future recruitment/training of current staff would take DEI into account.

Application #	DISC0-13750
Title (as written by the applicant)	Generation of cortical organoids with tunable areal identities by spatial engineering of morphogens
Research Objective (as written by the applicant)	This project is to identify molecular mechanisms for generating different cortical regions using a novel hydrogel platform and brain organoids.
Impact (as written by the applicant)	The hydrogel technology to establish localized signals and resulting enhanced cortical organoids would advance 3D human stem cell cultures as tools for biomedical innovation.
Major Proposed Activities (as written by the applicant)	• Hydrogel optimization for signaling molecule encapsulation. We will utilize a library of hydrogel materials and properties that incorporate signaling molecules as inks for a bioprinter. • Assessment of regional markers in cortical organoids interfaced with hydrogel bioscaffolds that encapsulate signaling molecules, using immunohistochemistry, RT-qPCR, and single-cell RNA-sequencing. • Precisely and focally localized signaling molecule presentation to cortical organoids, using a bioprinter, to reproducibly form different regions. • Characterization of enhanced cortical organoids, using single-cell RNA-sequencing to demonstrate if they better mimic the human fetal cortex, compared to spontaneously formed cortical organoids • Identification of novel gene regulatory networks that govern cortical regionalization, using scRNA-sequencing and bioinformatic analyses. • Verification of key hub genes identified in Activity 5 by knocking down and overexpressing these genes using electroporation and/or viral delivery methods in cortical organoids.
Statement of Benefit to California (as written by the applicant)	This project aims to enhance cortical organoids derived from human pluripotent stem cells. Although current cortical organoids recapitulate the developing human fetal cortex, they lack a controlled formation of different regions, including prefrontal and motor cortices. Many neurological disorders, including autism, are differentially affected in distinct cortical regions. Once established, enhanced cortical organoids that can better model disease and pave the way for therapeutic applications.
Funds Requested	$1,497,032
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	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	• Formation of cortical areas is critical for normal brain function, yet we still have very limited understanding of this process. This project offers to fill in the very important gap in our understanding of cortical development. • This proposal has a potential to fill in one of the critical gaps in our understanding of cortical development which, if successful, would push all the related fields forward. • The proposal has the potential to enhance the utility and reproducibility of central nervous system (CNS) organoids derived from stem cells. • The idea to mimic arealization of cortex is good and of high relevance in the field. • Arealization of organoids will provide better models of brain development. • Could enhance capacity to generate patterned organoids for other systems. • The expected/proposed outcomes of this project proposal are likely to have a significant impact, particularly in the field of neurodevelopmental disorders. • Low risk, high gain. • Innovation is limited but problem is highly relevant.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 15	• Project is based on sound developmental principles. • Project will address differences in mouse vs human brain development. • The rationale is very sound, well described and the project is well thought through with a good experimental set up. • The delivery of extracellular morphogens in hydrogels can be widely applied and have an impact also beyond the scope in the proposal.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 14	• Strong preliminary data on effect of two molecules but would be enhanced by data showing feasibility of the proposed hydrogel system for regionalized signaling. • New model would be applicable to basic studies of development and neurodevelopmental disorders. • Project should yield clear results on arealization. • The scRNA-seq data and gene regulatory network analysis will provide critical information on success. • Investigators may be able to integrate gene expression and spatial data. • Plans for tunable hydrogel construction are logical; different technical approaches are proposed. • Technique provides for a high throughput system. • The flow of experimental steps is logical and well laid out. • Problems with adjusting morphogen release and absence of a gradient is acknowledged. • The design is very good. Somewhat limited novelty in the approach. • A reporter line could simplify the assays.
No: 1	*none*
GWG Votes	**Is the project feasible?**
Yes: 14	• Based on the experimental plan, the project is feasible within the proposed time frame. • The project is feasible given the expertise of applicants that includes experts on stem cells, organoids, biomaterials and omics. It is an excellent team with relevant and complimentary experience. • Good team with experience in complementary techniques.

	• An interdisciplinary project. PI is an expert in 3D organoid models. A collaborator is a bioengineer with a very strong publication record across materials science and bioengineering. • Better evidence that these approaches can generate gradients of morphogens would help; not clear if covalently linked proteins will have the requisite activities. Screening of hydrogels might be simplified through use of reporter cell lines. • Direct costs for year one are $505K; stronger justification for purchase of a second bioprinter is required.
No: 1	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• Experimental set up and the sources of the hPSC lines clearly demonstrate this. • Diverse cell lines will be used. • Excellent, the best in this group of grants. • Excellent outreach and education activities. • PI has a track record in study of Zika virus congenital malformations affecting Latinx communities.
No: 0	*none*

Application #	DISC0-13816
Title (as written by the applicant)	Towards a trophectoderm stem-cell model representing human blastocysts of the highest implantation potential
Research Objective (as written by the applicant)	To define a new reference for embryos and stem cell lines of the highest developmental potential and work towards a trophoblast stem cell model to study factors important for successful implantation
Impact (as written by the applicant)	The molecular determinants of successful human blastocyst implantation remain unknown and trophectoderm stem cell models to study these embryo factors and improve implantation success are needed
Major Proposed Activities (as written by the applicant)	• To determine the pattern of gene expression expected in cells of each lineage—and the cell-cell signals between them--for blastocysts with the highest developmental potential • To identify the specific genes and cell-cell signals—among the thousands present--that are uniquely enriched in blastocysts of high compared low development potential • To identify candidate ligand-receptor signals between polar trophectoderm of the blastocyst and maternal endometrium important for successful implantation • To evaluate the developmental state and potential of current trophoblast stem cell models relative to blastocysts of high developmental potential • To determine the degree to which network of cell-cell signals predicted for trophoblast stem cell models with models for the EPI and PrE and for maternal endometrium resemble signals expected in vivo • To test whether signals from cells representing neighboring blastocyst lineages enhance expression of biomarkers of polar trophectoderm with high implantation potential
Statement of Benefit to California (as written by the applicant)	Failed embryo implantation represents one of the greatest obstacles in infertility. However, the molecular mechanisms required for successful implantation of the human embryo are poorly understood. We take a novel approach to identify factors most highly associated with successful implantation in human embryos and steps to develop an in vitro stem cell model to learn to optimize expression of these factors. We hope our findings will lead to increases in embryo quality and implantation success.
Funds Requested	$1,584,000
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	85
Median	85
Standard Deviation	4
Highest	90
Lowest	74
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 project targets one of the greatest obstacles in fertility treatment: failure of embryonic attachment and thus addresses a major gap in our current knowledge of human development. • As ~70% of lost of pregnancy in fertile women is due to implantation failure, the successful characterization of molecular factors required for high implantation and developmental potential will have a very high impact in regenerative and reproductive medicine. • A key cause for failure in assisted reproductive biology is failure of implantation. This grant may lead to a better understanding of why human blastocysts have a relatively high rate of implantation failure. • As implantation cannot be directly studied in humans, stem cell models are really the only means to explore this important biology. • High impact area of research with relatively sparse knowledge. • Research into human trophectoderm is very important. • A successful outcome will contribute to world class science. • Though this work will shed light on gene expression patterns and signaling pathways that differ between high quality and low quality embryos, the research plan does not directly test implantation itself.
No: 1	*none*
GWG Votes	**Is the rationale sound?**
Yes: 15	• The project is highly significant for human embryology and reproductive medicine as the factors that regulate implantation are not known. • The rationale is based on solving one of the greatest obstacles in infertility treatments, the identification of molecular factors, and the design of genetically tractable in vitro models for implantation is very sound. • This group has already performed RNAseq (not single cell) on each of the three cell types from embryos with good morphology as compared to poor morphology, and found differences, especially in signaling factor genes. • The preliminary results supports a model in which successful implantation primarily depends on signals from the inner cell mass to the trophectoderm and support the proposed project. • Bioinformatic and pathway analyses of the RNAseq comparative data suggest the importance of signaling pathways which may affect their ability to participate in proper implantation. • This group has already made trophoblast stem cells from naive hESCs, which may be useful in model creation.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 15	• The two specific aims of the project which explore the identification of gene expression and cell-cell communication in human blastocysts with different implantation potential and to assess the potential of trophectoderm stem cell models are very well planned and developed. • Very well written application and well designed experiments. • Pitfalls and outcome analysis is provided. • This represents an urgent priority in CIRM's mission.

	• The budget rationale is appropriately justified. • Aim 1 looks at signaling between different cell types in blastocysts (an understudied area) and their role in implantation, and aim 2 will yield insights into gene expression and signaling patterns found in high quality embryos. • Much will be learned about gene expression and signaling in polar trophectoderm. • It is not entirely clear how the Aim 2 experiments really test implantation. Though information about trophoblast stem cell function and gene expression will be obtained, there are apparently not any cells that correspond to endometrium, so Aim 2 studies are limited to trophectoderm behavior in the absence of anything that actually models implantation. • No functional assays for implantation.
No: 0	*none*
GWG Votes	**Is the project feasible?**
Yes: 15	• The project can be done within the proposed timeline. • The project is well within the expertise of the PI. • This group has expertise with culture of human embryos, and can do RNAseq and associated data analysis. • The proposed team is appropriately qualified and staffed. • The team has access to excellent resources at their institution. • The budget for the outlined proposal is appropriate.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• The donated human blastocysts to be studied will be drawn from a diverse population, however, as these are de-identified, it will not be possible to determine the ethnicities of individual embryos. However, male and female embryos will be determined by sex chromosome content. • The project outcomes would extend to underserved populations. • This project design adequately addresses and accounts for the influence of race, ethnicity, sex gender and age diversity. • PI has adequately described prior efforts to outreach and partner to inform the development of DEI. • Past work with the CIRM SPARK and Bridges internship programs have reached a diverse set of high school students.
No: 0	*none*

Application #	DISC0-13806
Title (as written by the applicant)	Development of universal off-the-shelf iPSC-derived dendritic cells for use as patient specific anti-tumor vaccine
Research Objective (as written by the applicant)	This proposal aims to develop a novel cell-based vaccine for the treatment of cancer. The vaccine would be derived from stem cells and be personalized to the genetics of the patient and their cancer.
Impact (as written by the applicant)	Success would provide proof-of-principle of a novel cell-based therapy for cancers broadly. It is expected to overcome limitations of current immune-based strategies in cancer therapy.
Major Proposed Activities (as written by the applicant)	• Differentiation of induced pluripotent stem cells (iPSCs) to antigen presenting cells (APCs) • Development of universal antigen presenting cells (UAPCs) • Transfer of patient specific tumor antigens to universal antigen presenting cells (UAPCs) • Optimization of cell presentation and activation of tumor antigen directed immune cells
Statement of Benefit to California (as written by the applicant)	If successful, the results of these studies will be translatable to a therapeutic that would be developed here in California. Such a therapy could be immediately beneficial to a very large number of patients here in California who suffer from currently deadly and incurable cancers. Furthermore, concepts learned will have utility in development of related strategies for an even broader range of disease types.
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	85
Median	85
Standard Deviation	3
Highest	90
Lowest	80
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	7
(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: 14	• Cancer remains a leading cause of death and tumor vaccines are a promising strategy to change that. This proposal aims to address some major limitations that have so far prevented the wider application of a vaccine strategy. • Yes, this could support a gap in the development of dendritic cells (DCs) as anti-tumor therapies, and have future applications in immune therapy. • Yes. If the applicant's premise is proven correct, this project has the potential to change immunotherapy in oncology. • Excellent and competitive. There are many groups working in this space, however. But this is a novel idea. • There is a great need for this type of approach in the field.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 14	• Figs 1-5 indicate that the applicant has the capability to create MHC-deficient iPSCs, induce differentiation of iPSCs to dendritic cells (DCs), and use extracellular vesicles (EVs) for transfer of antigens into their engineered DCs. However, planned methods for quantitation, the expected efficiency, and other biological challenges of moving the technology into humans are not sufficiently addressed. • Preliminary data support the ability of the applicant to generate of iPSC-derived DCs (according to staining and morphology). • The potential for generation of universal antigen presenting cells (UAPCs) is also demonstrated in preliminary studies: (i) B2B deletion and partial disruption of TAP1 is reported in human iPSCs, and (i) EV delivery to DCs is demonstrated in mouse experiments. • More preliminary data on the transfer of antigens via EVs are needed to motivate the aims. • The proposal leverages an existing FDA-approved technology - Sipuleucel-T. A comparison between the proposed approach and Sipuleucel-T would better place this proposal into context. • The proposal strategically combines a unique and novel combination of technologies and biology. It's difficult to determine whether this will be superior to current CAR therapies or not, especially as the proposal does not include in vivo studies. • Overall tumorigenicity of the product needs to evaluated, including genetic stability and in vivo tumor capacity of both iPSC parent lines and DC progeny. • The number of clones to be selected and tested is not provided. • Aim 4 seems to be unnecessary and unlikely to be accomplished. There are many existing methods of enhancement that could instead be attempted with the applicant's novel iPSC lines. • Significant to oncology in general. • Well written, clear, and concise.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 11	• Aim 1 will focus on the generation and (molecular and functional) characterization of DCs differentiated from human iPSCs. This will include optimization of the differentiation conditions with cytokines, and characterization of DCs using single cell multi-seq and FACs analysis. Functional assessment of DCs will include the presentation of antigens. • Aim 2 will generate universal iPSCs (via B2M deletion), and confirm HLA-A,B,C loss. • Aim 3 will evaluate the EV-mediated transfer of patient-specific antigens to DCs. • Aim 4 will focus on enhanced T-cell activation via genetic modifications. • No concerns.
No: 3	• According to the applicant, if under Aim 1 they fail to generate engineered DCs similar in profile to natural, de novo DCs, they will try overexpressing of transcription factors known to promote DC fate or DC maturation. This seems like an entirely new method and would change the course of the proposal and its resources, impacting Aims 2-4. • The basis for testing 2D versus 3D systems for iPSC differentiation to DCs is unclear. How the results will be prioritized for next steps is also unclear.

	• Cite-Seq will provide excellent biological data, but using healthy cells as controls these data will be difficult to interpret. Furthermore, the expense of this study may not be justified. • Aim 4 is insufficiently justified. • It's unclear why in vivo studies are not included in this project. Problems encountered in vivo may necessitate re-initiation of differentiation and engineering approaches. Depending on in vitro studies is high risk, with potential loss of time and resources.
GWG Votes	**Is the project feasible?**
Yes: 12	• All aspects require optimization (in particular the EV transfer), and efficiency will be a key question. But, all is feasible in principle. • No concerns.
No: 2	• Methods and cellular reagents are all in place. • The applicants team members are experts in the field.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• The section is mostly about the Principal Investigator and the proposed team, but universal DCs would benefit all. • No concerns.
No: 0	*none*

Application #	DISC0-13808
Title (as written by the applicant)	Development of a stem-cell based approach to interpret global effects of genetic variants contributing to neurodevelopmental disease risk
Research Objective (as written by the applicant)	We are developing a strategy to characterize the disease-relevance of hundreds of mutations across diverse genetic backgrounds using stem cells
Impact (as written by the applicant)	Understanding how mutations impact cellular function can identify treatments for genetic diseases, but currently less than 1% of identified mutations have a known function.
Major Proposed Activities (as written by the applicant)	• Identify optimal conditions for SNV library introduction in hiPCSs and characterize the global impact of individual mutations in ERCC2 and MECP2 on transcription, chromatin state, and mutational rates • Develop a computational pipeline to design SNV libraries and analyze data from our method, scBE-seq • Employ scBE-seq to study the impact of libraries of mutations in ERCC2 and MECP2 during the in vitro neurodifferentiation of hiPSCs into cortical organoids • Analyze scBE-seq data and compare with orthogonal datasets for clinical interpretation of genetic variation
Statement of Benefit to California (as written by the applicant)	An overrepresentation of European human genome sequencing data has generated inequities in regenerative and precision medicine efforts. We propose here to develop a more equitable strategy to characterize the disease-relevance of mutations from diverse populations. Our project will identify new preventative strategies, treatments, and cures for genetic diseases applicable to a variety of ethnic groups, and will therefore benefit the State of California and its highly ethnically diverse citizens.
Funds Requested	$1,518,982
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	84
Median	85
Standard Deviation	1
Highest	85
Lowest	80
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: 15	• Exciting technology to augment the use of iPSCs. • By avoiding the effect of varying genetic backgrounds, this project holds promise to enhance our understanding of brain disorders. The project has a potential to enhance our genome understanding while at the same time provide new treatment targets. • Pooled genome editing will be combined with single-cell genomic tools to systematically characterize the overall impact of single genetic variants on mutagenesis, transcription, and chromatin structure in a single experiment. The approach will yield novel multidimensional data. • Most studies are based on comparisons of cells from healthy and diseased individuals and while sibling studies can be done, the genetic background is still variable enough to generate noise and affects interpretation of outcomes - the approach of directly introducing libraries of mutations into isogenic backgrounds would overcome these issues. • High risk - high reward approach.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 15	• Considering that less than 1% of identified genetic variants have a defined clinical interpretation, the approach will have a potentially high impact for the field and will inform many studies. • The rationale is scientifically sound and backed up with appropriate methodology. • The focus on MECP2 needs to be sharper and better motivated with scBE-seq preliminary data.
No: 0	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 13	• Development of a novel high throughput approach seem promising. • Aim 1 is critical to success. • The scope of work is too ambitious for the timeline, and the aims are interdependent. Focus on Aim 1 would be sufficient for the funds provided. • Aim 2 is premature and is dependent on Aim 1. • Library preparation is quite critical and potential issues here should be addressed in more detail.
No: 2	*none*
GWG Votes	**Is the project feasible?**
Yes: 13	• Clearly described approach, well conceived. • The lack of preliminary data showing that the approach is feasible in iPSC cells makes this a risky proposal. There should be a clear demonstration of success of aim 1 before embarking on aim 2. • The library preparation with scBE-edited iPSCs must be demonstrated with appropriate quality controls. • While the aims of the project are logically presented, I fear that the project is overambitious for the proposed timelines which are also not described in detail.
No: 2	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• Clinically characterized variants seem to mainly cover European populations. The proposed high-throughput project would enable analysis of genetic variants from diverse populations. • Described in detail and well-thought through.
No: 0	*none*

Application #	DISC0-13937
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 - the ability of cells 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 set the basis for new regenerative dentistry avenues based on cell and molecular behavior, in turn facilitating the development of new tooth decay treatments.
Major Proposed Activities (as written by the applicant)	• Investigate cell reprogramming in the mouse incisor in response to mandibular denervation • Investigate cell reprogramming in the mouse incisor in response to physical clipping • Unravel the extent and limitations of cellular plasticity in uninjured mouse molars at the molecular and cellular level • Characterize cell reprogramming in the mouse molar in response to mandibular denervation • Characterize cell reprogramming in the mouse molar in response to drilling • Integrate mouse and human data by exploring biomarkers of dental regeneration in human samples
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	$1,346,851
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	76
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: 13	• This proposal aims to understand lineage plasticity during endogenous regeneration in the mouse incisor and mouse molar. It is unclear that this project will address a major bottleneck to the use of stem cell-based or gene therapies. • This project, if successful, will advance the field of dental regeneration, but have limited impact on other regenerative medicine fields. • The outcome from this project will have limited impact on regenerative medicine. • The aim of the project is to understand the role of cellular plasticity in dental regeneration by comparing the mouse incisor (which replaces itself continuously as it is worn down by erosion) with the mouse molar (which is rooted and does not continuously replace itself, having a reduced stem cell response). The degree of plasticity in human teeth will then be investigated in comparison. • The longer term goal is to develop a strategy for molecular stimulation of human tooth regeneration. There remain gaps in our knowledge of dental regeneration that would undoubtedly be addressed by the proposed studies. • The studies may have an important impact on the field of dental regeneration, however this is a somewhat narrow field and it is questionable whether the approach would ever be viable as a standard healthcare treatment. It could certainly become commercially realistic as a cosmetic treatment. • The applicant was awarded a $3 million grant in the past to explore the contribution of epithelial stem cells to tooth regeneration - according to the application, all aims were achieved. This proposal now extends these studies to mesenchymal renewal in the incisor. This is a logical extension of the work. • Massive omics analyses in different established injury models will characterize changes associated with regeneration after injury. • It is not clear whether the mutations in core amelogenesis-related genes that the applicant points out are addressed in the project.
No: 2	• The clinical relevance of the work needs to be stronger.
GWG Votes	**Is the rationale sound?**
Yes: 14	• The roles of epithelial and mesenchymal cell populations in maintaining the rodent incisor during homeostasis are established. This proposal aims to investigate mouse incisor lineage reprogramming in response to injury, and examine plasticity of the mesenchyme in the mouse and human molar during homeostasis and repair. • The applicant hypothesizes, based on preliminary data, that cellular plasticity and transdifferentiation are critical to the regulation of dental regeneration. Therefore they focus their studies on those processes. • The applicant provides relevant supportive data for this project. • The rationale for the proposal is to fill the gap in our knowledge of what limits human dental regeneration through comparison of mouse incisors with mouse molars. This is clearly a logical approach given that incisors regenerate through the rodent life, while molars have a limited capacity for regeneration. • The rationale for Aim 1 is that plasticity is triggered in the incisor epithelium and mesenchyme in response to injury to allow healing. This seems straightforward and consistent with other injury responses. • Extrapolation to human dental regeneration also seems to be a logical step, although it remains to be seen if the regenerative pathways are shared between mice and humans. While mouse molars are rooted and lack regeneration capacity, like human teeth, the mouse incisors are not rooted. • This project focuses on mouse models, with limited human cell studies. The applicant will dissociate human dental pulp and its surrounding tissues into single cell suspensions for transcriptomic analyses. As this is the only experiment with human cells, the project is not sufficiently relevant to human biology. • No concerns.
No: 1	*none*

GWG Votes	Is the project well planned and designed?
Yes: 10	• Injury models in mice are established and published data show that the applicant has expertise in the mouse model. • Aim 1: Investigate mouse incisor lineage reprogramming in response to injury. Two types of injuries - denervation and clipping - will be used. • Aim 1 is to investigate mouse incisor lineage reprogramming in response to injury, using a single cell multi-omic approach. The hypothesis is that in response to injury, adult mouse incisors will undergo increased cellular plasticity compared with the homeostatic state. Injury will be induced by denervation and by clipping in 8 week old mice with recovery assessed in the 8 to 16 week period. • Aim 2: Examine plasticity of the mesenchyme in the mouse and human molar during homeostasis and repair. These planned studies will generate meaningful results for understanding dental regeneration. • Aim 2 is to explore the mesenchymal response to injury in the mouse and human molar. Mouse and human molars lack epithelial stem cells and so can only respond to injury through the mesenchyme. • The injury model will be drilling of the mouse molar, or denervation, in adult 8 week old mice and single cell analysis undertaken in the same was as for incisors under Aim 1. • For Aims 1 and 2 the applicant states that they do not anticipate problems - though the proposed studies include injury procedures, tissue harvest, scRNA-seq, scATAC-seq, proteomics, histology, RNAscope assays, and µCT experiments. A thoughtful potential pitfalls plan should be proposed. • Analysis will involve a combination of single cell RNA sequencing by MULTI-seq, scATAC-seq, high resolution bulk proteomics using trapped ion mobility spectrophotometry, and dual EdU/BrdU labelling as well as cell proliferation assays, to assess proliferation dynamics. Data from across the platforms will be integrated. • Ex vivo analysis of single cell changes will be mapped and then any changes validated using in-situ hybridization/micro-CT imaging to follow differential gene expression and different phases of injury response in different injury models. • For human tooth studies, MULTI-seq technology will be used for single cell RNA sequencing, to compare normal 3rd molars (obtained at wisdom tooth extraction) with teeth displaying cavities. Data from the human teeth will be integrated with the mouse data set to allow detailed comparison. • The added value of ATAC seq is not clear - what is the rationale for including this analysis? • The timeline is reasonable. • No concerns.
No: 5	*none*
GWG Votes	**Is the project feasible?**
Yes: 13	• The proposed aims are feasible. • The team is qualified for this type of work. • The team has access to the necessary resources for this research. • The proposed budget is reasonable. • The project is feasible and well designed. • No concerns. • Yes, although the overall goal is not clear and how omics data will be used is not well described.
No: 2	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• Yes, mutations in core amelogenesis-related genes (such as FAM83H, FAM20A, ENAM, AMELX, MMP20, and others) are more prevalent in patients in the United States from Mexico, Turkey, Brazil, and Korea. • According to the applicant, oral health disparities disproportionately affect the Black, Indigenous, and People of Color (BIPOC) communities. • Dental regeneration will be relevant to underserved populations. • This project does not account for the sex/race/ethnicity of human samples.

	• The Principal Investigator has a record of DEI-related community efforts. • No concerns.
No: 1	*none*

Application #	DISC0-13784
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 hiPSCs 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 an unprecedented, multi-dimensional studies of the cellular complexity
Major Proposed Activities (as written by the applicant)	• Establish complementary two new 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 members in hiPSCs.
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 (iPSCs) 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	(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	83
Median	80
Standard Deviation	3
Highest	91
Lowest	80
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 aim of the project is to bring next generation protein and gene analyses into use for deeper understanding of stem cell biology. The proposed approach will allow state of the

	art studies of protein-protein and protein-DNA interactions over time and under a range of conditions. • The main properties of stem cells such as pluripotency, response to differentiation signals and regulation of chromatin structure are dependent on interactions between proteins (protein-protein interactions; PPIs) within complexes as well as the genomic localization of such protein complexes (protein-DNA interactions). However, current data is limited due to the inability of available methods to simultaneously detect the multiple interactions at scale. • In particular, as most of the current studies focus on a single or a handful of factors, they do not address the multi-factorial manner in which complexes affect the physiology of normal or disease conditions. This gap presents a major roadblock in advancing knowledge of stem cell biology and improving regenerative medicine, such as the study of the impact of genetic variations on stem cell properties, understanding the impact of aberrations in proteins on disease states or drug development. • To fill this gap, the applicants have developed a framework that enables simultaneous identification and characterization of multiple interactions: between proteins within functional complexes (Prod-seq), and between DNA associated-proteins and the genome (WhIP-seq). • High tech equipment is not needed to use the technology and so the approach can be used in any molecular biology laboratory. • Excellent idea that could be transformative. • High-risk and high-reward approach. • The project addresses questions of high relevance and broad applicability in the field.
No: 1	*none*
GWG Votes	**Is the rationale sound?**
Yes: 13	• The proposed methodology uses antibody-oligonucleotide conjugates to target dozens to hundreds of proteins. The methodology also uses a molecular detector that can identify DNA-tagged proximal entities. • Ideal detection of proximal nucleic acids requires capturing both of them on a single molecule. This presents a unique challenge, as both must be initiated in a 5' to 3' direction, and thus cannot both be captured using traditional methods. • To overcome this hurdle, the applicants have invented a specialized detector of molecular proximity. • The project will develop the proposed tools and test them in a system that has already been well described, for validation. Thus the outputs from the proposed project will be new tools/approaches rather than new stem cell knowledge. • There is a clear need to better study protein/DNA interactions in stem cell models • Lack of preliminary data that support the technology impact enthusiasm. • Not clear whether technology is superiors to existing methods • There is some concern that key proof-of-concept data is not present.
No: 2	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 11	• The methodology in essence is simple in concept. However the molecular techniques are themselves very complex and each antibody-oligo needs to be designed and tested/validated. • Under Aim 1, key metrics will be determined such as the % of reads containing valid barcodes; whether barcodes for group proteins occur in expected locations and frequencies. • Following the optimization steps, the applicants will use Prod-seq and WhIP-seq to detect PPIs, protein-DNA interactions and protein abundance in two iPSC lines (male and female) each in triplicate. • Under Aim 2, the applicants will study changes in protein-protein and protein-DNA interactions during neural differentiation of hiPSCs. The protein groups are known to be critical regulators of differentiation and therefore offer an ideal target of testing the new approach. • Analyses will be undertaken with neural progenitor cells and neurons derived from hiPSCs and changes from the undifferentiated cells determined. The generated data will

	then be compared with known changes in the group family proteins based on published literature. In this way, it will be possible to validate the approach based on previously described interactions as well as adding new observations using the current methodology. • The project is generally well designed and planned.
No: 4	• Unclear. No preliminary data at all.
GWG Votes	**Is the project feasible?**
Yes: 9	• This is undoubtedly a high-risk high-return project. As a new methodology, there are bound to be challenges in achieving the sensitivity and accuracy desired. However, if effective, a large amount of new data and biological insight will be possible. • This is a high-risk high-gain proposal. Only very limited preliminary data is provided but feasibility is supported based on competence of the team.
No: 6	• Preliminary data showing feasibility and proof-of-concept is needed. • Unclear. No preliminary data.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 12	• Diversity has been considered and may benefit from the approach in terms of detection of any ethnicity-related molecular pathway differences. • This is not so relevant for the proposed project.
No: 3	• Incomplete.

Application #	DISC0-13735
Title (as written by the applicant)	Decoding human embryo development 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.
Major Proposed Activities (as written by the applicant)	• 3D printing human embryo models • Understand the physical parameters that influence embryo development • Determine how environmental signals are read by embryos • Build a mathematical model to integrate our findings and make predictions
Statement of Benefit to California (as written by the applicant)	The citizens of California will benefit from the development of widely applicable, cutting edge tools for tissue engineering which will substantially advance the field. Understanding the mystery of how the human body builds itself will improve fertility treatments. The students trained in this project will work in biotechnology firms across the state, contributing to the economy and making untold future discoveries at the promising interface of Physics, Biology, and Materials Science.
Funds Requested	$1,000,000
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: 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	87
Lowest	76
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	4
(1-84): Not recommended for funding	11

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 13	• The project targets modeling of early human embryos with embryonic stem cells and thus targets a major gap in our current knowledge of human development.

	• Any contribution to our current understanding of early human embryogenesis will advance the field. • This proposal seeks to develop a more sophisticated model of the early stages of gastrulation using human embryonic stem cells in a 3D in vitro model combined with optogenetic signaling to deliver specific genetic signals with a much finer degree of spatiotemporal patterning than has been achieved to date. This approach will be used to study the origins of asymmetry in the early embryo. • While there have been many studies of early embryogenesis in the mouse, ethical considerations have limited the possibility for detailed study in the human. More sophisticated 3D in vitro models are needed to fill this gap in our understanding of human embryogenesis. • While the long term implications of the proposed studies are uncertain, there is no doubt that a better understanding of asymmetry development will be important fundamental biology with real potential to lead eventually to healthcare impacts. • This is really a very ambitious and interesting project that could lead to very high-level findings--the precise spatial positioning of cells and the importance of these positions can be addressed with 3D bioprinting, and this group can initiate and monitor key signaling and morphogen-driven events on a sub-embryonic scale using optogenetic approaches. • In the last two to three years there have been some significant advances in making blastuloids and gastruloids, which do indeed break symmetry and even undergo gastrulation. It is not clear why these approaches could be not used, and whether the 3D printing approach will be superior to these recent advances to model the very early post-implantation embryo. • If successful, the project will allow modeling of aspects of early development, which in the absence of in vivo comparative analysis, will be at best speculative.
No: 2	• There is a lack of in vivo validation, so the findings may have limited value.
GWG Votes	**Is the rationale sound?**
Yes: 12	• This research plan is based on sound rationale, and also the preliminary data shows that they can use optogenetics to induce key signaling events in subregions of stem cell based embryos. • This group has used these approaches successfully in the preliminary data, and show that they can control signaling using precise light exposure in H9 hESCs (Figure 2). This shows that signaling can be induced with a resolution that should be able to induce signaling in a subregion of an embryo model. • The group has also developed fluorescent read outs of signaling and can see waves of signaling responses that are induced by precise ontogenetic light pulses. • The natural tendency of stem cells to aggregate in culture has been used in the past to stimulate the early stages of embryogenesis. However the effect is very inefficient and unreliable. More recently, microfluidics has been used to apply signaling molecules with some degree of specificity, however, the fluid channels themselves place limitations on growth. • This project will use an integrated 3D bioprinting system to deliver precise, freeform deposition of cells and delivery of signals. the system includes integrated, long-duration, high speed and high-resolution imaging. The system enables high throughput analysis. • Complexities of signaling pathways will be investigated using optogenetics. Triggering occurs within milliseconds, bringing it close to or within the time-frame of natural signaling waves. This has not been possible until now using traditional ligand-based signaling. • These approaches are largely based on sound scientific rationale. However, it is difficult to know if a model of this type, even if complex and refined, can accurately reflect natural embryogenesis. No work is proposed to compare the findings of this study with changes on the early embryo itself. A further concern is the choice of the signaling pathways for aim 2. Whilst the literature may support their critical role, it cannot be certain that they are sufficient for normal and complete embryogenesis.
No: 3	• The rationale is very strong. • The subject is relevant to human biology. • The preliminary data is compelling but unfortunately suffers from a serious lack of referencing. Most of the results presented as original have been published and

	developed by many other groups. There is also an obvious lack of understanding of basic embryological concepts. • Most of the biological questions do not require these types of tools.
GWG Votes	**Is the project well planned and designed?**
Yes: 11	• This is a very well designed project with a clear goal - to figure out how a spherical blastula of cells can break symmetry and establish embryonic axes using a human model of early pre- and peri-implantation development. • The applicants hypothesize that two signaling pathways are key for breaking symmetry and have created molecular reagents to explore this hypothesis. If unknown pathways (other than these) turn out to be crucial, results may not materialize as expected. This is probably the biggest concern in this grant. • The project is way over ambitious and could benefit from focus on specific questions. Some of the proposed experiments have already been done and published, but not referenced. A vast array of technological tools are used to answer basic question which could be answered by much more simplified, and perhaps more precise means. Nevertheless, the application has tremendous potential and will get stronger after revisions. • Applicants have addressed potential pitfalls to some extent. However, with so many different variables it is near impossible to identify all pitfalls. • This project is in line with CIRM's overall mission. • The budget rationale is appropriately justified.
No: 4	• The project is ambitious but logical and the scope of exploration within the proposed complex model has been appropriately limited to manageable challenges.
GWG Votes	**Is the project feasible?**
Yes: 11	• The plan is appropriate and feasible. • This is logical grant with two aims. The first having to do with further improvement of their already solid advances with this technology, and the second to ascertain mechanisms that lead to embryo asymmetry and polarity. • This is a talented team that has good expertise in early developmental biology, the set-up and use of ontogenetic systems, 3D bio-printing, and also custom device engineering. Overall, this is a powerful combination. • This team has an excellent set of cellular, optogenetic, and imaging resources, and have shown that they can use these effectively to induce and monitor key early development signaling events and their responses.
No: 4	• The institution provides an excellent environment for the execution of the project. • The budget outlined in the proposal is appropriate. • Unrealistic to finish the project within the timeline. • The vast arrays of experiments defined in the two specific aims are unlikely to be finished in a statistically coherent manner within the proposed timeline. • A team or collaborators have not been defined. There is only one student and one postdoc both to be named, which even then would still be short staffed.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• PI has adequately addressed and accounted for the diversity of race, sex, gender and age in the proposal. • These factors have been considered for the future impact of the work. Sex differences will be explored using stem cells from different embryos. • The outcomes of this project would have application to regenerative medicine discovers in underserved population including racial and ethnic communities. • PI sufficiently describes plans for outreach, partnership activities to inform the development of DEI with the research project. • There is a reasonable statement of prior work with outreach and education to underserved communities, and the institution is well positioned for this work.
No: 0	*none*

Application #	DISC0-13822
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 (GI) 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)	• Standardizing the generation of enteric neurons from stem cells of diverse backgrounds • Screening for and 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	(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	1
Highest	82
Lowest	80
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 14	• The project is highly significant, because it will allow efficient generation of enteric neurons to be used in transplantation into patients affected by diseases of the enteric nervous system (ENS). • The project could have a major impact on applications of stem cell research in treatment of various gastrointestinal (GI) conditions with impaired motility. • Yes, this could support a major knowledge and technology gap for ENS and GI problems. • May provide a paradigm shift in our approaches in therapies for GI disorders. • Novel, excellent and competitive in this space. • No concerns.
No: 1	*none*
GWG Votes	**Is the rationale sound?**
Yes: 14	• The general premise is sound. The project proposes the use of patient-derived induced pluripotent stem cells (iPSCs) for derivation and transplantation of autologous enteric nervous system (ENS) grafts. • Aim 1 (standardizing the generation of enteric neurons from stem cells of diverse backgrounds) demonstrates the applicant's clear understanding of the true challenges to applying iPSC technologies - i.e., differentiation, lineage specification, and heterogeneity of iPSC lines. • The rationale for the scRNA work up in Aim 1 is unclear. How will this data be utilized? • Aim 2 (screening for and developing methods to speed up the process of enteric neuron production from stem cells) is not aligned with the rest of the proposal. • If Aim 3 (evaluating the ability of stem cell derived enteric neurons to survive in the host tissue and rescue the disease in mouse models) is successful, this would be a significant step towards developing an effective cell therapy approach for treatment of gastrointestinal (GI) motility disorders. • Preliminary data support feasibility of Aim 3, but the aim is still risky. The details, controls, and parameters should be more clearly outlined. A study providing evidence of functional human ENS post-transplant would be ideal. • The applicant has a related research article under review. It shows that human iPSC can generate ENS and ENS progenitors that engraft in the colon of adult mice. This work supports the current proposal - e.g., it includes a protocol for differentiating human iPSC, into enteric ganglioids, and characterization of these ganglioids by RNA-seq. • In Table 1, it is not clear if the iPSC lines described are clones or bulk lines. Although three to five independent experiments are suggested, clone determination would assist in efficiency related future work to increase numbers of ganglioids generated. • This is a unique and novel combination of technologies and biology, put together strategically. • The project is based on a previously published work and promising preliminary data. • No concerns.
No: 1	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 7	• Aim 1 is a rational extension of prior work - examining additional PSC lines. This aim is feasible based on published and preliminary work from this laboratory. • The single nuclei RNA-seq data show high similarity between PSC-derived enteric ganglioids and mouse and human primary enteric neurons. • Comparing additional lines would be ideal if the applicant could use techniques for quicker assessment, but these eight lines will provide important and meaningful results. • Increased efficiency of enteric neuron production from stem cells is an important goal, but Aim 2 is not adequately detailed. How will the results from the studies proposed in Aim 2 be used to increase efficiency of the process, while not altering the composition of the differentiated neurons? • The design is logical and the project is well-planned. There might be scientific overlap with published work and/or work supported by another award.

No: 8	• Chemical screening for acceleration of differentiation (Aim 2) seems unnecessary to this project. Aim 3, and other aspects that seek to achieve an understanding of ENS transplantation, are more valuable. • The screen in Aim 2 is not sufficiently justified. • Aim 2 needs to be better developed. • The models for engraftment need further refinement. • The applicant proposes studies in NSG and NOS1-/- mice. Why are two mouse lines proposed, and how will NSG inform the project goals or downstream studies of function? • The Aim 3 in vivo work is presented in detail, but alternative plans for poor engraftment or function of transplanted cells are not provided. I.e., if observation suggests things need to changed, how will this be decided? • Previous and preliminary engraftment data are the most impressive aspect of the proposal. However, functional evidence beyond phenotype is lacking. • I have some confusion about what is proposed here versus what has already been done.
GWG Votes	**Is the project feasible?**
Yes: 15	• The laboratory is technically and scientifically well-equipped to achieve the goals of the proposed specific aims. • The applicant has extensive expertise in this area and the appropriate tools. • Yes; the applicant is a rare expert in the field of ENS. • There are limitations in Aim 3 due to the use of Nos1 full knockout mice as there will be non-ENS effects of Nos1 loss. The requirement for immune suppression in the transplant studies in mice will require key controls for analyses. While acknowledged, controls should be more adequately described. • There is some missing information, and it's not always clear what was submitted for publication versus what is proposed. • Overall, yes, though I have some concerns about the experimental design. • No concerns.
No: 0	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• The proposal includes generation of iPSC lines from donors representing different racial and ethnic groups. • Racial and gender diversity are accounted for to some degree in the hiPSC lines; age is not discussed. • No concerns.
No: 0	*none*

Application #	DISC0-13810
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 how mutations in chromatin regulatory proteins leads to neuronal defects in monogenic Intellectual Disability Syndromes (IDS).
Impact (as written by the applicant)	Our study of IDS will determine links between mutations and neuronal dysfunction.
Major Proposed Activities (as written by the applicant)	• Develop three in vitro human pluripotent stem cell (hPSC) derived models of three distinct Intellectual Disability Syndromes (IDS) • Determine which IDS show neuronal senescence in vitro • Identify the best culture method to recapitulate findings from IDS 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 IDS neurons
Statement of Benefit to California (as written by the applicant)	The project described here will bring great benefit to 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 1:10,000 live female births, so in a state like California, thousands of families are suffering with no treatment options.
Funds Requested	$1,500,337
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	3
Highest	86
Lowest	70
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	1
(1-84): Not recommended for funding	14

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 14	• Yes. This project uses induced pluripotent stem cell (iPSC)-derived neurons from human donors with monogenic intellectual disability syndromes (IDS - MECP2, KAT6A/B, CDKL5, etc.) to test the hypothesis that DNA damage and neuronal senescence play an important role in the development of these IDS. This represents a major gap in our knowledge - how do these mutated genes cause IDS, and are there shared mechanisms (such as DNA damage and/or neuronal senescence) that are targetable? • There is compelling preliminary data suggesting that a shared, targetable pathway underpins several neurodevelopmental disorders. The identification of shared mechanisms in monogenic IDS is arguably the major bottleneck in the field. • I have some concern about the hypothesis that DNA damage and cell senescence are key drivers of monogenic IDS. Similar genetic pathways appear to be involved in overgrowth syndromes (EZH2, NSD1, BWS, etc.), which are unlikely to be driven by DNA damage or cell senescence. • Generally, this is an excellent proposal to study the pathogenesis of monogenic IDS. The project could lead to major breakthroughs in the field and new approaches to treatment. • Yes. Understanding the link between these genes and their mechanisms of action would advance the field considerably. There are also some major side questions built into the proposal that would advance science beyond testing the applicant's hypotheses. • The applicant addresses the interesting question of whether induction of stress and senescence in neurons represent a common cause for a variety of IDD pathologies. If so, stress and senescence processes could be exploited for therapeutic intervention. • The applicant has a novel hypothesis that addresses a bottleneck - not knowing how specific mutations cause specific IDD pathologies - in the field. • This approach could lead to a unified target for many IDDs where the specific causative mutation is known, but its contribution to the phenotype remains unclear (e.g., MECP2 and Rett syndrome). • Yes. Despite high and increasing prevalence of IDS worldwide, we still have very limited understanding of the molecular pathophysiology of these syndromes. This project holds promise to fill some important gaps in this knowledge. • The impact is likely to be significant (it may significantly impact our understanding of other neurodevelopmental disorders), but not highly impactful. • No concerns.
No: 1	*none*
GWG Votes	**Is the rationale sound?**
Yes: 13	• Yes. The applicant previously identified a correlation between transcriptional and cell physiological aberrations and premature induction of senescence and induction of p53 in human iPSC derived Rett syndrome neurons - they now propose that premature senescence is causative and is a hallmark of IDD even if causative mutations have not been identified. • Yes. There are strong preliminary data from multiple models that support of the proposed hypothesis. This is the next logical set of experiments to test the hypothesis further. • There is a strong element of rigor built into the proposal. It is evident that the applicant has experienced the pitfalls of iPSC research and has been thinking deeply about experimental design to address these issues. • Overall, yes, although some statements in the proposal background are inaccurate to my understanding ("discovery of at least 80 monogenic ID syndromes", "zero effective treatments for ID syndromes" etc.). That said, this project will yield directly relevant data with strong translational potential for the specific diseases studied. • While the overall rationale for proceeding with these studies is sound, the rationale for the applicant's hypothesis is not clear - why should DNA damage build up in quiescent (not dividing) neurons? In addition, Ataxia Telangiectasia (AT) - a disease defined by DNA damage and premature aging - is not known to induce an IDD. AT is involved in P53 regulation and should, according to the applicant's hypothesis, cause a classical IDD. • Yes - the rationale is very clear and logically presented and the preliminary data are supportive of the current proposal. • No concerns.

No: 2	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 8	• Yes, very well-controlled, excellent hypotheses with well-designed studies that should yield important results regardless of the outcome. I was especially impressed with the Aim 1 multiple neuronal-derivation experiments - they have a high degree of rigor. • The hypothesis that IDD genes are involved in stress response and senescence will be tested in Bohring-Opitz, KAT6 and CDKL5-Rett Syndromes which have mutations in chromatin regulatory genes (like MECP2). This is reasonable. • It would be import to include an IDD disorder that does NOT involve a epigenetic regulator as a control. • Yes. MECP2- neurons in the Rett Syndrome brain show mitochondrial dysfunction and unregulated stress response to DNA damage blocking P53 activity; senescence can reverse neuronal defects in Rett Syndrome organoid cultures. • Yes, although it is not very clear how applicant will meet their goal of describing the direct link(s) between mutation, dysfunction, and activation of stress pathways in each IDD. How will those links be established? • Pitfalls are described for Aims 1 and 2, but not for Aim 3. The pitfalls described for Aims 1 and 2 are not very substantial. • Limited discussion of pitfalls, and no detailed alternate approaches. No technical pitfalls presented. It seems that the experiments were designed with a deep knowledge of the potential pitfalls, but these were not explicitly stated.
No: 7	• Additional central nervous system (CNS) disorders could be included.
GWG Votes	**Is the project feasible?**
Yes: 12	• The aims are quite logical but the experimental plan is difficult to follow (and not necessarily presented in the most logical order). This is a very ambitious project, but achievable by a large team of dedicated scientists. The team is very qualified to carry out this proposal. • Yes, given that the iPSC lines have already been generated. There is strong institutional support to make the timeline feasible. • Patient lines are already in hand and preliminary data suggest feasibility. • Good collaboration between the Principal Investigator and an MD/PhD pathologist. • There seem to be experienced technicians/trainees already in place. • No concerns.
No: 3	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 8	*none*
No: 7	• Only superficially - the applicant states that the pathologist collaborator will ensure diversity, but does not provide specifics about how that would be defined or checked by the Principal Investigator. • This is vaguely (almost like "in passing") mentioned with a statement indicating that the samples collected for the production of hiPSC lines will cover a diverse group of patients. More details are needed here (e.g. what is a diverse group, inclusion of different genders, etc.). • The applicant has not described prior efforts or proposed plans for outreach, partnership, or educational activities to inform the development of DEI within the research project - diversity among the lab members is not what is looked for here. • The applicant includes a brief DEI statement centered on diverse trainees, but does not describe prior efforts or future plans. • Needs major reconsideration. • Further thought on DEI is needed. • Short, not very informative section.

Application #	DISC0-13823
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 induced pluripotent stem cell (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 Parkinson's disease (PD), due to the lack of effective therapeutic targets. The success of proposal will help better understand a potential target and develop therapeutics.
Major Proposed Activities (as written by the applicant)	• Characterization of disease relevant phenotypes in induced pluripotent stem cell (iPSC)-derived neurons. • Evaluation of the disease modifying activity of the compound series. • Reveal the pathogenic mechanisms Parkinson's disease (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	$1,578,001
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: 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	84
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: 15	• The project is designed to address disease mechanisms in Parkinson's disease (PD); these are still largely unknown. Therefore it addresses an unmet need. It has the potential to materialize into new therapeutic strategies and targetable mechanisms.

	• This proposal is based on data generated with prior CIRM funding that revealed that the mitochondrial outer membrane protein Miro1 accumulates on damaged mitochondria in neuronal models of both genetic and sporadic PD. The PI has also reported that reducing Miro by either RNAi, or using a priori identified compounds, rescues some aspects of the PD phenotype, and suggests that functional impairment in Miro degradation and mitophagy may be common to several forms of the disease. • While dysregulation of calcium flux has been reported in PD models extensively, data from this proposal may additionally shed light on the role of Ca+2 in Miro1 accumulation and the interaction of Miro1 with other proteins. • A shared mechanism in familiar and sporadic cases with a druggable target would be a major advance. • This could have a clear impact on PD.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 10	• The overall motivation and rationale is good and based on solid preliminary data. • Yes - strong preliminary data motivate this proposal.
No: 5	• I would advise the applicant to incorporate more cell lines and provide more detail regarding the cell lines from patients with sporadic PD. PD has a wide spectrum of symptoms and, possibly, causes. Using two sporadic cells lines is too restrictive to gain insight, in my view, into the role of Miro1 in development of PD. Sex as a biological variable also needs to be considered. It seems from Table 3 that the selected cell lines are exclusively female. • The exclusive use of induced pluripotent stem cell (iPSC)-derived neurons may dampen the relevance of the data, given that aging, critical to neurodegenerative disease, cannot be taken into account in an iPSC-derived cell line. • Data provided in Figure 5 seem to relate to the prevention of deficits when the treatment is applied prior to degeneration. This is clearly less relevant to the clinical context as diagnosis is made when symptoms have appeared and degeneration is well underway. • The main concern is whether or not the proposed model will be relevant for PD. The basic insights gained will likely be very valuable, though. • The claim that this proposal will lead to the identification of the first disease-modifying approach is overstated.
GWG Votes	**Is the project well planned and designed?**
Yes: 9	• In aim 1, the applicant will characterize disease related phenotypes in PD neurons and evaluate the effect of the applicant's selected of bioactive compounds, using cell death as a read-out. Compounds with the most compelling profiles will be used in further mechanistic studies. Aim 2 will address the applicant's hypothesis that Ca dysregulation may be a mechanism of Miro1 accumulation in PD. • Overall well planned and designed, but a major weakness is the cell model of choice. iPSCs are now well known to reset cellular age; thus, one of the major risk factors (age) for late onset neurodegenerative diseases such as PD is not modeled. This may affect the applicability of these study results to human disease. • Moreover, in their model, the pathology develop after exposing the cells to stress - this will make it uncertain if findings are related to PD pathology or stress generally. • The data generated from this project would be relevant and highly complementary to previous work by the applicant, providing a more global view of mitochondrial issues related to all forms of PD. • Potential pitfalls are partially addressed for Aim 1; the discussion of potential pitfalls for Aim 2 is inadequate. • The timeline is well thought out and feasible.
No: 6	• The applicant needs to include a means of dissecting the role of stress versus PD in their study model.
GWG Votes	**Is the project feasible?**
Yes: 14	• The team is well poised to carry out this project. • Yes, no concerns. • Overall, the work envisioned in this proposal is ambitious but feasible within the timeline (given the preliminary data and team expertise).

	• The documents available for review do not include Biosketches for co-investigators nor letters of collaboration. The involvement of two post-doctoral fellows is mentioned and the role of collaborators is described elsewhere in the text. • At first glance, there does not seem to be direct overlap between the proposed grant and currently funded work - however, there is a relatively close relationship between all ongoing projects in this laboratory. • The in vitro models and compound exploration are reasonable strategies, in particular for the mechanistic investigation.
No: 1	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• Yes. • Yes, no concerns. • The application and the project plan cover this well.
No: 1	• The applicant mentions that the experimental design will include both sexes and iPSC cell models be matched for age. The applicant does a very good job at describing how, within the laboratory or through committed efforts of the institution, they will pay particular attention to this. However, there is no other mention as to how race or ethnicity will be considered in the choice of cell lines.

Application #	DISC0-13697
Title (as written by the applicant)	Targeting adipocyte progenitor cells to treat age-associated obesity
Research Objective (as written by the applicant)	To obtain new understanding of why a newly identified population of human adipocyte progenitor cells (CP-As) differentiate unstoppably into fat cells during aging, and determine whether targeting CP-As might prevent or treat age-related obesity.
Impact (as written by the applicant)	Why visceral fat expands during aging is unknown. CP-As - a highly adipogenic set of progenitor cells - have been identified but not completely characterized. There is an urgent need for safe and effective obesity treatments.
Major Proposed Activities (as written by the applicant)	• We will determine the capacity of primary human CP-As to proliferate and differentiate into adipocytes and other cell types in 3D culture and in vivo transplantation assays. • We will use our newly generated NewFAT-ATTAC mouse model to determine if killing new adipocytes generated during aging would prevent obesity and improve whole-body insulin sensitivity and metabolism. • Investigate if LIFR, a gene enriched in human CP-As, plays a critical role in promoting CP-A differentiation into adipocytes and if targeting LIFR would inhibit CP-A differentiation and fat expansion. • Determine if the macrophages in human visceral adipose tissue stimulate the transformation of human adipocyte stem cells into CP-As. • Screen for small molecule compounds that inhibit CP-A differentiation into adipocytes. Initial screening with primary mouse CP-As, and secondary screening with primary human CP-As. • Lead compound selection in aged mice based on the efficiency of preventing visceral fat expansion during aging and improving whole-body insulin sensitivity and metabolism.
Statement of Benefit to California (as written by the applicant)	California has the largest number of older residents. Many underserved communities reside in "food deserts," located in many large cities in California. For older, less-mobile individuals living in underserved communities, food deserts impose an additional barrier to healthy food, leading to a high obesity rate. Despite the urgent medical need, effective and safe obesity treatments are limited. This proposal is devoted to finding preventive medicine for age-related obesity.
Funds Requested	$999,924
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	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: 15	• The project defines a new paradigm for treating or preventing age-associate obesity by targeting adipocyte progenitors and inhibiting adipogenesis. This is a novel and innovative approach with very high significance to impact the tremendous burden of obesity. • The knowledge gap defined here is limited to a very specific, although potentially important, cell type in adipose tissue. It isn't yet clear that this gap is related to the application of stem cells as a therapy. • The applicant has identified a novel age-associate adipocyte progenitor population (CP-A) with potential for major impact on our understanding of age-associated obesity. CP-A potentially provides a new therapeutic target for prevention of obesity. • This is a high-risk, high-reward project. It is not clear that mechanisms identified in the mouse model will extend to humans. It is also not clear whether the in vitro characterization of human cells will provide a relevant context for development of obesity. • This project aims to characterize a special type of adipose progenitor cell and screen for compounds that inhibit adipogenesis in this cell. • The relevance of this proposal to CIRM's mission to promote stem cell or genetic research is questionable. • Obesity is a burden to modern society. The applicant proposes to target a special adipocyte progenitor cell population to prevent age-associated obesity. This could have a big impact on the obesity research field (if successful). • The applicant primarily proposes the use of human cell culture to study the differentiation of progenitors to mature fat cells. It is unclear how relevant this will be to the development of obesity in vivo. • This new application seeks to combat age-related obesity by modulating the behavior of adipocyte progenitor cells. Since the accumulation of white visceral fat in aged individuals is very prevalent, any approaches that could directly target fat deposition are very welcome. • This grant could lead to pharmacological agents that inhibit the ability of adipocyte progenitor cells to differentiate into mature adipocytes. • In addition to the medical impact, a good bit of information about adipocyte biology in the context of aging may be forthcoming (if successful). • Intriguing problem to explore. • No concerns.
No: 0	*none*
GWG Votes	**Is the rationale sound?**
Yes: 11	• The concept of targeting obesity by prevention of new adipogenesis is novel and sound. It's an idea worth further exploration. • This proposal is based on the assumption that white adipose tissue expands with age through adipogenesis from adipocyte progenitor cells. • The proposal presents compelling preliminary data suggesting that adipogenesis associated with aging is linked to an LIFR-expressing (LIFR+) adipocyte progenitor cell population in mice (CP-A). • Preliminary data from mice indicate a direct role of LIFR in CP-A cell adipogenesis - thus, LIFR is both a potential target and a CP-A marker. • LIFR was found as a CP-A marker, and inhibition leads to decreased differentiation to mature adipocytes. • Preliminary data in human cells are limited to single cell sequencing-based evidence of a LIFR+ population of adipocyte progenitors in white adipose tissue. The proposal would benefit from additional preliminary data from human cells.

	• The proposal suggests that 1) CP-As exist in both aged humans and mice, 2) LIFR is a surface marker for both mouse and human CP-As. • However, the evidence that humans also have this adipogenic progenitor cell population is based on RNA expression data (RNA-seq). More evidence from protein level analyses is required. • Overall, the preliminary data show that age does indeed affect adipocyte progenitor cell proliferation and differentiation (currently largely unpublished). However, there is a large amount of reasonable preliminary data in this grant that supports the approach. • The cited reference showing adipocyte proliferation (reference 18) is central to the rationale, but appears to be at a preprint stage. This preprint contains the bulk of the preliminary data presented in this proposal. • Fig. 5 shows that older APCs outcompete younger APCs in a transplantation model, suggesting that older APCs either divide more or survive better than younger ones. • scRNA-seq studies have identified CP-A populations (committed pre-adipocytes) and candidate markers for these cells. Transplantation studies of young versus old CP-As into contralateral sides of the same recipient mice suggest higher proliferative activity of the older CP-As. • This project is relevant to human biology and disease. • Interesting preliminary data. • No concerns.
No: 4	*none*
GWG Votes	**Is the project well planned and designed?**

Yes:
9
	• The aims are logically designed to provide a deeper characterization of human CP-A cells in vitro and in vivo, to explore the role of LIFR and macrophages in CP-A adipogenesis, and to screen for compounds that inhibit CP-A adipogenesis.
	• Overall, the research plan is to see if the findings obtained in mice are consistent in human cells. The source of human cells will be human cadaver adipocyte progenitors.
	• The complementary use of mouse and human models, and in vitro and in vivo analysis, is a strength.
	• Screen design is well-considered in the murine reporter line, with validation in human cells.
	• LIFR gain and loss of function studies are well-designed.
	• Macrophage interactions with CP-A cells are tangential to the overall goals and cursory in their design.
	• Overall, yes. However,
	  ◦ Under Aim 1, the applicant plans to validate the adipogenic capacity of human CP-As; but then in sub-Aim 1b, the applicant proposes to use a new mouse model to eliminate CP-As in mice. This is a bit confusing.
	  ◦ The planning in Aim 2 is also unclear. First the applicant proposes to test the role of LIFR in human CP-A adipogenesis; then in Aim 2b, the applicant will study the role of macrophages in the generation of CP-As in mice.
	• Alternatives are not addressed if conservation of mechanisms across mouse and human are not observed, especially in Aim 1.
	• The alternative approaches are insufficient. For example, the proposal does not address the potential problem that a LIFR inhibitor may have broader off-target effects.
	• Potential pitfalls of consequence are insufficiently addressed in Aim 1. For instance, what if human cells do not behave as mouse cells?
	• Aim 3 is only relevant if Aims 1 and 2 are successful, so Aim 3 may be somewhat preliminary at this stage.
	• The timeline is reasonable.

No: 6	• LIF inhibitors may have off-target effects that need to be considered. • The work with macrophages needs to be better designed.
GWG Votes	**Is the project feasible?**
Yes: 13	• Aims are logically designed and feasible in the proposed three-year timeframe. • The team has the expertise needed for the proposed study. Many of the model systems are already in place and they have expertise in the relevant assays. • The applicant should re-work their aims - e.g., they might put all the work related to mouse in one aim, and then extend to human studies in subsequent aims.

	• The team is qualified for this type of work. • The team has access to necessary resources for this research. • The proposed budget is reasonable. • Yes. This looks achievable in three years. • Overall, yes, but the screening in 384-well format may be daunting in terms of feasibility. I am concerned that that robotics, liquid handling, automated cell culture, and other high throughput approaches may prove difficult. • No concerns.
No: 2	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• The project accounts for age and sex in the experimental design. • Obesity affects broad populations, including racial/ethnic underserved communities. Outcomes have the potential for broad impact. • The proposal describes efforts at the institution for outreach, particularly in cancer, but the role of this team and this project is not clear. • Overall, yes, but this project did not account for race/ethnicity. • Obesity affects underserved populations. • Yes, but it is unclear to me what the applicant's prior DEI-related efforts are. • Yes - obesity is highly prevalent in underserved communities, and the research plan, if successful, could lead to interventions available for these individuals. Overall, the diversity, equity, and inclusion statement is strong and demonstrates a recognition of the problem of obesity that is felt especially among these groups. • White adipose tissue will be obtained from cadavers that are diverse in origin. • Yes, but there doesn't seem to be much discussion of outreach and education in the grant. • No concerns.
No: 0	*none*

Application #	DISC0-13706
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	$1,472,857
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	83
Lowest	74
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 14	• Cystic Fibrosis is a monogenic genetic disorder that affects the airway mucus layer due to mutations in the CFTR gene. There are no therapeutics allowing permanent corrections of the gene defect in all patients, in particular due to difficulties to deliver gene therapy to airway basal stem cells (ABSCs). This proposal aims is to develop a gene therapeutic strategy that results in effective delivery to ABSCs using gene-editing tools, packaged into nanoparticle-based carriers. • If successful the impact of the project will be high. If successful, the outcomes of this project will not only be applicable for delivery of gene editing reagents for the correction of CF, but also for other lung diseases and for other diseased epithelia and organs that require the targeting and modification of long-lived stem/progenitor cells of the epithelium. • The study addresses delivery of gene therapy for cystic fibrosis to airway basal stem cells. Delivery is a major barrier to success. • If the new delivery system were successful the study would have a major impact. • New technologies for gene delivery might be the most important outcomes of this project. • The project is highly significant, it has a potential to generate an effective strategy for treatment of Cystic Fibrosis.
No: 1	*none*
GWG Votes	**Is the rationale sound?**
Yes: 13	• The project is based on a strong scientific rationale that introduction of a wild type gene into long-lived ABSC will result in sustained alleviation of CF pathology. • Project is very relevant to an important genetic disease. • Mucus which builds up in the airways presents a serious barrier to gene delivery that need to be overcome and it is important to target long-lived ABSCs responsible for the renewal of the airway epithelium. • Delivery systems, surfactant excipient and gene editing tools are convincing. • The underlying premise is sound, but the model may not capture the key features of the disease-the model is normal epithelium from the upper airway, not CF epithelium or distal airway. • Data on model do not show gene editing, not clear if submucosal glands are exposed.
No: 2	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 6	*none*
No: 9	• The proposed aims are logical and based on the previous track record and expertise of the investigators. However, it is very important to include experiments where epithelial injury and efficacy of the resultant gene therapy product are tested in the models of airway tissues and Air-liquid cultures derived from CF patients. Tracheal tissue explants are not sufficient as bronchial epithelium is the target tissue here. • Ex vivo upper airway model does not capture critical aspects of the disease phenotype - it is a tracheal model. Air-Liquid cultures suffer the same limitations, and do not model submucosal glands. Not clear if excessive mucus is deposited in CF model. • While the overall project strategy is reasonable, the applicants failed to account for complications related to additional mucus accumulated in CF airways and inflammatory milieu of CF airways on the delivery of CFTR gene to the ABSCs. • No study with patient derived tissue provided. • Plans to develop nanoparticle carriers are sound, however gene editing will not be assessed directly in the airway model. • CFTR null airway stem cells are assessed in aim 3 but no preliminary data are shown. This should be the primary model for the entire study and it should be validated. • Investigators do not acknowledge limitations of the model. • A key functional experiment is needed.
GWG Votes	**Is the project feasible?**
Yes: 13	• The preliminary data show feasibility and capability of the research team to deliver such project.

	• Interdisciplinary team, strong expertise in gene therapy and materials science.
No: 2	• The project is feasible for the delivery of CFTR gene to normal airways, but the applicants need to demonstrate the feasibility of the strategy to CF airways.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• CF predominantly affects Caucasians but the study takes into account a significant Hispanic patient population. • CF affects people of different races and ethnic groups, and the outcomes of the project will benefit a broad range of patients, including underserved communities. • The applicants state that they will ensure that they will obtain human samples that represent all racial and ethnic groups, although it is not clear how they are going to account for differences between different patient populations.
No: 0	*none*

Application #	DISC0-13748
Title (as written by the applicant)	Role of eIF4G2, a putative regulator in translation initiation, in pluripotent and somatic stem/progenitor cells
Research Objective (as written by the applicant)	To reveal novel mechanisms of gene regulation important for multiple stem/progenitor cell systems 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 (iPSCs) to reveal novel mechanisms in gene regulation in multiple stem/progenitor cells. • We will analyze mouse models to reveal and fully understand novel mechanisms of 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: 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	4
Highest	84
Lowest	70
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	14

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 13	• This project will test the hypothesis that eIF4G2, a putative translational regulator, plays a fundamental role in translation, especially in stem/progenitor cells. This hypothesis shifts the paradigm that most mRNAs are translated by the eIF4G1 complex, and eIF4G2 is involved in exceptional cases of non-canonical translation. • If successful, this project will significantly change our understanding of the mechanisms of protein translation. The outcome will significantly advance world class science. • The proposal is highly biochemical and biophysical in nature; the fundamental biology is well described in full detail. • Little is known about eIFG2 in stem cells. • Feasibility is an issue, as is prioritization of the vast number of experiments proposed. • Specific relevance to stem cells is not clear. • The applicant seeks to understand the role of a ubiquitous protein (eIF4G1) that is involved in protein translation and biological function (including in stem cells). The proposal therefore does address a biological gap. • In terms of project significance, it's not clear why CIRM should prioritize funding research on this particular protein over many others that are also necessary for proper maintenance of pluripotency during development. • No concerns. • Partially.
No: 1	*none*
GWG Votes	**Is the rationale sound?**

Yes: 10	• The hypothesis is based on a strong rationale:
	  ◦ eIF4G2 is evolutionarily conserved,
	  ◦ eIF4G2 is more abundant than eIF4G1 in the majority of tissues, and
	  ◦ it is unlikely that such an abundant and evolutionarily conserved protein exists just to translate a small number of target mRNAs.
	• In mouse embryos, eIF4G2 deficiency results in embryonic lethality during gastrulation. In pluripotent stem cells (PSCs), eIF4G2 deficiency shows complex phenotypes depending on the pluripotency state (naïve vs. primed) of the PSCs.
	• The applicant's preliminary data provide compelling evidence that in addition to its critical role in PSCs, eIF4G2 is necessary for maintenance of adult somatic stem/progenitor cells.
	• This fundamental research project is of high significance to human biology.
	• The rationale is sound, but not well described in the proposal.

No: 4	• Preliminary studies shows that a mouse with conditional knockout of the eIF4G2 gene has anemia (a decrease in red blood cells) and histological abnormalities in the small intestine (both in vivo and modeled in intestinal organoids). • The conditional eIF4G2 knockout mice have a hematopoietic and gastrointestinal phenotype. However, the applicant does/will not provide sufficient evidence that these phenotypes derive from a stem or progenitor stage. • Binding partners of eIF4G2 have been identified in preliminary studies, but their spatiotemporal distribution is not established. • It's unclear how the role of eIFG2, whatever it turns out to be, will be distinct in pluripotent stem cells (PSCs), progenitor cells, and somatic cells. The applicant does not propose detailed experiments to provide such distinctions. • Although the proposed studies are likely to lead to important fundamental understanding of protein translation, it is unclear how this will impact the field of stem cell biology. • It is unclear how the study findings will be applied to stem cell biology or stem cell applications. E.g., will the findings be relevant to inducing differentiation? Specifying lineages? Drug targeting of somatic stem cells? • The project is not stem cell-specific. • In Objective 2, the applicant assumes that gene regulation by eIF4G2 will manifest in human as it does in mouse PSCs. The rationale for this assumption is unclear. • Evidence for candidate gene regulation by eIFG2 would have strengthened the basis of Objective 2. • Objective 3 - to delineate the mechanism of action of eIFG2 - is not related to stem cells, nor do the proposed studies use stem cells.

	• Redundancy between the function(s) of eIFG1 and eIFG2 should be evaluated in knockout or other eIFG1 or eIF4G2 mutant cells. • The applicant should propose isolating a stem cell fraction from the conditional eIF4G2 knockout mice and using CRE induction to observe the effects of eIF4G2 depletion during stem or progenitor stages, and outside of niche environments. • The work is unnecessarily dense and each aim could be a proposal in itself. • The basic science rationale is sound but not of the highest priority for the field. • It's not clear to what extent this will influence priorities in human biology.
GWG Votes	**Is the project well planned and designed?**
Yes: 7	• Overall, yes, but the project plan detail is insufficient.
No: 7	• The project is designed relatively well. It is mouse-centric, with a few experiments in human cell culture systems. • The proposed project is descriptive and includes great deal of omics characterization - including but not limited to DNA-microarrays, scRNA-seq, proteomics, global mass spectrometry, and CRISPRi library. The results might be meaningful, but tremendously large in volume and lacking a strong explanation or rationale on how to prioritize. • The design of this project is not sufficiently focused. The rationale for, and expected outcomes of many proposed experiments, are not clear. • The proposal needs focus. • There are known and well-documented human-specific signatures of both pluripotency, iPSCs, and progenitor cells as well as a dynamic of differentiation not adequately addressed here. Incorporating this background knowledge might inform the applicant's priorities for mouse versus human experiments. • In some cases, it's unclear why a proposed study will be conducted using mice, mouse PSCs versus human PSCs. • The rationale for studying PSC in both naïve and primed pluripotency states is unclear. The applicant should include experiments early in the proposal to establish a rationale for using PSC in one state, or the other. Alternatively, if there is a rationale for differences in eIFG1 function in naïve vs. primed states, how would this be identified or incorporated in the studies? • Details of the CRISPRi screen are insufficient; also, it's not clear how the screen will or will not be used in conjunction with the gain-of-function and loss-of-function experiments. • How will the gain-of-function and loss-of-function experiments be prioritized and done? How will the results be incorporated into our understanding of the function of eIFG2? • No validation of RNA/protein ratio is suggested. How will this be prioritized? • Many of the experiments are not needed to prove the hypotheses. • The project is not extremely urgent to CIRM's mission. • The budget rationale is appropriately justified.
GWG Votes	**Is the project feasible?**
Yes: 8	• The project can be achieved within the timeline. • Two postdoctoral fellows plus a research investigator (staff scientist) might be borderline for the vast array of omics and analysis (especially when done in duplicates or triplicates). • The institution is perfectly suited for the successful accomplishment of this project. • The budget is appropriate for this project.
No: 6	• My concern is that the amount of work proposed here cannot be accomplished by the personnel (40% Co-Investigator, 50% Postdoctoral Fellow, 15% Project Manager, three 10% Research Assistants) in three years. • The budgeted staff will not be able to complete the aims. • It is unclear how the depth of scRNA versus proteomics work will be determined to ensure that a large enough subset is analyzed and cross-matched. Is there a pre-set extent of discrepancy or ratio, or, how will these be determined and validated? • Given the proposed work and lack of detail in the project plan, it is difficult to envision this being completed in three years. This is a massive undertaking and requires prioritization. • The team is appropriately qualified and staffed, but experts in somatic stem cells should be included. • This is a massive and unnecessary undertaking.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**

Yes: 14	• The applicant states that they will be able to utilize "iPSC cells derived from multiple donors covering different race, ethnicity, sex, gender and age diversity," however they do not provide more detailed information or power calculations. • The project does not outline any efforts for outreach or educational activities to inform the development of DEI. • The project's plan minimally addresses the influence of race, ethnicity, gender and age diversity. • The project outcomes do extend to some underserved populations. • Well described and suitable. • No concerns.
No: 0	*none*

Application #	DISC0-13780
Title (as written by the applicant)	Neural Stem Cell Aging and Neurodegeneration
Research Objective (as written by the applicant)	Dissecting the mechanism of neural stem cell aging will advance our understanding of the biology of stem cells with implications in treating neurodegenerative diseases.
Impact (as written by the applicant)	Successful completion of the proposed studies could lead to the identification of a novel pathway regulating neural stem cell aging and a druggable target for treating Alzheimer's disease.
Major Proposed Activities (as written by the applicant)	• Determine the role of SIRT7 in neural stem cell maintenance and cognition. • Determine the mechanism by which SIRT7 regulates neural stem cell maintenance and cognition. • Determine the role of SIRT7 and the mitochondrial unfolded protein response in neural stem cell aging, cognitive aging, and Alzheimer's disease. • Determine the therapeutic potential of sirtuin activation to reverse neural stem cell aging, cognitive aging, and Alzheimer's disease.
Statement of Benefit to California (as written by the applicant)	The proposed research will advance our understanding of the biology of stem cells that is relevant to human biology and disease and provide Californian students a training opportunity for stem cell research.
Funds Requested	$1,574,807
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	1
Highest	80
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: 15	• This proposal is based on a significant amount of preliminary data suggesting that SIRT7, essentially is involved in suppressing mitochondrial protein folding stress – could serve as a meaningful target to enhance neurogenesis and consequently, cognitive function. • Given the absence of treatments for disorders of the brain which present with prominent cognitive decline, the implications of such findings are particularly important to the field of Alzheimer's Disease (AD). • The proposal has potential for generating knowledge that can be impactful for future therapeutic potential but the link to Alzheimer's is weak. • AD needs more treatments.
No: 0	• This project has not been proven to address a bottleneck, because despite excellent evidence for SIRT7 involvement in aging and Alzheimer's, analogous or stronger data in related model systems is available for many other molecules, all of which have presently failed to address cognitive decline in humans. The narrative presented here could be written for other sirtuins or other families of molecules. • There is no use of human omics data to prioritize targets, and then pursue those prioritized molecules, instead of what you already are studying. If you use the large and growing collections of single cell multi-omics and find SIRT7 is the nexus of decline in Alzheimer's disease, that's fantastic - then go ahead with this study. If this is about the basic biology of sirtuins and refining the mechanism of action of SIRT7 the same point above goes - there is no use of time-series data or molecular networks - mainstays of understanding development on a global scale, which are all pointing to SIRT7. Moreover nicotinamide riboside is already in trials, without huge success, though granted not powered for that. • What's proposed is precise, beautifully imaged molecular investigation but I don't see real input from disease biologists and from anyone with omics experience, which is what is now driving many molecular investigations, making this fine work very incremental. • If we spend this much time and money on hundreds of molecules - each with their own backers for relevance to Alzheimer's disease - we're not going to find a solution for a very long time. The proposed research is world-class but inefficient at this stage.
GWG Votes	**Is the rationale sound?**
Yes: 12	• Mechanistic studies of adult hippocampal neurogenesis is well motivated. Metabolism and mitochondria are a good focus. • The immunohistochemistry results shown are compelling for a role for SIRT7 in models of Alzheimer's disease. However, other molecules are not tested. A major scientific development of the last 20 years is in understanding the complexity of cellular responses and the insufficiency of the one-gene-at-time approach for having a transformative effect. • There seems to be some confusion on the limitations of C.elegans and mouse models of Alzheimer's disease. Showing effects in mice have repeatedly failed to translate to humans. Many molecules have passed the tests of relevance they propose. Human data integration is needed - ideally from diverse aged human brains with Alzheimer's disease. • While the use of the APP mouse model may well be justified here, I would have liked the applicants to present some arguments for this choice. In particular, describe the timeline of cognitive decline progression in these animals and the general pathology that is found in their brains. This would also help justify the time points chosen for intervention/treatment. • Along these lines, I see that only the Morris water maze was performed in preliminary data collection. What other tests have been planned and explain how they specifically relate to dentate gyrus function/anatomy? • I would have liked the applicant to provide more information on the AD patients targeted for the study of post-mortem tissue. I understand that the tissue has not been requested/obtained but knowing the profile of the patients targeted would have been wise (ex: stages of disease, cognitive tests and scores, co-morbidities to be excluded). • Along these lines, are the applicants planning to include tau and Ab staining in the analyses? Having some sort of idea of pathological load may be important to understand their data. Additionally, are statistical correlations between cognitive test scores and their immuno findings planned? There is no mention of these important factors/analyses in the proposal.

	• One overall weakness of this grant is that the justification for a number of experimental elements is too superficial. Because the proposal is so dense (with four proposed aims), it lacks important details that should have been mentioned to the reader/reviewer.
	• I felt that at times, the data was weak. Examples of this include:
	  • Figure 4a where the staining of SIRT7 is really not convincing. It also seems to be a rare event.
	  • Figure 5 – it is rather difficult to see what has been quantified on the images provided. This does not seem to demonstrate a strong effect.
	  • Figures 6 and 7 show statistical differences that are less than 20% (Figure 6) and 10-15% (Figure 7). I would consider this rather subtle effects.

No: 3	• Alzheimer's link is weak. • The link to AD is weak.
GWG Votes	**Is the project well planned and designed?**
Yes: 9	• One of the strengths of this grant is that it is extremely well laid out and the hypotheses are very clear. Figure 1 is an excellent example of this. The illustration of the aims into a schematic of the mechanisms studied is very clever. The sequence of work is logical, thorough, feasible. All experiments include a variety of approaches using cells, animal models, human tissue, KO models, imaging, pharmacological treatments to investigate the research question. • The technical side of imaging, cellular and animal models are adequate. • The project is appropriately planned and designed in terms of models and methodology, but group sizes seem small, both for behavioral studies and intervention studies. • Potential pitfalls are superficially discussed as the applicant rather argues that problems should be minimal by justifying their choices or by referencing back to their expertise rather than directly raising potential pitfalls and how these would be overcome if indeed encountered.
No: 6	• Primary human tissue data is needed.
GWG Votes	**Is the project feasible?**
Yes: 13	• Overall, the work envisioned in this proposal is very ambitious but still feasible within the timeline provided given the amount of preliminary data accumulated, the group expertise and the established collaborations. • The applicant has provided letters of support from an individual very well known in the field of aging and neurogenesis and who will assist the group with the characterization of the knockout models as well as one letter from an individual who will share expertise of 2-photon imaging. • The preliminary data is a very strong demonstration of the 2-photon microscopy they plan to employ, and results on related disease models. • The PI is very well published and externally well-recognized within this topic and (normalizing for career length) possibly one of the best to carry out this project. • The project is feasible but challenging in terms of sourcing aged mice.
No: 2	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• AD affects both men and women and the models take this into account. • All of these parameters are taken into account and explicitly stated into the DEI section as well as when describing the experimental design. • The PI has taken advantage of the space dedicated to DEI to provide several examples as to how they have already significantly contributed to DEI efforts within their laboratory practice, teaching but also by advising on various university committees and taking on a leadership role. The section of the proposal is very strong. • For this type of research, the main thing is to test in both genders. Possibly you could explore race effects on the mechanism, but then you'd need humanized/engineered mice and the budget swells tremendously and that's probably premature of this stage of research for this target. • You could sample human brains from diverse backgrounds, and there is one sentence related to this but no other mention of this that I can find, so it is unclear how it may fit in the plan.

	• Possibly extends or validates the applicability of regenerative medicine discoveries to underserved populations, but not specifically targeted to do so to the exclusion of well-served populations. • PI has a definitely above average and possibly excellent track record for educating diverse scientists. The activities listed in this regard require time and energy, so the personal commitment is quite strong, indicating the proposed work will come to pass.
No: 0	*none*

Application #	DISC0-13705
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)	• Develop scalable genomic technologies in human cells • 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	$1,577,425
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	78
Median	80
Standard Deviation	4
Highest	83
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	• A major bottleneck in the field of autism research is the task of evaluating the effect(s) of many of thousands of genes that affect neurodevelopment. This proposal uses a pooled technique to overcome this limitation. • This work uses 3D organoids derived from an induced pluripotent stem cell (iPSC) line that has been genetically modified to incorporate ten known autism spectrum disorder (ASD)-associated risk mutations. RNASeq and ATAC-Seq (Aim 1) and spatial transcriptomics (Aim 2) will provide insight into shared molecular mechanisms. There is a major gap in our understanding of the mechanism by which specific ASD genes affect neurodevelopment and function. This would be a hypothesis-generating study on this topic. • The spatial transcriptomics (Aim 2) have the potential to have a major impact. The studies in Aim 1 are more incremental as they are repeating common studies (scRNAseq/ATACseq) - just for ten new disorders. • The combination of ASD risk gene perturbations and spatial transcriptomics is powerful, and represents an advance in the field. • This more high-throughput system could accelerate development of targeted therapies. • This is the next obvious step following the applicant's excellent preliminary study. These data will likely provide novel insights into ASD biology that will be impactful across multiple fields. • This work would set the stage to develop many new hypotheses for additional avenues of exploration - not just in the applicant's lab but throughout the world. • ASD seems to involve a cell population that is not present in murine brains - the use of human organoid models is critical. • This is a potentially interesting project, however, in its current form it does not provide enough novelty as similar approaches have already been attempted. I am afraid that, even if successful, this project will not have a major impact on the field. • No concerns.
No: 2	*none*
GWG Votes	**Is the rationale sound?**
Yes: 13	• Yes, there is strong rationale for the proposal, although I'm not clear on the advantage of making an iPSC line with a collection of ten mutations when some of the corresponding single mutation iPSC lines have already been published. • It is clear that there will be data of interest to many people generated by this study. There are appropriate controls, with a high degree of rigor to the experimental design. • I have some concerns about the specifics of the iPSC modeling. the application may be improved by consultation with an iPSC/neurodevelopment specialist. • Strong preliminary data, I have no concerns that the project is technically feasible. Much of the work is predicated on a strong publication by the applicant from 2020. • The overall hypothesis that ASD risk gene perturbation may disrupt the expansion, migration, and lineage specification of progenitor cell types and contribute to disease is not new. It has been validated in a number of ASD organoid models. • The project will mainly accelerate already acknowledged approaches - no new concepts. • Rationale is sound, however the rationale for choosing these particular ten mutations is not entirely clear. This is particularly true for ASD. • Preliminary data are supportive of the current proposal. • No concerns.
No: 2	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 7	• Yes. This project includes hypothesis-generating experiments, which are likely to give meaningful results (and not as likely to be funded through traditional NIH mechanisms). There is significant thought to controls and statistical analysis. I appreciate the level of detail presented for the more novel/esoteric experiments proposed. • The pitfalls identified are somewhat limited, especially for Aim 1. A more robust set of potential pitfalls and approaches would increase my confidence.

	• Per the timeline there will be significant progress within the three year period, with translational potential evident within that time. • The applicant assumes that the selected ASD risk genes affect mainly cells in dorsal regions of nervous system. The potential impact of these genes on the function of ventral neurons, and/or indirect impact on dorsal neurons, is not considered. • The applicant will be able to identify cellular changes but the hierarchy of such changes may be difficult to interpret. For example if migration is affected, is that due to changes in the cells that are migrating, is it a consequence of other cells that affect migration in a paracrine manner, or are migration changes a consequence of altered differentiation or proliferation? • The flow of experimental steps is logical and well laid out, however, the potential pitfalls are not sufficiently well described. Also, confocal microscopy is not a high throughput methodology.
No: 8	• The organoid assay is not well defined. • The initially sequencing experiments require further refinement.
GWG Votes	**Is the project feasible?**
Yes: 12	• This is a very ambitious project for a three year period, but the applicant has a strong record of success with the required techniques. Given the strong record and preliminary data, feasibility seems likely. • Strong institutional support and a strong environment are evident. • The Principal Investigatory is a junior investigator (faculty as of 2021), but has plans to recruit a technician and student for the proposed work. My main concern is that there appear to be a small number of available people in the primary laboratory at this time, but given it is a new lab there should be growth. The supporting environment suggests that hiring/training people to perform the experiments is feasible. • Overall yes, although the applicant states that confocal imaging is inherently high throughput. This rational is not clear. • The project is feasible with a larger team of dedicated scientists, but here this does not seem to be the case. • No concerns.
No: 3	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 9	• Adequate.
No: 6	• Use of diverse materials is addressed, but the project will not particularly benefit an underserved population. Use of ethnic background cannot be used in making hiring decisions in California. • Yes - although as a Diversity and Inclusion Committee member and a Gender Equality Champion at my University, I must note that DEI is not about "prioritizing-" but rather, actively encouraging - the recruitment of trainees from historically underrepresented groups. • Further work is needed to uphold these principles.

Application #	DISC0-13726
Title (as written by the applicant)	Stem Cell-Based Bioengineered Therapies for Kidney Failure
Research Objective (as written by the applicant)	The objective of this proposal is to develop methods which promote the differentiation, organization, and maturation of 3D human pluripotent stem cell (PSC)-derived bioengineered kidney tissues to treat kidney failure.
Impact (as written by the applicant)	Our work will provide critical insight into the ways in which we can use biology and engineering to significantly advance stem cell-based therapies for patients living with kidney failure.
Major Proposed Activities (as written by the applicant)	• Generate kidney progenitor lineages from human pluripotent stem cells (hPSCs) and integrate into bioprinting workflow • Biofabricate 3D patterned kidney tissues using kidney progenitor populations • In vitro tissue maturation and vascularization via perfusion culture; assessment via implantation in vivo
Statement of Benefit to California (as written by the applicant)	Chronic kidney disease (CKD) and kidney failure (KF) have a profound impact on California. More than 105,000 Californians are living with kidney failure and more than 75,000 require dialysis. There are more than 16,000 new cases of kidney failure diagnosed each year in CA. In 2018, 16,179 patients went onto dialysis, while only 323 received a kidney transplant. Our work aims to directly address this crisis by developing the knowledge and methods needed for a stem cell-based bioengineered therapy for these patients.
Funds Requested	$1,280,388
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: 79

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	79
Standard Deviation	8
Highest	90
Lowest	65
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: 13	• The project holds high significance. If successful, it would provide valuable information for generating morphologically and functionally competent bioengineered kidneys for transplantation. • Kidney disease is a major health issue. The derivation of stem cell derived mature tissues would be a key advance. • No concerns.
No: 2	• The proposed technology is bioprinting of human pluripotent stem cell (hPSC) derivatives that express features of kidney progenitor cells. • The project aims to develop in vitro differentiation, organization, and maturation of 3D human PSC-derived kidney tissue. • Bioprinting is a broad area - it is not clear which aspects of 3D tissue will be improved and most need improvement. A clear hypothesis for improving bioprinted structure is not advanced. • While it is true that there is a long waiting list for donor kidneys, the reasons for this are complex. This is not dealt with, nor is it clear what the applicant's vision is to address this challenge. • The proposal is vague as to which improvements are planned or expected. Each of the technologies proposed - differentiation of the stem cell pools, bioprinting, and fluidics - has been previously described. Individually, none of these has succeeded in meeting the challenge. I'm skeptical that the combination of these existing technologies will close the gap.
GWG Votes	**Is the rationale sound?**
Yes: 11	• The project is built on a strong scientific rationale: that recapitulation of the structure of nephrogenic niche will facilitate a structural assembly of bioengineered kidney. • Clear rationale as to both why and how. • No concerns.
No: 4	• The rationale is based on combining existing approaches that are flawed on their own. It's not clear this will result in a major advance. • The proposed efforts to compare the functionality and morphology of the partially-bioengineered structures with their current counterparts (as controls) are not adequate. • Much more sophisticated constructs (mouse metanephros) have failed to produce any substantial improvement in kidney function after implantation, despite decades of attempts. This fundamental challenge is not addressed.
GWG Votes	**Is the project well planned and designed?**
Yes: 8	• Aim 1 aims to derive progenitor lineages and incorporate bioprinting. Aim 2 will use 3D bioprinting with the Aim 1 derived progenitors (NP and UB). Aim 3 will take the best 3D prototypes from Aim 2 and undergo shear stress and maturation. Prototypes will be implanted under the renal capsule in mice and progression/maturation assessed. Aim 3 is rather ambitious to expect function, but overall the aims should be informative to advance the field. • The proposal is clearly written, the experimental plan is logical and is based on a strong preliminary data.
No: 7	• The project is broken down into three major aims, including generating the cell types (inks), trying out different patterns with extrusion bioprinting, adding flow on top of the structures, and implanting them into animals. There is some overlap and dependency between these aims. For instance, if the suggested ureteric bud structures are not functional enough to induce branching morphogenesis, which is expected based on the literature, then it will be very difficult to achieve meaningful results. • The perfusion aspect of the project (Aim 3) involves running media over the structures (superfusion), rather than through them as occurs in vivo. The publication on which this is based (Homan et al) fails to prove a substantial improvement in the resultant structures. The vascularization aspects are immature, there is no flow through the tubules, and glomeruli do not form. • In terms of branching morphogenesis (Aim 2), it is unclear why printing these cell mixtures in various patterns should result in a fundamentally different outcome. These cell mixtures have been mixed in various ways in the past, but none of these has resulted in the desired outcome. There is something fundamental missing in the formulation and

	that is not addressed. The idea of using artificial patterns to suggest branching morphogenesis (without achieving it) is concerning. ● Quantitative metrics and rigorous comparison of the findings to the existing state of the art are lacking. The preliminary data are focused on representative images rather than controlled comparisons (which must be carefully designed). ● The system may be difficult to scale up for the field. ● Comparison to the state of the art is needed. ● Focus on the largest need for the field is needed.
GWG Votes	**Is the project feasible?**
Yes: 10	● The rationale is based on a series of recent papers, one of which involves two members of the current application team. The individual technologies involved can be adopted, although they are relatively new and have not been widely reproduced. ● The scope of the work is rather ambitious for the timeline, which is two and a half years. A more focused and detailed proposal might be more realistic and produce more meaningful information. ● All three aims are highly linked. Everything depends first on the generation of the progenitors cells and then the proper 3D assembly. Hence there are quite a few risks, but the principle approach is logical, well supported and technically feasible. ● The specific aims are well outlined and are achievable. ● No concerns.
No: 5	● The project will not provide sufficient proof of concept that physiologically functional kidney bioengineering is feasible, but may lead to improvements.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	● DEI is discussed and clearly relevant given the disproportional effect of the indication on minority populations. ● Kidney diseases disproportionally affect underrepresented minorities. The project will include derivation of iPSC lines from individuals of different racial and ethnic backgrounds. ● No issues.
No: 1	● Kidney disease disproportionately affects racial minority populations, which is acknowledged by the applicant. But it is unclear how this work will address this problem, nor are efforts clearly being made to generate a diverse cohort of iPS cells or derived structures. ● Plans for outreach or education are not described. As a for-profit company, the group may not publish its results, and trainees are not anticipated. These issues are not addressed.

Application #	DISC0-13804
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 MCHS but also other forms of 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	$1,842,358
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	77
Median	77
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	• This proposal is based on the unique expertise of the group in differentiation of human induced pluripotent stem cells (hiPSCs) into microglia. The overall goal is to develop a 3D organoid model incorporating microglia to better reflect the true physiological context, and thus allow the applicant to study the role of microglia in complex disorders such as MEF2C haploinsufficiency (MHS). • The broad goal of this project is to study the role of a genetic defect - MEF2C haploinsufficiency (MHS) - in microglia immune responses and in the development of autism spectrum disorder (ASD). Both of these are interesting. • The project is relevant to understanding MEF2C haploinsufficiency (MHS) forms of ASD, and may have broader relevance for microglial function. • The development and analysis of microglia-containing organoids in a potentially disease-relevant model has the potential to be interesting. • This group has made very interesting and important contributions in the past, and this project has innovative aspects that are very interesting. However, the proposal is currently problematic because the precise goals are often unclear. • The role of MEF2C is an important area of investigation, but a more focused approach would be good. • This is relevant to the field and the applicant is well positioned to significantly contribute to the development of such models. • Yes, no concerns.
No: 2	• This proposal is difficult to follow. The title states that they will investigate the role of microglia in autism spectrum disorder (ASD), but in Aim 1 the applicant says they will investigate whether human-induced pluripotent stem cell (hiPSC)-derived microglia cells from MHS patients contribute to MHS-associated phenotypes. The proposal should be clear about whether MHS or ASD is the focus of study. • Not all MHS patients are autistic. Therefore, it is important to describe how autistic patients will be recruited - clinical diagnosis should be used.
GWG Votes	**Is the rationale sound?**
Yes: 12	• I found this a bit difficult to follow, as there seems to be two projects. Nonetheless, the role of MEF2C haploinsufficiency (MHS) in microglia is an interesting problem to study. • The rationale for this project is unclear. It seems that this project actually consists of two projects (Aim 1 and Aim 2) with different objectives. • The preliminary data are largely supportive. • Preliminary data support the current proposal. • Preliminary data depicted in Fig. 5 show increased Iba-1 levels in MHS microglia, suggestive of an inflammatory phenotype that would support the applicant's hypothesis. • Fig. 2 shows that microglia colonize cerebral organoids, suggesting the feasibility of Aim 2. • Figure 6 shows a single cell RNA-sequencing plot, suggesting that the team has access to a sequencing and bioinformatics platform/expertise. • The investigators already have experience culturing all the cell types and organoids to be used in this proposal, according to a preprint from 2020. • While the overall preliminary data indicate that the team has the tools to produce the model, Fig. 2 and 3 were not so convincing. Quality of the staining is arguable and the actual phenotype of the microglia cells indicate that they may not be that healthy. The effect of MEF2C haploinsufficiency on neurodevelopment also remains unclear.
No: 3	• It's not clear how autism spectrum disorder (ASD) will be defined in the study. Clinically, or by genotype? • The phenotype the applicant describes for the MHS microglia is not adequately supported by the data shown.

GWG Votes	Is the project well planned and designed?
Yes: 6	• This project is largely straightforward, in terms of generating cell lines and exposing them to the proposed conditions. But precise outcomes to be studied are somewhat vague, making it difficult to evaluate design details. • I'm also not certain what will be conducted in 2D studies, what will be conducted in 3D studies, and what data the different approaches are expected to generate, or how the results will be interpreted to build hypotheses. • The proposal includes a good discussion of potential pitfalls.
No: 9	• The proposal seems to have two foci. I have some uncertainty about what outcomes will be measured. • The proposal needs clearer delineation and should be reduced. Right now it seems to be two divergent proposals. • I am afraid that it is very difficult to follow the narrative and experimental protocol of this project. The aims are described but the experimental set-up lacks necessary details. Experiments are not sufficiently described, e.g. what exactly and how will be done, what are the outcome measures, etc. • The concept of this project is relatively straightforward, i.e. production of iPSC-derived cell types and organoids and characterization of mutation-induced changes to cellular phenotypes. However, the investigator did not explain in much detail the readouts that will be measured. For example, they mention immunostaining and qPCR, but what are the target proteins and genes? How were these targets chosen? • The same is true for experiments focusing on inflammation, phagocytosis etc. Is this ELISA? Flow cytometry? Imaging? The methods and measured outcomes are unclear. • In Aim 1, the investigators discuss the use of 2D cultures with or without astrocyte/neuron co-cultures, but it is unclear if this is the main approach, or if it is an alternative. Overall, the proposal would benefit from a clearer, detailed and systematic description of the methods, readouts and alternatives. • In Aim 1, the investigators discuss a mix and match approach. While I see the benefits of such approach, I would have liked to know what hypothesis will be tested in these experiments. • The investigator discusses screening of compounds. Is this a sub-Aim or something that will be done in the future, as a follow up project? It is unclear. • It is proposed that Aim 2 will help understand if microglia grown within organoids acquire in vivo-like features when compared to monolayer cultures. While this is a very interesting question, it seems out of scope for the main goal of the proposal, which is to understand the role of MEF2C haploinsufficiency in microglia. When reading the proposal, one wonders why there is no Aim 3 that would focus solely on studying MEF2C haploinsufficiency using the organoid model from Aim 2. • A strength of the proposal is that the team mentions that all the necessary iPSC lines are available. The combination of experiments with 2D monolayers and 3D organoids is also a strength, as these are complementary approaches to study disease mechanisms. The organoid model is particularly interesting to study microglia-neuron environment in a native-like tissue. • An additional strength is the use of microglia derived from yolk-sac progenitors instead of bone-marrow-based protocols. However, there is not enough information or preliminary data to convince the reader that the results produced using the yolk-sac protocol will differ from that of the bone-marrow protocol. • Outcomes are not well-specified. • There is no description of statistical methods.
GWG Votes	**Is the project feasible?**
Yes: 12	• This project seems overly ambitious for the proposed timeline, but this is a very talented laboratory. In addition, the team has a good record of meeting past milestones. • I feel that there are two separate projects here and therefore the experimental plan is not well described. Furthermore, it may not be feasible in the proposed timelines (which were also not clearly presented). • Ambitious but likely feasible. • The time line is well laid out and the project seems feasible given the expertise of the group.

	• Milestones of prior CIRM funding have almost all been met which gives reassurance that the proposed work will also be brought to completion. • The Principal Investigatory has extensive experience in hiPSCs, particularly in the differentiation of brain microglia. • A number of the Principal Investigator's drug discoveries have led to clinical trials. His/her publication record, high H-index, and awards received testify to the impact of his/her work.
No: 3	• The outcomes are not well specified.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• Yes. the authors use hiPSCs obtained from diverse populations, and both sexes, ensuring representatives of the population of California. • The applicants do take these parameters into account in their choices of cell lines. This is explicitly stated, as details for each cell line are provided. • Overall, yes, but the applicants do provide a clear plan as to how they are currently and will continue to pay attention to DEI in their research activities. • Yes, no concerns.
No: 2	• Very sparse DEI content.

Application #	DISC0-13918
Title (as written by the applicant)	Decoding Firefox: A New Stem in Cancer Stem Cells
Research Objective (as written by the applicant)	Investigate the firefox (FFX)-MYC axis, which facilitates the propagation of cancer stem cells, an essential subpopulation of cells within tumors promoting metastasis, and resistance to cancer therapies.
Impact (as written by the applicant)	Understanding the mechanisms by which the firefox (FFX) peptide enhances MYC function will provide new avenues to target MYC-driven cancer stem cells
Major Proposed Activities (as written by the applicant)	• Investigate the prevalence of CircPVT1 and FFX in human medulloblastoma • Determine the functional significance of FFX in MYC-driven medulloblastoma • Investigate the molecular mechanism by which FFX augments MYC expression
Statement of Benefit to California (as written by the applicant)	Pediatric brain tumors are responsible for the highest number of cancer-related deaths in children in California. Medulloblastoma is the most common malignant brain tumor in children. Among these, the most lethal form is one that is characterized by overexpression of the MYC oncogene. We propose to identify and understand the unique and novel features driving cancer presentation to improve outcomes in otherwise lethal childhood medulloblastoma.
Funds Requested	$1,399,886
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: 76

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	76
Standard Deviation	6
Highest	83
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: 10	• Yes. There has been a long pursuit to tackle myc in cancer.

	• Yes, impactful to the field of cancer research. The cancer stem cell (CSC) element is nearly absent. • This project could potentially contribute to the advancement of world class science, but based solely on the success of the proposed PDX/pre-clinical modeling. • This project's relationship to cancer stem cells (CSC) is not clear. • Overall, yes, but this lacks a regenerative aspect. • Interesting approach.
No: 5	• The applicant has good preliminary data that point to a role of a small peptide (firefox) that comes from exon 2 of the PVT1 ncRNA. The work has relevance for a subset of medulloblastoma, but little to no relevance to stem cell biology or regenerative medicine. • There is concern that this is really mostly a cancer grant without a strong connection to stem cell research or regenerative medicine. Therefore there is considerable concern that this grant is not responsive to the mission of CIRM. • The proposed work may not contribute to the development of gene therapy or stem cell therapy. • Not directly relevant to CIRM priorities.
GWG Votes	**Is the rationale sound?**
Yes: 13	• Yes, while the fit to the program is questionable, the overall rationale as well as step-wise approach to pursue FFX is logical and supported by the preliminary data. • There is a strong rationale to target PVT1 based on the importance of MYC in cancer. We currently lack strategies for targeting upstream regulators or downstream effectors of MYC. Innovative, new, and fresh approach. • However, the rationale for the use of cell lines (rather than, e.g., an animal model) for this study is difficult to follow. It may or may not be based on technical issues, but this is unclear. More importantly, I don't see a clear basis for interpretation of the findings from these cell-based studies. Aims 2.2 and 2.3 do use animal model studies and could be the core of the project. • It's unclear how this work is related to cancer stem cells (CSCs)? The applicant does not show or suggest any differences in hierarchical expression. • It's not clear whether cell heterogeneity is being adequately addressed, e.g. between stem, progenitor, or other cells in medulloblastoma. • The applicant has strong preliminary data indicating MYC is downregulated indirectly via PVT1 targeting. • Interesting preliminary results show that the open reading frame (ORF) circular PVT1 produce a 1044 aa protein. Reagents such as an antibody against FFX are available. • The role of FFX in MYC-driven cancers seems solid - e.g., in Fig 4 and Fig 5. • This is highly relevant given the impact of MYC in human cancers. • Strong rationale. • No concerns.
No: 2	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 9	• Aim 1 will explore the prevalence, Aim 2 the functional role and Aim 3 the mechanism. This is a logical and good approach to explore FFX. • Overall, yes, though some techniques seem redundant and not well-rationalized. • Innovative approach. • No concerns.
No: 6	• To be suitable for the program, the project plan would need a stem cell element or gene therapy pathway. • The variety and extent of the technical approaches proposed in Aim 1 are unjustified. The combination of RT-PCR, Westerns, RNA-FISH IHC and use of public databases is redundant. • Annotation of resistance, metastases, or relapsed medulloblastoma for all groups (not just group 3) would facilitate this project's contribution to understanding FFX's role in medulloblastoma biology and potential for therapeutic development. • The relevance of Aim 3 (mechanism of action studies using CRISPR-integrated flag tagging) is unclear in terms of value added, especially if Aim 2 fails.

	• It's unclear what the diagnosis measures of medulloblastoma tumors in the animal models are providing? Why include the additional staining when primary samples can be evaluated? • Pitfalls and alternative strategies are not adequately discussed. This discussion should include acknowledging timing, optimization, and missing controls in animal model experiments, plus how the cell line studies may challenge and not aid the animal model approaches. • The timeline and urgency are well in place and clear.
GWG Votes	**Is the project feasible?**
Yes: 14	• Very well-constructed proposal where each objective is straightforward, important, and in place over the timelines suggested. • Yes. Team members are experts in both the subject area and the core technologies. • Overall, yes, though the aims are dependent on access to patient tumor material. • Tools are in place, e.g., antibodies, constructs, and cell lines. • No concerns.
No: 1	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• Yes. • No concerns.
No: 1	• This is only partially addressed; their plan is to get some diversity from the models.

Application #	DISC0-13926
Title (as written by the applicant)	Establishing an organoid-based preclinical model of epilepsy and neuronal network dysfunction
Research Objective (as written by the applicant)	We will model SCN2A-driven infant epilepsy by measuring cell types and neural network activity in brain organoids derived from patient biopsy cells. This model will allow discovery of novel therapies.
Impact (as written by the applicant)	Establishing brain organoid models of neural network dysfunction will enable preclinical research into treatment options for genetically based brain disorders beyond SCN2A-caused infant epilepsy.
Major Proposed Activities (as written by the applicant)	• Establish cerebral cortex organoids from diverse iPSC lines for neural network activity characterization using multielectrode array measurements. • Establish cerebral cortex organoids from six SCN2A mutant (patient) iPSC lines for neural network activity characterization using multielectrode array measurements. • Perform extensive MEA-based neuronal network characterization of the organoids developed in activities 1 and 2 to identify quantitative neuronal network dysfunction phenotypes in SCN2A mutants. • For two SCN2A lines with neuronal network dysfunction phenotypes, generate a panel of control lines including the corrected patient line and engineered SCN2A mutations in two, diverse iPSC controls. • Perform neural network characterization using the quantitative phenotypes identified in Activity 3 on the cell lines developed in Activity 4. • Perform molecular characterization of the organoid cellular and molecular phenotypes associated with neuronal network dysfunction using scRNA-seq on relevant patient and control organoids.
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 over 25% of the funds for brain and CNS projects. SCN2A mutations are highly penetrant and cause neuronal network dysfunction leading to a spectrum of neurological phenotypes including epilepsy and autism. The preclinical models we develop to benefit SCN2A patients will likely be generalizable to other disorders associated with neuronal network dysfunction and neuropsychiatric phenotypes.
Funds Requested	$1,224,585
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	76
Median	75
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: 10	• The use of iPSC-derived organoids to model human brain development represent an exciting and emerging field. Organoids recapitulate several aspects of brain development and offer the promise to unlock fundamental new human-specific biology. • In the current proposal, it is unclear what new knowledge will be uncovered using these approaches with SCN2A disorders. The hypotheses are largely that there will be changes in the underlying network of neuronal activity as a result of SCN2A mutation and that these will be due in part (or potentially result in) changes in gene expression. Overall, this is observation would not be surprising. The second major hypothesis is that the restoration of the SCN2A mutation will resolve this. • The project is significant, because it has a potential to develop an effective iPSC-based approach for screening and identifying therapeutic targets for epilepsy. • The work is focused in generating more iPSC lines and organoids to capture more diversity. To date only six lines are available. While useful, the lack of clear rationale for how diversity might matter (i.e., specific mutations associated with specific demographics) makes this an incremental tool. • Current work (see Bisogno et al, 2020) has suggested that ancestry-dependency is mainly seen in reprogramming efficiency. There is little appreciation of reprogramming as a potential ancestry-dependency factor.
No: 5	• It is unclear why diverse backgrounds will affect the phenotype.
GWG Votes	**Is the rationale sound?**
Yes: 11	• The rationale is valid - it states that self-organized iPSC-derived organoids will mimic electrophysiological behavior of neural networks in vivo. • The autoculture microfluidics system, combined with monitoring of organoid growth and morphology using a sensor-per-well parallel system for longitudinal imaging (brightfield and fluorescence) of 3D organoids, is well conceived and will minimize variation. • No disease-specific organoid phenotype is presented.
No: 4	• The scientific rationale itself is solid; there is solid evidence from animal models and clinical data that mutating a critical voltage-gated sodium channel will lead to alterations in neuronal physiology and gene expression. • Preliminary data using SCN2A patient-derived iPSCs are absent. • Preliminary data are needed to show the phenotype(s) of the organoids.
GWG Votes	**Is the project well planned and designed?**
Yes: 9	• The approach is clearly stated and will use both patient-derived iPSCs and control donors with diverse backgrounds. • The project is well-planned, but a demonstration that SCN2a organoids will exhibit aberrant electrophysiological phenotype is not presented. This lack of evidence increases the risk of the project. • Aim 1 is to address whether established risk variants in SCN2A are preserved in organoids. The applicant will using high-throughput assays including molecular and physiological measurements. • Defects in mutant organoids have not been established through preliminary data. • Aim 2 depends entirely on Aim 1.
No: 6	• More lines are needed for these experiments.
GWG Votes	**Is the project feasible?**
Yes: 9	• The lack of specific preliminary data with regard to SCN2A patient-derived cells reduces the chance that the proposed aims will be successful.

	• The aims are feasible provided that SCN2a organoids exhibit aberrant electrophysiological phenotype. This, however, has not been demonstrated. • The pitfall and mitigation strategy described for Aim 1 is insufficient. The applicant proposes to use single cell patch electrophysiology recordings if MEA recordings are inconsistent. While this might confirm sodium channel function changes in patient-derived organoids, it may not be a suitable method to unmask and resolve subtle variation across genotypes. • The team is outstanding; they have expertise in all relevant areas. • No concerns.
No: 6	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• The current plan proposes the use of iPSCs from a diverse set of donors as experimental controls. In addition, the team has established a collaboration with a patient-oriented foundation that will connect them with additional donors for future studies. • The current number of iPSC lines is limited to six, but the applicant has established a close relationship with a patient-oriented foundation (see the Letter of Support) that represents over 1,000 affected families worldwide who are interested in getting involved. However, I'm not sure whether this would specifically target undeserved populations. • The applicant proposed to derive organoids from people of different racial and ethnic backgrounds. • No concerns.
No: 2	*none*

Application #	DISC0-13795
Title (as written by the applicant)	Characterization and Evaluation of Gene-Editing Efficacy to Correct RAG2-Dependent Omenn Syndrome
Research Objective (as written by the applicant)	How a limited V(D)J recombinase can support partial T-cells but no B-cells development, drive elevated serum levels of IgE in the absence of B-cells, inhibits AIRE expression in thymus in RAG2-OS.
Impact (as written by the applicant)	Our studies are designed to validate the effectiveness and safety of our CRISPR/Cas9-AAV6 gene correction for human RAG2-OS disease as a pre-requisite in the path to clinical translation.
Major Proposed Activities (as written by the applicant)	• Engineer and characterization of a human RAG2WT-B6 mouse model. • Optimization of the gene-editing tools and transplantation regimens using RAG2WT-B6 mice. • Engineering and characterization of hRAG2R229W-B6 Omenn Syndrome (OS) mouse model. • Identify percent functional alleles to correct disease phenotype in hRAG2R229W-OS mice. • Evaluate ex-vivo gene-editing correction strategy for the hRAG2-OS disease. • Transcriptomics analyses to study hRAG2-OS disease and for evaluating the therapeutic efficacy.
Statement of Benefit to California (as written by the applicant)	RAG2-deficient patients develop SCID, autoimmunity, and granulomas. Current treatments are either refractory or hold a great risk of morbidity and mortality. Our goal is to develop a gene-editing therapy that is effective at correcting the whole spectrum of disease presentation and is accessible to all patients. Our study will support California's lead in driving innovative stem cell research for studying disease mechanisms and developing innovative stem cell-based gene therapies.
Funds Requested	$1,578,000
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	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	• The experiments proposed in aim 3 are the biggest strength of this proposal. These would directly lead to a therapeutic clinical trial for patients with Omenn Syndrome.
No: 4	• Greater knowledge of this rare disease is needed. • The proposed work will generate a new mouse model for studying gene defects in the Recombination Activating Gene (RAG) 2 that lead to immune deficiencies and autoimmune dysfunction seen in Omenn Syndrome (OS) patients. • The major strength of the proposal comes from aim 3. However, this aim is fully dependent on aims 1-2, and could be more feasible if the PI uses the already available RAG2 mutant mouse model to test the outcomes of HSCT in mice with OS pathology. • Nevertheless, a new mouse model to study RAG2-dependent OS immunopathogenesis may advance our understanding of this rare disease, and also provide a way to test gene editing approaches to correct the disease.
GWG Votes	**Is the rationale sound?**
Yes: 10	• While I agree with the rationale for the experiments proposed in aim 3, I am not convinced that investigators need to generate a new mouse model for this. The main rationale for aims 1 and 2 is to develop a potentially better fully human correlate of RAG2-OS disease. I am not sure why the currently available RAG2 mutant mice could not be used to test whether gene editing and hematopoietic reconstitution would be feasible.
No: 5	• Potentially yes, as the proposed work will add to already developed mouse models of RAG2-OS immunopathology. The main advantage, i.e.., to use a fully human RAG2 gene rather than a mouse RAG2 gene to model the disease could offer a better model. However the PI points out the high level of gene homology/identity between mouse and human versions of RAG2, which seriously weakens the rationale, making the use of a mouse RAG2 mutation potentially the better way to pursue aim 3. • The need for the human knock-in model needs to be motivated better. Much of the biology could be explored with existing mouse models. • Why are the applicants not performing aim 3 in already existing mice? Not clear why a new mouse needs to be generated.
GWG Votes	**Is the project well planned and designed?**
Yes: 5	• Aim 1 and 2 seem unnecessary. Currently available RAG2 mutant mouse models would likely suffice and save investigators a lot of time and effort. • Expansion in Aim 3 would be exciting.
No: 10	• Aim 3 should be expanded, and aims 1-2 should be performed already as preliminary data. • The proposed work is outlined as three main Aims. The first aim is focused on the generation of a new human RAG2 knock-in mouse model, which will be contracted out. Of note, this aim will also optimize hematopoietic stem cell transplant (HSCT) approaches in these mice. Aim 1 would highlight the fact that human and mouse RAG2 genes are functionally complementary and thus making the need for a fully human model system less important. • Aim 2 will address the generation of a mouse model of RAG2-OS. This will provide a mutant version for comparison to mice carrying the wild type human RAG2 gene. • A key aspect of Aim 3 is the use of mouse strains to compare the immunopathology expected in the RAG2-R229W-OS mice, as well as determine how this would impact HSCT approaches. As pointed out above, this aim is the main strength of the proposal, albeit the current mouse models could be used to tackle this question. • In Aim 3, the PI discusses the need to study metabolic changes in lymphocytes from RAG2-OS mice, something that is not clearly justified or explained in the rationale section.
GWG Votes	**Is the project feasible?**

Yes: 10	• I have no doubts about the capability of the contracted and PI labs to introduce the gene knock-in or the gene mutation. Collaborators provide the PIs the expertise in primary immunodeficiencies as well.
No: 5	• The main rationale for the work is based on the need for a potentially better fully human correlate of RAG2-OS disease models. Nevertheless, it is unclear whether the current mouse RAG2 mutant mice could not be equally used to test whether gene-editing and hematopoietic reconstitution would be more feasible. • The proposed team is extremely well qualified to direct and carry out the work. The co-PI provides complementary expertise, as does the inclusion of a named key personnel, who is a leading expert in primary immunodeficiencies.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• The PI points out and addresses these factors in the proposal. • The PIs represent a diverse and inclusive group.
No: 1	*none*

Application #	DISC0-13707
Title (as written by the applicant)	Fundamental disease-driving features of human astrocytes
Research Objective (as written by the applicant)	We will gain new insight into the validity of stem cell-derived astrocytes as a platform for modeling neurological disorders and for drug discovery.
Impact (as written by the applicant)	We will address the question of whether and how disease-associated changes in astrocytes cause neuronal defects and reveal the potential of astrocyte-targeting stem cell therapies in central nervous system (CNS) disease.
Major Proposed Activities (as written by the applicant)	• Determine how disease-relevant stimuli affect human astrocytes • Determine whether disease-relevant stimuli-treated human astrocytes contribute to neuronal dysfunction • Explore molecular mechanisms of human astrocyte morphology • Determine the molecular mechanisms of reduced human astrocyte morphology following expression of mutant Huntingtin • Determine the neuronal and synaptic consequences of human astrocytes with reduced morphological complexity
Statement of Benefit to California (as written by the applicant)	Central nervous system (CNS) disorders such as Alzheimer's disease, Parkinson's disease, Huntington's disease, stroke, CNS trauma, autism spectrum disorder, and depression impose a heavy health and economic burden on the State of California and its citizens. Most CNS disorder research in the past has focused on neurons and overlooked the potential for targeting astrocytes. We will identify disease-driving features of human astrocytes that can be targeted with stem cell-based and gene therapies.
Funds Requested	$1,560,000
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	74
Median	75
Standard Deviation	3
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: 10	• Yes; no concerns.
No: 5	• Extending our understanding of the complexities of astrocyte biology is an important contribution to the field of neuroscience, and the utilization of new approaches is always welcome. That said, it is unclear how this proposal will fill a gap in knowledge as the applicant appears to present a simplified view of astrocyte biology as the starting point. • The project does define a key knowledge gap in our current understanding of human astrocytes during normal development and in disease state. However, while human derived astrocytes may represent an advantage, the impact of the knowledge obtained from these in vitro studies - using standard tissue culture conditions - remains unknown. • The proposal will analyze changes in human astrocyte morphology (form) in response to a variety of methods and insults. While informative, the approach does not address whether astrocytes are disease driving - it rather tests how astrocytes respond to specific set of insults and/or influence co-cultured neurons. Astrocyte-neuron interactions are well studied and not a novel concept. • The comparison of induced pluripotent stem cell (iPSC)-derived astrocytes with primary astrocytes is interesting, but limited to cortical astrocytes. • Unlikely, as the approaches in this proposal are standard to the field of stem cell research.
GWG Votes	**Is the rationale sound?**
Yes: 5	• Understanding the basic biology of human astrocyte development has a strong rationale. • If validated functionally, the project might be relevant to human biology - however, it's not clear how findings from these studies will be mapped to a particular stage of human development. • The preliminary data support the feasibility of the aims.
No: 10	• The conceptualization of morphological changes is too simple. It's unclear how any findings will relate to the real world, where subtle perturbation in astrocyte morphology are the norm. The proposal gives little attention to how astrocyte morphology may vary based on the cells' brain region of origin, or to the analysis of subtle changes in astrocyte morphology. • Moderate, rather than extreme changes in astrocyte morphology, should be measured. • Regional differences needed to be accounted for. • It is not clear whether the applicant appreciates that morphological appearance is dependent on region. For example, the "bushy" morphology of astrocytes described by the applicant is thought to represent grey matter astrocytes, while long relatively unbranched processes are typical for white matter astrocytes. Thus it is not clear whether loss of complexity is necessarily a sign of pathology
GWG Votes	**Is the project well planned and designed?**
Yes: 4	*none*
No: 11	• The project is well designed to get results, but the question is open as to how meaningful those results will be. • There is minor discussion of pitfalls but the subtle question of whether or not the core hypothesis is correct is not adequately considered. What will happen if the core hypothesis turns out to be wrong? • Some aspects of this descriptive project are not well presented, and there is limited innovation in the alternative approaches. • Astrocyte function depends on the function of other cells that are not represented in the organoids. For example, in Alzheimer's disease (AD) the presence of dysfunctional microglia induces astrocyes to adopt a phagocytotic activity that might be associated with morphological changes. In such a case changes in morphology are an adaption to a pathology rather than the cause. The approach proposed will not differentiate pathophysiology from response to pathophysiology. • Although in line with CIRM's mission, this would not qualify as the most urgent project. • Unfortunately, cell morphology is not a very robust experimental endpoint. • Quantitative metrics of morphology are needed.
GWG Votes	**Is the project feasible?**

Yes: 12	• The team combines highly qualified and world class expertise in primary cell culture (the Principal Investigator), iPSCs (a co-investigator), and astrocyte function (another co-investigator). • Yes. The institution provides an excellent environment for the successful execution of this project. • Highly feasible and technically straightforward, though conceptually simple. • The project is feasible within the period of funding. • The budget is appropriate. • Yes, no concerns.
No: 3	• I am concerned because the proposal does not demonstrate a clear understanding of potential complexities of astrocyte biology, including the different astrocyte populations and lineages.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• This project adequately addresses and accounts for the influence of race, ethnicity, gender and age diversity. • The project outcomes would extend to the application of regenerate medicine discoveries to underserved racial and ethnic communities. • Yes; a diverse set of cells will be studied. • Yes, no concerns.
No: 1	*none*

Application #	DISC0-13789
Title (as written by the applicant)	Single stem cell polymeric encapsulation to improve and elucidate mechanisms of stroke recovery
Research Objective (as written by the applicant)	The ability to control stem cell activity after transplantation is limited. Our proposal seeks to understand how modulating stem cell activity post-transplantation improves stroke recovery mechanisms.
Impact (as written by the applicant)	Our method of coating single cells in polymer increases each stem cell's effect by 1) improving survival, 2) increasing beneficial factors produced and 3) targeting cells to stroke tissue.
Major Proposed Activities (as written by the applicant)	• Develop our method of coating each individual stem cell with a protective polymer so that it can be utilized across multiple stem cell types; determine the effect on stem cell survival. • We will test, using our method, if the stem cell's environment can increase the beneficial factors released to improve stroke recovery therapeutics. • Using stem cells derived from a patient's own skin, we will determine if our method improves cell survival and how this affects functional stroke recovery in our animal model of stroke. • Using our method to increase beneficial factors that the transplanted stem cells release, we will evaluate the impact on VEGF-B, an important molecule for stroke recovery, specifically. • We will evaluate the changes in new blood vessel formation and the inflammatory response after stroke with modulation of stem cell activity after transplantation using our method. • We will incorporate important molecules that allow stem cells to target injured areas such as stroke into our method to determine whether this improves efficacy and functional stroke recovery.
Statement of Benefit to California (as written by the applicant)	Stroke is a common and devastating disease across California. Stroke disproportionately affects certain races and ethnicities - with stroke risk being almost twice as high for African Americans compared to whites, and Hispanic death rates increasing. Currently, almost no therapies exist for stroke recovery. While stem cells show promise, our method increases each stem cell's therapeutic impact and improves the likelihood of successful stem cell-based stroke therapies to help all Californians.
Funds Requested	$1,577,996
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: 13	• Stroke is one of the leading causes of disability worldwide - yet, we still have very basic (if that) understanding of potential mechanisms for recovery of damaged/lost function. Stem cells have been used in previous research. However, as the applicant correctly states, there are some limitations/bottlenecks to their use. This high-risk (potentially high-reward) project - if successful - may offer new insights into mechanisms and therapeutic approaches. • The applicant proposes approaches to alleviate bottlenecks in the development of transplanted stem cell therapies. These bottlenecks include (i) reduced viability of cells upon transplantation (addressed by coating them with PEG polymers), (ii) problems in controlling individual cells, and (iii) control over release of trophic factors such as VEGF, BDNF and other support factors. • The impact of this work would primarily be in the field of biomaterials – i.e., approaches to allow transplanted cells to release trophic factors that are involved in recovery from stroke. All proposed work will be in a rat stroke model. • Overall, this work, if successful, may yield a cell therapy approach for stroke treatment, likely mediated by transplanted cells as sources of helpful factors such as VEGF, BDNF, and CTNF, which can assist stroke recovery. • Strengths: The single cell encapsulation method may provide key insights into the mechanism by which stem cells provide therapeutic benefit in stroke. • In addition, single cell encapsulation may enhance grafted cell survival and function. • Weaknesses: The applicant previously published that standalone VEGF is not an effective therapeutic and must be combined with MMP. This contradicts the proposed use of VEGF alone. The deviation from combined (VEGF and MMP) to single (VEGF) growth factor approach is not well rationalized. • Overall, yes. However, stroke is now considered a neurovascular unit impairment rather than vascular damage or neuronal cell death. In this proposal, the focus in the outcome assays on vasculogenesis and angiogenesis disregards the importance of recovery of neurons. • This approach has the potential to probe mechanism of action and enhance the functional benefits of grafted stem cells in stroke. • This single cell approach is quite novel. I believe the technology is a significant advance in the stem cell field. • Promising technology.
No: 2	*none*
GWG Votes	**Is the rationale sound?**
Yes: 10	• The overall rationale to test single cell encapsulation technology for stem cells is logical. However, the rationale to focus on VEGF alone, disregarding the applicant's own published report that VEGF and MMP combination is needed to enhance the therapeutic effects of stem cells, is not well-developed. • Preliminary data and publications by the applicant generally support the proposal but the combination VEGF and MMP should be pursued. • Yes. If proven effective, the single cell encapsulation may have direct clinical application. • The rationale is very clear and scientifically sound. It is backed up by extensive literature and it represents and obvious next step. Preliminary data support the current proposal. This project holds promise to enhance our understanding of mechanisms inhibiting regeneration after central nervous system (CNS) injury or stroke, while at the same time offering a possible new therapeutic approach. • Yes - the proposed research is based on the findings that post-stroke (after the acute phase) recovery is aided by the presence of trophic factors such as VEGF, BDNG, CTNR

	and others. These factors can be supplied directly via preparations of biomaterials that secrete these products, or by stem cells. • The applicant claims that the individual cell coatings can allow greater control over the cells. They show that alterations of PEG stiffness has effects on trophic factor transcription and levels of secretion, which may be further modulated by electrical stimulation. These seem to be the only aspects of 'control' that are considered. • The single coated cells are not directly compared to bulk coated cells, so it is hard to assess if single cell coating is superior. In addition, it is not clear that release of trophic factors from cells is superior to release from biomaterial preparations. In fact, Fig. 4 appears to show that a hydrogel releases VEGF and MMP-9 just as effectively as cells. • What are the environmental cues that lead to changes in gene expression in Fig. 9? These data are difficult to interpret without a description of the manipulation. • No concerns.
No: 5	*none*
GWG Votes	**Is the project well planned and designed?**
Yes: 4	• The main weaknesses are the need for stroke expertise, outcome measures that do not recognize the full neurovascular unit, and the unclear rationale for using VEGF alone rather than in combination with MMP. • Generally, this is a thematically relevant set of aims that will probe the therapeutic effects of single cell encapsulation. • A few oversights are noted: (i) combined VEGF and MMP should be the focus of Aim 2; (ii) the outcome assays should consider the neurovascular impairment in stroke; (iii) a stroke expert should be enlisted as a consultant. • There are stroke-related procedures that were not presented well, e.g., stem cells will be transplanted via a syringe, but the reference provided for details did not describe this more fully. Does the applicant mean stereotaxic injection? Also, what do the brain coordinates refer to - stroke penumbra or infarct area? • In addition to determining the status of the neurovascular unit in Aim 2, Aim 3 needs to assess whether the enhancement of SDF-1 expression allows increased homing of stem cells to the brain areas enriched in the SDF-1 receptor (i.e., CXCR4). This would directly test the hypothesis that the SDF-1-CXCR4 migration pathway is solicited by the stem cells. • There are pitfalls and alternative approaches identified and discussed. • The project milestones are logically presented. • The budget is appropriate.
No: 11	• One might think that in general, cells that are coated with a foreign substance would be impeded in their ability to home to sites of injury, as their surface receptors are likely occluded by the matrix. This proposal does not clearly describe what is known about neural homing mechanisms, or how cell coating might affect this process. • The applicant does not discuss any potential downsides to polymer coating of cells. For instance, this treatment may inhibit useful cell-cell contact signaling or may inhibit the ability of transplanted cells to respond to endocrine signals. • There are some flaws. For example, behavioral experiments, which are necessary to demonstrate functional recovery from stroke, are not well described nor do we know in which order they will be performed (which is quite crucial, as the literature shows) or exactly when. Also, why isn't a food pellet grasping/reaching task included in functional recovery studies? • Overall, the aims are logical and can be completed by this group, though the proposal could benefit from improvements to the experimental design. • The potential pitfalls and alternatives in this proposal are adequate, though they could be developed further. • The experimental plan needs to be designed better. • What brain region is targeted? • Overall, insufficient stem cell differentiation and characterization data are provided. • Cell sources need to be clearly specified. • Stroke expertise is not apparent on the project team.
GWG Votes	**Is the project feasible?**

Yes: 7	• Project is feasible except that the applicant's own publication argues that combined VEGF and MMP is needed to enhance stem cells, contradicting the proposed VEGF alone enhancement. • Potential pitfalls are identified, However, I wonder about the animal numbers. While the power calculations look fine, and the n=16 per group is fine, dividing each group into n=8 males and n=8 females is highly likely to result in data that is difficult to interpret. This is because n=8 is the very smallest number of animals needed for a behavioral experiment - if there are any dropouts (which is very likely in such studies) the applicant may not achieve meaningful results. • The applicant has good biomaterial experience, as well as animal and stroke expertise. There is some concern that stem cell knowledge is largely contained within the co-investigator's lab, as the grant does not contain much information about the cells to be using in the project. • There is limited information about exactly what the stem cells are, how they are made, how their quality is assessed, or whether they are to be made by the Principal Investigator's group or obtained through collaboration. • This applicant also wants to try single stem coating of additional cell types, but again, there is no description of the cells, their source, quality, or culture methods. For example, the applicant states they will coat undifferentiated iPSCs (why?), MSCs, iPSC-derived neurons (what kind?) and astrocytes. Overall, the cell culture and stem cell biology in this grant are not well explained. • The applicant team has expertise in stem cell biology and engineering. A stroke authority is needed. • The team has access to the personnel and equipment needed for this study. • The budget is appropriate. • No concerns.
No: 8	• The animal numbers are not sufficient to generate informative data.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 8	• A great discussion on diversity and inclusion is provided starting with the research team, then the envisioned target patient population, and finally the overall scientific and clinical rewards to the community. • The outcomes are envisioned to introduce a novel technology that may enhance the therapeutic effects of stem cells in stroke. • There is ample discussion of outreach, partnership, or educational activities. • Not including progenitor cells from diverse backgrounds seems a missed opportunity - unless there is a specific reason for not doing so. • Sex is addressed (male and female iPSCs will be used), but I didn't see a plan to determine the effect(s) of differing genetic backgrounds. • The applicant acknowledges that women face additional strokes recovery roadblocks, and expresses a commitment to bring treatments to underserved communities. • The outreach plan should focus on community outreach.
No: 7	• Further work is needed to uphold these principles. • There is little talk about DEI in this proposal - with the exception of plans to use rats of both sexes, which is often as much as can be done in preclinical research. • The applicant states that stroke disproportionally affects underserved racial and ethnic communities, as well as women. However, it is not very clear if/when these communities will benefit from this new approach. Of course, that will eventually happen, but new therapies rarely reach underserved populations quickly.

Application #	DISC0-13834
Title (as written by the applicant)	In vivo engineering of immune cells for cancer therapy
Research Objective (as written by the applicant)	These studies will advance our ability to use targeted gene therapy approaches to engineer or reprogram patients' immune cells to target refractory cancer.
Impact (as written by the applicant)	These studies aim to provide better, cheaper and more accessible therapies for ovarian cancer, hepatocellular carcinoma and potentially other refractory malignancies.
Major Proposed Activities (as written by the applicant)	• Utilize targeted lentiviral vectors to express chimeric antigen receptors (CARs) in T cells, NK cells and macrophages in vitro and test anti-tumor activity • Utilize mRNA containing lipid nanoparticles to express CARs in T cells, NK cells and macrophages in vitro and test anti-tumor activity • Direct in vivo engineering of immune cells to test for anti-cancer activity using immune competent mouse models and murine tumor cells • Direct in vivo engineering of immune cells to test for anti-cancer activity using humanized immunodeficient mice with human immune cells and human tumor cells
Statement of Benefit to California (as written by the applicant)	These studies aim to develop a novel gene therapy approach to better treat ovarian cancer and hepatocellular carcinoma - malignancies with few good treatment options. This approach will potentially be more accessible, cheaper and more effective than current regimens. Therefore, these studies can reduce the cancer burden for California residents. Also, since these malignancies disproportionately impact medically underserved populations, advances for these patients will be especially valuable.
Funds Requested	$1,584,001
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: 73

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	72
Median	73
Standard Deviation	3
Highest	75
Lowest	65
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: 10	• The proposed work will take advantage of targeted in vivo gene editing to express anti-tumor chimeric antigen receptors (CARs) in endogenous T cells, NK cells and/or macrophages. The work is visionary and thus a high-risk/reward project. The main bottleneck the project seeks to address is the need for more efficient and less expensive ways to re-target immune cells to attack tumor cells. • The major weakness of the work is the approach itself, i.e., broadly targeting immune cells would not only target cells with effector function, but also those with regulatory/suppressive function - Tregs, NKreg, MDSCs - thus indirectly supporting tumor growth. • Another weakness is the lack of direct preliminary evidence for the approach. The applicant provides indirect preliminary evidence in support of the main approach, showing that in vivo targeting can work, using hepatocytes (not immune cells) as targets. They also show that macrophages can be non-specifically targeted with lipid nanoparticles (LNPs). • Nevertheless, the proposed work has a potential to define a game changing approach: in vivo gene editing to engineer a patient's immune cells to express tumor-specific CARs. • Yes; targeting immune cells in vivo is highly impactful, in theory. • This is a cancer immunotherapy project. It is not stem cell related. However, it is clearly relevant to gene therapies. • The approach is to use modified mRNA to program immune cells for killing cancer. • If successful, this project will significantly advance the field of cancer immunotherapy. • If successful, the outcome from this project will contribute to the advancement of world class science. • This is a gene therapy application that pursues a potentially great idea. • No concerns.
No: 4	*none*
GWG Votes	**Is the rationale sound?**
Yes: 8	• Yes. The use of LNP and mRNA to program immune cells in vivo has a sound scientific rationale. • Overall, yes, but the applicant did not provide compelling preliminary data. According to Fig. 4, they were not able to effectively transfect NK cells with LNP and mRNA in vitro. It is questionable that they can achieve their proposed objective in vivo. • Yes, this project is significantly relevant to cancer research and treatment. • The rationale is based on the applicant's prior work and their expertise in the field.
No: 6	• The rationale is not supported by preliminary data; i.e., the transfection seems not to work well according to the data shown. • Stronger preliminary data are needed to justify funding this proposal. • The main rationale for the work is based on indirect preliminary evidence that supports (i) the use of lineage specific CAR constructs, and (ii) the use of LNPs to non-specifically target macrophages. • The preliminary data are somewhat compelling and also highlight the need for the proposed work, as it would be good to know that either lentiviral or LNPs can be effective in targeting different immune cells.
GWG Votes	**Is the project well planned and designed?**
Yes: 4	• Aim 1: Development of novel vectors to optimize engineering of immune cells in vitro. • Aim 2: Direct in vivo engineering of immune cells for anti-cancer activity. • It is not clear why the applicant will use lentiviral vector first in Aim 1 when the ultimate goal is to use mRNA as the vector for delivery. It is totally feasible to transfect mRNA into immune cells. • The applicant does not provide convincing alternative approaches in case they cannot deliver mRNA into specific immune cells with their LNPs. • The timeline is reasonable.

No: 10	• The proposed work is outlined as two main Aims. The first aim is focused on testing whether lentiviral or LNP constructs can be modified with antibodies that target T cells, NK cells or macrophages. The antigens to be used for targeting are likely good, lineage-specific candidates. • For Aim 1, different cell lines will be used to test functional targeting by the engineered constructs, which makes sense. However, it would also be useful to test specificity of cell targeting in admixtures of cells, as the applicant proposes later for testing with peripheral blood mononuclear cells (PBMCs). • Aim 2 will address whether targeting immune cells in vivo with the constructs defined in Aim 1 will enable successful gene-editing and CAR expression. A key aspect of Aim 2 is the testing of in vivo anti-tumor activity in both a mouse model and a humanized mouse system. • Pan-targeting of immune cells might lead to unintended outcomes. • The pan-targeting of T cells could lead to expression of CARs in Tregs, antagonizing the anti-tumor effect. • Unintended targeting of Tregs needs to be considered. • Preliminary data and contingency plans are insufficient. • No experience with modified production.
GWG Votes	**Is the project feasible?**
Yes: 8	• No concerns.
No: 6	• Yes, the proposed aims are well structured and feasible within the outlined timeframe. However, the lack of real preliminary evidence raises serious concerns about the feasibility. • The proposed team is extremely well qualified to direct and carry out the work. The collaboration will be pivotal for the LNP-based approaches. • The proposed Aim 2 may not be feasible if applicant cannot develop suitable LNPs suitable for the specific immune cells. • Aim 2 may not be feasible. • The team is qualified for this type of work. • The team has access to the necessary resources for this research. • The proposed budget is reasonable. • Insufficient preliminary data to support feasibility.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• Overall yes, though this project does not account for the sex/race/ethnicity of human samples. • Yes, the applicant points out and addresses these factors in the proposal. • Yes, though the applicant did not include description of any DEI-related efforts. • No concerns. • Acceptable.
No: 1	*none*

Application #	DISC0-13769
Title (as written by the applicant)	Expression of Extremophile Genes for Increased Stress Tolerance of Stem Cells
Research Objective (as written by the applicant)	Tardigrades ("water bears") are microscopic animals that can survive in extreme conditions. We will engineer stem cells to express tardigrade genes, making them more resistant to damage.
Impact (as written by the applicant)	Stem cells are inherently fragile, making them difficult to use or store for research and patient treatment. This research will make stem cells less fragile, lowering cost and increasing access.
Major Proposed Activities (as written by the applicant)	• Create adult stem cell models that express tardigrade stress tolerance genes. • Determine if tardigrade genes protect stem cells during freezing and other damage. • Determine if tardigrade genes change how stiff the cell membrane is, which is important for stem cell behavior. • Determine if tardigrade genes disrupt stem cell behavior, especially differentiation into specific cell types.
Statement of Benefit to California (as written by the applicant)	Stem cell research and treatment is expensive and difficult because the cells are fragile. This research will decrease cell fragility, lowering costs and increasing access to regenerative medicine in California. Additionally, the research will be conducted at our institution, one of the nation's most successful institutions for graduating students from underrepresented groups. These students will be included in the research, encouraging diverse STEM leadership.
Funds Requested	$1,178,539
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	72
Median	70
Standard Deviation	6
Highest	85
Lowest	60
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: 7	• The aim is to develop methods for enhancing cryopreservation of stem cells through the use of tardigrade extremophile genes. In this way, the applicants intend to rescue barriers to production, storage and transport of stem cells and thus lower the barriers to access in areas where high level cryopreservation facilities are not available. • The proposed project will certainly address a bottleneck (cryopreservation) and at the same time produce new and important biological information relating to the effects of tardigrade genes on human cell survival. • Perhaps, but significance and potential impact are difficult to assess based on the information provided in the proposal.
No: 8	• Cryopreservation of stem cells is an important problem in the field. • The proposed work will examine the possibility of taking advantage of genes expressed by extremophiles, like tardigrades, as a way to increase human stem cell robustness and protection from stressors such as cryopreservation. • The applicant provides intriguing preliminary evidence in support of the main approach, showing that tardigrade intrinsically disordered proteins (TDPs) can be expressed in adipocyte stem cells (ASCs) and increase their viability after DMSO exposure. However, whether this potential benefit is outweighed by disruption in ASC function or differentiation remains to be tested. • The knowledge to be gained from the proposed work could be used to help develop new compounds to increase stem cell viability after cryopreservation. However, the use of purified proteins or compounds based on these genes is not directly addressed. • This project does not carry sufficient significance.
GWG Votes	**Is the rationale sound?**
Yes: 7	• The rationale is clearly described and very strong. There is now quite good information on which genes contribute to tardigrade survival in extreme conditions and these extremophile genes could therefore be exploited for use in protecting human cell therapies. • Accordingly, Aim 1 is to determine if tardigrade genes transferred into human ASCs can increase the stem cell resistance to stresses associated with cryopreservation. Aim 2 is to determine if tardigrade genes have negative impacts on cell membrane mechanical properties. Aim 3 is to determine if the trilineage potential of ASCs is maintained or disrupted by tardigrade gene transfer. • This approach is straightforward and logical and will provide important information.
No: 8	• Unclear if this is even a real problem or limitation, especially in abundantly available ASCs. • No experimental test to determine if this is specific to ASCs and not progeny or other stem cell that are likely to be a greater gain should robustness work. • The other effects, long term etc. of expression of these genes has not been considered, nor the costs and labor to express them in the first place. • Aside from concerns regarding the importance of this study, no fundamental knowledge is likely to be generated about stem cells, and TTG MOA • Unclear why OE of TTGs would be welcomed to alter lineage development of ASC as hoped by the applicants. Why is this beneficial or supportive to the use of TTGs ? • Although there is demonstration of expression in human endothelial cells, the data is weak to show effects on viability or membrane integrity in Fig 3 or 4. Fig 3a is yield, not viability and as each test is controlled internally to 0% DMSO, the effects and interpretation is challenging and not supportive. • No data to support lenti or AAV expression and optimization and maintenance in mature cells generated from ASCs e.g., Aim 3. • The type of stem cell used in this study may not have significant issues with cryopreservation. • The main rationale for the work is solidly based on preliminary results and emerging understanding as to the function of TDPs. One key aspect of the proposed rationale is that stem cell use or implementation is hampered by their fragility. This point, albeit a concern, may not be as big a roadblock as envisioned by the PI. • The proposed work does not directly address human biology or a disease model, rather it may provide a way to increase the viability of stem cells after cryopreservation.
GWG Votes	**Is the project well planned and designed?**

Yes: 8	• No concerns • Under Aim 1, different tardigrade genes will be tested for their ability to protect human ASCs from cryopreservation induced stress after transient (adenovirus) or constitutive (lentivirus) expression. • Under Aim 2, tardigrade genes will be examined for their effects on ASC cell membrane mechanics. Established techniques will be used to test membrane stiffness and the coefficient of friction. In the same studies, the cells will be put under pharmacological or osmotic stress and the effects on cell membrane determined with or without the tardigrade genes. • Under Aim 3, ASCs with or without tardigrade genes will be tested for their ability to undergo adipogenesis, osteogenesis or chondrogenesis at 14, 21 and 28 days. It is expected, based on known biomechanics, that tardigrade genes will slow adipogenesis but not chondrogenesis osteogenesis. • This is a simple and clear experimental design that will undoubtedly deliver results.
No: 7	• Needs to focus on cryopreservation • The proposed work is outlined as three main Aims. The first aim is focused on whether TDPs, ectopically expressed from a lentiviral (constitutive) or adenoviral (transient) construct affect the recovery of ASCs following cryopreservation. Aim 1 outlines a comprehensive set of analyses for cell viability, and mitochondrial function. • Aim 2 will address the effect of TDPs on membrane mechanics in cells expressing TDPs and subjected to a number of stressors. A key aspect of Aim 2 is the notion that changes to membrane mechanics will underpin the effects caused by TDPs, which would increase membrane resistance to microcrystals, and/or affect the behavior of mechano-sensing receptors. • Aim 3 will determine whether ASCs expressing TDPs have defects in their ability to differentiate into adipocytes, osteocytes or chondrocytes. Preliminary results point to a defect in adipogenesis. One is left wondering whether this or some other unknown cellular dysfunction will disadvantage cells expressing TDPs. • One aspect of the proposed work that is not considered is the fact that expression of a foreign protein, like TDPs, would make modified stem cells more readily rejected by the immune system.
GWG Votes	**Is the project feasible?**
Yes: 12	• Yes, easily feasible based on the proposal. • Highly qualified, fully capable team who are experts in the area. • Yes, the proposed aims are well structured and feasible within the outlined timeframe. The research environment at the institution is well equipped and supported to carry out the work • The project is simple and easily feasible. • No concerns.
No: 3	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• Yes, the applicant points out and addresses these factors in the proposal. Notably, the potential to address disadvantaged communities is a major rationale for the proposed work. • The project is designed to lower barriers to accessibility by reducing cost and reducing dependence on sophisticated lab technology for provision of stem cell therapies. • The project will account of sex differences in donor cells. • Yes; evidence based. • No concerns.
No: 2	*none*

Application #	DISC0-13899
Title (as written by the applicant)	Elucidate the role of MSH3 in repeat instability and neurodegeneration in Huntington's disease.
Research Objective (as written by the applicant)	We will determine the potential of MSH3 as a therapeutic target for Huntington's disease based on its role in repeat instability and neurodegeneration using hPSC-derived neurons.
Impact (as written by the applicant)	MSH3 modifies disease phenotype in HD but the disease mechanism remains unknown. We will elucidate the role of MSH3, a potential therapeutic target, in repeat instability and neurodegeneration.
Major Proposed Activities (as written by the applicant)	• Determine MSH3 protein dynamics in CAG-expanded hPSC-derived neurons in basal conditions and upon DNA damage exposure. • Determine MSH3 dynamics associated with an increase in neurodegeneration and repeat instability in CAG-expanded hPSC-derived neurons. • Analyze the change in repeat instability and neuronal disease phenotype by MSH3 knock out using CRISPR. • Validate the role of MSH3 in repeat instability and neuronal disease phenotype upon re-introduction of MSH3 in MSH3 knock-out cell lines.
Statement of Benefit to California (as written by the applicant)	Huntington's disease is one of the most studied neurodegenerative diseases and our study outcomes could translate to other nucleotide repeat diseases such as myotonic dystrophy, spinocerebellar and Friedreich's ataxias and amyotrophic lateral sclerosis. There is no treatment that slows the progression of these diseases and the slow decline in patient health requires continued hospitalization and care. This is a devastation to patients and their families, and it becomes a burden to health care.
Funds Requested	$1,651,428
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	72
Median	70
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: 12	• This proposal intends to evaluate MSH3 protein dynamics in healthy and diseased conditions and to further expand the arena of MSH3 as a therapeutic target, aiming to knock out this gene using CRISPR technology to assess repeat instability and disease phenotype. • This will be done, in part, using a microscopy technique that the applicants have developed; a unique imaging technology that has already shown to be very useful in the field when they reported that inclusion bodies, initially thought to be toxic to cells, were in fact neuroprotective. • Yes, the project could lead to better understanding of Huntington's disease (HD). If successful, it could lead to future developments of new therapies for HD where there are very limited treatment options. • This proposal deals with an important issue in the field of Huntington's disease – i.e. that despite the autosomal dominant genetic nature of this disorder, the resulting CAG repeats cannot account for the disparity in the age of onset nor symptom severity. Indeed, patients with the exact CAG repeats may present with different clinical profiles.
No: 3	• DNA mismatch repair is of major recent interest in the context of Huntington's (even for post-mitotic neurons) as molecular components of that pathway may be contributing to somatic CAG expansion and associated neurodegeneration. The authors never directly come out and say it, but what's different in what they're doing compared to many in the field is they're actually looking at the canonical function of MSH3 in DNA repair and possibly neurodegeneration, versus its role in repeat expansion. • It should almost go without saying that this research is also operating in the framework of essentially trying to catch/limit the effect of mutant Huntingtin. There is substantial variability in disease onset, so if we were able to make the worse cases closer to the most mild cases, that would have a substantial improvement on disease-free life, though still wouldn't fundamentally prevent morbidity. There is no guarantee or consensus that such ability to shift the course of Huntington is certain or that MSH3 is the key to doing so. • I think this project is related to a concept and specific molecule which has the potential to be significant in the treatment of Huntington's, which would be a major application of genetic research, however this particular approach is unlikely to be the one that actually delivers on addressing that major knowledge and application gap. • Given that MSH3 is already under clinical development with multiple ligands targeting it, this proposal doesn't address any less-studied Huntington Genome-wide association studies (GWAS) hits, and the proposed mechanism of MSH3 isn't novel, the question of impact really comes down to if the microscopy aspects of this provide additional details. Even if it does pin down MSH3 to a cellular compartment, I don't see this proposal as having a major impact on the field. • It's possible it might attract more interest in DNA repair mechanisms in Huntington's, but I don't know how there could be any MORE interest in that given the latest data, so really the impact comes down to the mechanism they establish, especially as they're not contrasting MSH3 to other proteins in the pathway. I don't see how the results are more than incremental, despite the cool imaging tech. • This is more about codifying, replicating and moderately extending what is already known than proposing some revolutionary concept or target. Another way of weighing this issue in light of the criteria of CIRM for this round is asking what action a drug-development entity would take if all results were positive. I can't see the delta motivation for MSH3 being much higher than it already is, after the proposed study. • Some aspects of this study are world class, but in terms of advancement, when considering the current and envisioned future list of major contributions to HD, I don't think this will be on the list.
GWG Votes	**Is the rationale sound?**
Yes: 12	• The project is well motivated by background and preliminary data. • The proposal is not entirely novel, but builds on what is already known on the role of MSH3 protein in repeat expansion. The PI proposes to make use of their platform to explore, in real-time, the direct role of MSH3 in CAG repeat instability linked to neurodegeneration in HD.

	• Overall, the findings obtained from this proposal may provide more mechanistic insights into the role of MSH3 and altered DNA damage response in the progression of HD. • This proposal is based on the use of IPSC-derived cells of three different CAG repeat lengths. One concern relates to the fact that the lower CAG repeat they intend to use is still much higher than most common HD cases. Accordingly, the mechanisms identified by the applicants may not relate to most clinical cases.
No: 3	• I don't think that anyone doubts that MSH3 has a role in mismatch repair and DNA repair is implicated in neurodegeneration in many contexts. My reservations have to do with impact, and to a degree, implementation, rather than the basic premises involved. While looking at MSH3 DNA repair recruitment is novel, it gains that novelty in contrast to what I think most researchers in the field are most excited about, which would be its role in a somatic expansion framework. • More broadly, in terms of regenerative medicine MSH3 is a strong candidate for a modifier of the onset and severity of disease for some people with fewer CAG repeats. However, it is not the only such candidate - the other MutS proteins are options, although arguably less optimal due to cancer risk, as well as FAN1. • In this system with relatively high-throughput capacity, you want sources of cellular variability, I wonder why they don't insert the naturally occurring variant in MSH3 that appears to be protective. • The preliminary data need to support the rationale further.
GWG Votes	**Is the project well planned and designed?**
Yes: 8	• Overall, the proposal builds on strong preliminary data accumulated by the applicant with previous CIRM or other funding, some of which has already been published. • The project is well planned and makes use of forefront technology. • This proposal builds on new imaging technology developed by the group. This is a very elegant approach to study the proposed research question. • The strengths of the proposal are the use of imaging for neurodegeneration in tandem with engineered markers for MSH3. There is supposed to be some image analysis that provides subcellular localization of MSH3, but that's not been demonstrated, although it should have been possible given preliminary results shown for analogous molecule that are already available. • There is a major unanswered question as to the power of the proposed imaging+analysis+cell lines to determine MSH3 alterations in kinetics or localization. • Aim 2 will determine the potential of MSH3 as a therapeutic target, and for that, gene knockout will be performed using CRISPR/Cas9 instead of knockdown. Safety concerns associated with MSH3 abrogation (instead of lowering which will permit the partial maintenance of its role in DNA repair) should be discussed/presented. • It would be important to consider the effects of polymorphisms of MSH3 in determining CAG somatic expansion in HD. • How was the transfection and expression efficiency of a plasmid(s) confirmed to ensure that it is truly delayed DNA damage response (recruitment of the protein at the site of a lesion) and not the inefficient transfection and expression? • How was the endogenous protein effect eliminated in the cell lines? Or how was their contribution to the DNA damage response nullified? • How will dynamics study add value to MSH3 targeting strategy for disease treatment? Even if the response to DNA damage is altered in HD, ultimately this will lead to somatic expansion. In fact, slower recruitment of MSH3 will result in a slow repeat expansion process.
No: 7	*none*
GWG Votes	**Is the project feasible?**
Yes: 10	• I believe the work to be feasible within the timeline mentioned here given the preliminary data and expertise. • The PI's track record in neurodegenerative disorders is exceptional, including in HD. • The PI is a decorated and well-published researcher in the field, and they are putting substantial FTE in the project. • They're very well equipped for the microscopy. The deep learning is limited and vague, but they apparently are doing something along those lines in the lab, so that's probably okay.

	• The project is well designed and planned for the most part. A weakness is that age is a major factor in disease onset and progression, yet this can not be modeled in the iPSC model of choice for this application. • The aims are achievable, but the outcomes are uncertain. Major components of the pipeline are not demonstrated, for example, showing significant changes in protein localization can be distinguished by a TBD deep learning algorithm and these are related to propensity to neurodegeneration. • One concern that arises upon reviewing the documents is that PI has received two previous grants from CIRM, of which the latest could not meet any of the three proposed aims – as disclosed by the PI. This was a $2M grant and will led to no measurable (data, publications, patents) outcome. • At first glance, there does not seem to be direct overlap between the proposed grant and currently funded work but there is definitely a very close relationship between funds provided by another organization to the PI lab. • Concerns with application overlap.
No: 5	• Concerns about overlap with other grants.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 10	• Both male and female lines will be used.
No: 5	• The PI briefly addresses these issues in his experimental design and incorporates, as per availability of cell lines, gender, age and ethnicity. • They do say they will use both male and female lines, but there is a missed opportunity to specify they will use equal numbers of each. In fact it seems they've already picked the lines they're going to use, but don't specify the sex. • There is less prevalence of Huntington's in non-white populations, so the cell lines they plan to study do not cover multiple races. Although they claim to be open to using such lines, it appears unlikely they will be used. They say they considered lines from minority groups so they seem to exist and seem feasible to try but then a grammatically odd sentence says they're using lines from whites, because donor origin isn't recorded? • They have already selected the 4 clonal lines they're going to use, so rather than talk about how they theoretically could be of minority origin, please clarify if they are or not, or if you don't know. There is some mention of additional HD lines on page 10, but not clear how they will be selected or how many. If only for the science, this is key information that is important but never clarified. • There is no clarification whether the MSH3 mechanism known to be disrupted in a minority haplotype will or will not be represented in the lines they use. • More work here is needed to uphold these principles. • There is no mention anywhere in the dedicated space or elsewhere in the proposal on how DEI is applied to the research and educational efforts.

Application #	DISC0-13763
Title (as written by the applicant)	Targeting pluripotency-related DPPA2/4 cancer functions
Research Objective (as written by the applicant)	Important new insights into the relationship between cancer cells and stem cells will be gained, including as yet unclear pluripotency mechanisms that contribute to human tumor development.
Impact (as written by the applicant)	It will have strong impact both on understanding and targeting many cancers where stem cell mechanisms are at work and in making safer stem cell therapies with a lower risk of cancer as a side effect.
Major Proposed Activities (as written by the applicant)	• Determine how the pluripotency genes DPPA2 and DPPA4 are abnormally turned back on to trigger cancer • Use a novel human embryo model system to define how forcing DPPA2 and DPPA4 genes to remain on outside their normal time frame leads to cancer-like changes • Determine the cofactors and effectors of DPPA2 and DPPA4 as they cause cancer through stem cell-related mechanisms
Statement of Benefit to California (as written by the applicant)	The proposed research will benefit California by providing fundamental new insights into cancer development and specifically how cancers use stem cell-related mechanisms to be more aggressive. This new knowledge can catalyze future, more effective cancer treatments. At the same time, the research will yield novel insights into why stem cells sometimes form tumors, advancing potential impact by making many diverse types of stem cell therapies safer.
Funds Requested	$1,609,873
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	69
Median	70
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: 7	• This proposal aims to understand the role of Developmental pluripotency factors 2 and 4 (DPPA2/4) genes in cancer because these two genes are helpful markers for iPSCs, and when overexpressed, can aid in reprogramming to make more iPSCs. • The PI does not make it clear the connection of this study to regenerative medicine. • Even if successful, new knowledge generated from this proposal will have limited impact in the stem cell/regenerative medicine field. • The PI focuses on study DPPA2/4 re-activation during oncogenic gene activation. This only incrementally advances the field.
No: 8	• The role is limited to a subset of cancers and the gene activation model needs further support. • The proposed work will attempt to investigate the potential role of DPPA2/4 in oncogenesis and tumor progression. • It is possible that the proposed work will further advance our understanding of how tumors hijack the pluripotent gene network to enable a self-renewal program. Nevertheless, the lack of overt phenotypes from mice lacking these genes makes it difficult to appreciate their role in normal physiology, and the proposed work could have taken advantage of these mice to determine whether they fail to form tumors with similar kinetics, using mouse models for tumor formation/progression. • The knowledge to be gained may extend our understanding as to how DPPA2/4 are regulated, but it is not clear that it will directly affect how iPSC are generated, maintained, or utilized. Also, the proposal does not directly address whether oncogenesis (emergence or progression) is directly affected by DPPA2/4. • Relevance to CIRM mission not clear.
GWG Votes	**Is the rationale sound?**
Yes: 6	• This study of these two genes in the context of cancer may be relevant to cancer, but it is unclear whether we can utilize this knowledge to treat cancer. • This project proposes the relevance of a transcription factor on the activation DPPA2/4. However, expression of this transcription factor is not common in human cancers. • The PI provides some data on the role of DPPA2/4 in mouse ESC and embryos. The relevance of these data to human cancer is unclear.
No: 9	• The preliminary data highlight the need for the proposed work. The results show that the transcription factor appears to play an important role in driving the expression of DPPA2/4, which serves to validate aspects of the proposed work to detail how DPPA2/4 expression is regulated. • However, the rationale of whether DPPA2/4 play a role in oncogenic gene activation is not well characterized or directly addressed by the outlined aims. • The overall rationale to study DPPA2/4 further makes sense in ESCs. Other aspects including the role of the transcription factor are less clear given that it is not frequently active in human cancers. • Many other aspects are not supported and the rationale for the transcription factor activation model, blastoid perturbation and overexpression is not clear.
GWG Votes	**Is the project well planned and designed?**
Yes: 2	• The timeline is reasonable. • It is confusing that the PI uses a human stem cell embryo model to study oncogenic gene activation. Would cancer cell aggregate models be better? • The alternative approaches are superficial and weak. For example, in aim 1, the PI said if it does not work, they will use a different cancer cell line. No explanation of why the second cancer cell line will make it work.
No: 13	• The proposed work is outlined as three main aims. The first aim is focused on detailing how DPPA2/4 gene expression is regulated. The method to identify transcription factors at the DPPA2/4 promoter is well justified, as is the use of sequencing approaches. • The use of inducible overexpression approaches in aim 2 adds some elegance to the proposed work. • A key aspect of Aim 2 is the use of blastoid cultures, which will allow the PI to directly test the effect of DPPA2/4 overexpression in early human development, and see whether an oncogenic gene activation network is apparent. Although overexpression of DPPA2/4

	beyond its normal stage specific timeframe is clearly artificial, it may shed light of their function to maintain a pluripotent state. ● Aim 3 will examine the role of a transcriptions factor and others as cofactors for DPPA2/4. One critical caveat is the removal of the transcription factor from iPSCs, which the PI points out will affect their survival and PSC function. ● The hypotheses are not being tested directly. ● Hypothesis is not directly tested. ● Major weakness in the design and plan. ● Aim 1 to 3 all have major issues.
GWG Votes	**Is the project feasible?**
Yes: 12	● The proposed aims are well structured and feasible within the outlined timeframe. The research environment at the institution is well equipped and supported to carry out the work. ● Straight forward experiments. ● The proposed aims are feasible. ● The team is qualified for this type of work. ● The team has access to necessary resources for this research. ● The proposed budget is reasonable.
No: 3	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	● Yes, the PI points out and addresses these factors in the proposal. ● This is basic science research and the relevance to regenerative medicine is unclear. ● This project did not account for race/ethnicity. ● It is unclear to this reviewer what the PI's prior efforts are related to DEI.
No: 2	● Applicant points to a resource but not how this project addresses DEI.

Application #	DISC0-13689
Title (as written by the applicant)	ESPRESSO Phenotyping enables the study of neural stem cells phenotypic heterogeneity and its link to spinal cord injury transplantation efficacy
Research Objective (as written by the applicant)	We will identify the set of properties (organelle landscape and gene expression profile) that characterize an efficacious neural stem cell lines for transplantation in spinal cord injury treatment
Impact (as written by the applicant)	The method we will validate will enable immediate, cheap and highly informative phenotyping that can be applied to living cells, enabling the characterization of stem cells from diverse donors
Major Proposed Activities (as written by the applicant)	• Ten neural stem cell lines with known transplantation efficacy will be studied to reveal what characteristics (organelle landscape and gene expression) make them efficacious • A new microscopy technology will be developed that enables the profiling of tens of thousands of cells, increasing throughput and applicability of our novel method • Eighteen cell lines from donors of diverse background will be studied to determine common phenotypes to develop a patient-agnostic, highly efficacious cell line for treatment of spinal cord injury
Statement of Benefit to California (as written by the applicant)	Our research team has developed a series of neural stem cell lines that have proven to be highly efficacious for the treatment of spinal cord injury. However, immunosuppression for extended periods of time is typically required as the body tends to fight foreign cells. The knowledge that will be acquired from this study will identify how to manufacture a neural stem cell line from patient-derived pluripotent stem cells that is highly efficacious and doesn't require immunosuppressors
Funds Requested	$1,366,077
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	4
Highest	75
Lowest	60
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

KEY QUESTIONS AND COMMENTS

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

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 8	• A previous grant by this group was focused on the same question and apparently all goals were achieved- it is not clear how the previous work informs this work and how the previous screening tool needs this kind of additional screening • The limitation and bottle neck are not clear- i.e. what were the weaknesses of the previous approach? The proposal is focused on developing a screening platform that allows single-cell phenotyping of live induced pluripotent stem cell (iPSC) lines and embryonic stem cells (ESC) with minimal interference • Focus on using Environmental Sensors that Phenotype Subcellular Structures and Organelles (ESPRESSO) and "connect " the data to sRNA seq data might provide a comprehensive map of cells and their potential efficiency as transplantation targets for spinal cord injury • Non-destructive live cell phenotyping presents a significant bottleneck in predicting therapeutic efficacy of stem cell therapies.
No: 7	• The goal of this project is to develop a rapid screening tool for determining whether or not particular neural stem cell populations are likely to be effective in transplantation for repair of nervous system damage. Having a means of rapid identification of potentially useful cells would be a valuable addition to the field. • As will be discussed later, it is not clear however, whether this approach offers anything beyond the gene expression analyses that already have been funded by CIRM for this group of investigators. • Development of an incremental tool with a limited impact in the field. • The research may not be aimed at solving a major bottleneck and thus impact is somewhat modest.
GWG Votes	**Is the rationale sound?**
Yes: 7	• The premise that cell phenotypes maybe informative of their therapeutic efficacy is valid and is based on strong scientific rationale.
No: 8	• The ability to rapidly phenotype live cells is certainly interesting if applied at a metabolic level. That said, it is unclear how much of this actually differs from what can be obtained by existing flow cytometry approaches, Including ones that are able to provide resolution at the level of intracellular structures. • There are insufficient data required to determine whether this is the case. Specifically, they have examined two cell lines, one of which was potent in transplantation and one of which was not. The question is whether this is just a difference between these cell lines, because they did not do the key experiment of examining another cell line from each of these categories to determine whether or not the profiles observed actually are predictive. • On a more general level, it appears that they can address the technical goals they have set out for themselves. What is not clear is whether or not this information is useful for the predicted utility of being able to identify cell populations more or less useful for transplantation purposes, for example. The differences seen in pilot experiments might be due, for example, just to being from different people. • There is also a weakness in the failure to consider how changes in the phenotypes studied might be relevant to understanding cellular function. For example, mitochondrial function and lysosomal function vary in cells at different times and in different circumstances. Because the cells are growing in vitro, the question of how the information obtained is going to apply to the in vivo transplantation circumstances is completely speculative. • Correlative studies may not be informative of the mechanism. • The explanation of the parameters that are shown is not very informative as to the function - for example, what is the biological relevance of the slight increase in mitochondrial size in one cell line versus another (Fig3)? Is the applicant proposing that the size change relates to transplantation efficacy or could size simply not matter? • Cells will encounter after transplantation a highly inflammatory environment- it is not clear to what degree these conditions are mimicked to identify a pro survival signature for suitable graft cells

	• No- a previously funded CIRM grant that focuses on the same problem has been completed - the previous work is not integrated and additional needs for this project are not described • Some concerns that the preliminary foundational data is sufficiently robust (only two cell lines used).
GWG Votes	**Is the project well planned and designed?**
Yes: 5	• Biological relevance of the readout remains unclear although the measurements seem feasible
No: 10	• The project is well designed from the point of view of its internal logic. The design questions relate to applicability to the broader field of stem cell science. • The project as well designed to obtain the data they propose, but there is a lack of design features in regards to analysis of complex data sets and the development of testable hypotheses. • There is no discussion of potential pitfalls, which is an enormous concern particularly in regards to the failure to test their hypothesis that the parameters that they are studying do have predictive value when carried out on more than two cell lines already known to be of different character. • There is also lack of a clear rationale as to how the proposed imaging is an improvement over existing imaging technologies. • There was no discussion of pitfalls. • Many items are not in place. • The design of the project has limitations and the project is overly risky, because the proposed specific aims are interdependent on each other.
GWG Votes	**Is the project feasible?**
Yes: 7	• The screening can be achieved although there is no clear sense of the time required of analyzing one cell line
No: 8	• There is also a concern that the second aim is focused on building a microscope that appears to be critical in order for the work to be carried out. • Although the team is qualified to carry out the analysis, there is no evidence that they are capable of analyzing the complex data in a manner that will provide interpretable information and testable hypotheses. • There is also a striking lack of cell biologists on this proposal, which is striking in terms of the focus on biological information. • Aims are interdependent. • Major revisions are required. • Parts of the project are feasible, but given the interdependence of the specific aims, it is unlikely that all the goals will be achieved.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15	• This is actually an interesting question on this proposal as there is a selection here for cell lines of Caucasian origin, with no explanation as to why other ethnic groupings were excluded in the first analyses. Nonetheless, the proposal says that they will include cell lines from all communities. • In addition, a key person on the team has excellent experience in regards to outreach. • Yes, no concerns. • Addressed although it is not clear what will be expected. Is there an expectation that racially diverse iPSC cells are different than the general diversity of any iPSC cell pool? The power might not be high enough to address that specific point • The iPSC lines will be derived from individuals of different races and ethnic groups.
No: 0	*none*

Application #	DISC0-13869
Title (as written by the applicant)	Dissecting the molecular determinants of metastatic breast cancer stem cells
Research Objective (as written by the applicant)	The object is to identify unique molecular signatures of metastatic breast cancer stem cells (CSCs) and to dissect the molecular mechanisms regulating metastasis competency of CSCs.
Impact (as written by the applicant)	The impact is to reveal the molecular signatures of metastatic CSCs in circulating tumor cells from human breast cancer patients for effective monitoring of disease progression and therapy response.
Major Proposed Activities (as written by the applicant)	• To perform multi-omics analysis of metastatic CSCs to a mouse breast tumor metastasis model. • To reveal the molecular signatures specific to CSC sub-populations that have high metastatic potential. • To perform multi-omics analysis of CSCs from circulating tumor cells isolated from breast cancer patients. • To generate the human breast metastatic CSC marker panel that define metastasis competency of CSCs. • To perform spacial mRNA and protein expression analysis to further define the key human metastatic breast CSC marker panel. • To test the functional impact of inhibiting key signaling pathways in metastatic CSCs in breast cancer metastasis.
Statement of Benefit to California (as written by the applicant)	American Cancer Society estimates that ~31,000 Californian women will be diagnosed with breast cancer and ~5,700 will die from metastatic breast cancer in 2022. Our research aims to identify why some breast cancers become metastatic . It also aims to determine why/how metastatic breast cancer stem cells are chemoresistant and can lay dormant for years before re-emerge to deadly distant metastases. Our research could reduce the mortality associated with metastatic breast cancer in California.
Funds Requested	$1,519,219
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

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Mean	68
Median	70
Standard Deviation	4
Highest	75
Lowest	60
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 10	• Strengths of this project include that it addresses gaps in knowledge about metastatic cancer stem cells and more broadly, circulating tumor cells. • Unique aspects of the proposal include addressing the technology gap of characterizing long-dormant cancer stem cells (CSCs) and further development of a novel mouse model to study circulating tumor cells (CTCs). • CSCs themselves have been debated, and this work could advance our understanding of the prevalence and transient nature of CSCs and/or other CTCs, too. However as CIRM reviewers pointed out in the discussion, the work starts with the assumption that CSCs will be identified. Identifying the diversity of the CTCs themselves and changes over time, may yield a more unbiased, different view of what cells constitute 'aggressive' cells that later contribute to metastasis.
No: 5	• Yes, little is known about metastatic cells from tumors. Profiles could be informative. • Good science, competitive. • Potentially major impact, if novel markers that are applicable arise. • Triple-Negative Breast Cancer is clearly a health concern that affects minorities disproportionally. The focus is on CTCs which makes it only potentially relevant for CIRM depending on how strong the evidence is that these contain actual CSC rather than other types of tutor initiating cells.
GWG Votes	**Is the rationale sound?**
Yes: 6	• Yes, the rationale for studying CTCs is strong. The PIs will be able to characterize the potential of subpopulations of dormant CTCs to contribute to tumor metastasis. • Metastatic disease is the main cause of death in cancers. • Figure 3 data suggests a robust model is in place. This showed more metastasis and more CTCs. • Broadly characterizing CTCs' role in cancer metastasis and their spectrum of 'stemness' may be more valuable than focusing on a more narrow definition of cancerous stem cells. • Many assumptions are made that drive the rationale. A more agnostic approach would perhaps be more informative. • No bioinformatics or proof of principle of CSC markers and profile of transcription or chromatin accessibility is provided, e.g. level of heterogeneity or similarity of CTC vs. known markers of CSCs or primary tumor. • Aim 1 hopes to analyze CTC, primary, and lung metastasis. The bioinformatics to be used for comparisons and define cell clusters is unclear and not described. How will the fractions be compared at the cluster level? • The use of prospective isolation is not explored. This is an immense depth of sequencing technology, and a pilot study would have best determined the correct methods to conserve resources, e.g. time and money, and to assure signal depth of transcription is met. Controls? Thresholds? Statistics to define profile? • Unclear why mouse work is being done. Why not just move straight to human CTCs from patients? How the murine results are being used beyond "cross-species comparisons" is unclear, and could have been applied to probe set the human instead of discovery based exploration in human CTCs. • An inducible system in PDX models could provide more powerful demonstration of the proposal ideas, and translational future. • Rationale to weigh epigenomics results in the case of heterogeneity in breast cancer patients is not supported. • Reduction of 10-20 markers for mouse CSCs in breast cancer is convoluted and likely to generate known markers as well, and basis of using PDX models for this reduction is unclear. • Patient data in Figure 5 is compelling, but statistical relevance is lacking.

No: 9	• The rationale to explore CTC at single cell resolution is sound, the use of mouse models for molecular profiling is not. • The preliminary data do not fully support the rationale of the proposal.
GWG Votes	**Is the project well planned and designed?**
Yes: 6	• Overall, the project is planned and designed well, with the exceptions that it hinges on (1) significant overlap between cancer-contributing genes in human and mouse without substantiating this overlap and (2) identifying a specific subset of cancer stem cells. There may be minimal to no overlap in the top tumorigenic genes between human and mouse within the CTC population. • The patient sample size is overall small when considering the diversity and rarity of specific cell types within CTC and correlating these to metastatic outcomes. • The PIs bias their approach towards identifying a sub-type of CTCs that are independently key contributors to metastasis. Allowing for a way to test for a spectrum of cells' stemness with the ability to study transience across cell fates and markers could prove more impactful and sustainable as a framework. That is, studying the diversity of cells within CTCs as a metric may together contribute to cancer metastasis, not independent CSCs.
No: 9	• The use of a mouse genetic mouse model is unlikely to be meaningful for molecular signatures in human. Aim 2 is well suited but rather vague on how the data will be analyzed. In particular, the value of the epigenetic sequencing is not clearly presented. The rationale for spatial RNA seq in the mouse model for Aim 3 is not very strong. • In Aim 2 alone, 100 samples will be collected for analysis, the budget of which is near half a million for the kits alone in one sub-aim. Concerned about budget limitations that would be exceeded. • Alternatives are provided, but mainly additional experiments to substitute for those proposed vs. using the results or reducing overall experiments in terms of the approach and applying these to other aims. • No plan to connect and use expensive data.
GWG Votes	**Is the project feasible?**
Yes: 8	• Yes, the models exist and the proposed work is technically not too challenging. The collection plans are in place. • While overall the project is feasible, the translational rationale for the full multi-omics approach is not clearly justified given the sequencing and analysis cost for large patient groups. The ability to perform all modalities within the budget and proposed timeframe is questionable. • "Astronomical" amount of sequencing - feasibility is not clear. • Extreme heterogeneity makes interpretation difficult.
No: 7	• Team well designed and recruited for success. • More resources would be required. • Unlikely given the amount of work suggested, and collection of patient samples for Aim 3. • Expensive.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 12	• Yes, given the disproportionate effect on certain populations the study will take DEI into account. • The PI notes that aggressive breast cancer is more prevalent among Hispanics (23%) and black (23-30%) patients, however recruitment in these populations is not emphasized or specifically addressed within the broader outreach plan. The study follows the anticipated racial/ethnic composition of the breast cancer population at the institution, which is cited as 71% Caucasian, 13% Hispanic, 8% Asian, 6% African American, and 2% Other. • The work would be relevant for underserved racial/ethnic communities however itself does not make an effort to extend the applicability to underserved populations.
No: 3	*none*

Application #	DISC0-13783
Title (as written by the applicant)	Cell Biology of Induced Pluripotent Stem Cells (iPSCs)
Research Objective (as written by the applicant)	This proposal addresses a major, essential, yet underexplored aspect of pluripotency, the role of clathrin-mediated endocytosis in dictating receptor levels that control pluripotency.
Impact (as written by the applicant)	The proposed studies promise deeper understanding of the pluripotent state and, by example, will inspire wider adoption of stem cells, moving basic science researchers away from tissue culture cells.
Major Proposed Activities (as written by the applicant)	• Genome editing of induced pluripotent stem cells (iPSCs) cells to express fluorescent fusion proteins at native levels, avoiding over-expression artifacts. • High-speed, state-of-the-art, live-cell imaging of membrane trafficking and cytoskeleton dynamics. • Gene knockdown studies using both RNAi and CRISPR • Functional studies of the Allen Institute for Cell Science's resource of genome-edited human iPSCs to study diverse cell structures. • Use of iPSCs derived representing differences in gender and ethnicity.
Statement of Benefit to California (as written by the applicant)	The proposed research continues efforts by our laboratory to develop human stem cells as an ideal basic research subject. Traditionally used tissue culture cell lines are derived from tumors. iPSC lines used for proposed studies represent normal physiology and can be differentiated into many fates isogenically. This research will improve fundamental knowledge of human stem cells, increasing their utility for human disease therapy, and will attract wider stem cell adoption for research.
Funds Requested	$1,574,808
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	3
Highest	70
Lowest	55
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	14

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 5	• Identifying new regulators of stem cell differentiation is of high impact.
No: 9	• The proposed study will address an understudied basic biological feature of human pluripotent stem cells, namely their cellular mechanisms for clathrin mediated endocytosis; and how this is modified during differentiation. • The study would enhance our knowledge of human pluripotent stem cell biology. • The project will provide tools and data that will be of general use to the scientific community. • This project will contribute to the advancement of science. • The significance of the project to stem cell biology and regenerative medicine is low. The project uses human induced pluripotent stem cells (iPSCs) as a platform to study clathrin-mediated endocytosis on the premise that iPSC are superior subjects when compared to Hela or 3T3 cells. • While of general biological interest, this study would have incremental impact in the stem cell / regenerative medicine field. • The writing of this grant needs revision.
GWG Votes	**Is the rationale sound?**
Yes: 2	*none*
No: 12	• It would better to have some underlying rationale for why clathrin-mediated endocytosis (CME) should have unique properties in pluripotent stem cells. These properties will likely change on differentiation, but the studies proposed will not demonstrate functional significance. The rationale is rather general and lacks specifics. • CME is a fundamental biological process. It would be useful to know if the applicant intends to look at any disease-causing mutations among the genes known to be important for CME, or if there are specific known developmental roles for the process. • The pathway (CME) that the applicant is studying could very well have an impact on stem cell differentiation. However, lacking preliminary data from studies using iPSCs or embryonic stems cells (ESCs), it is impossible to evaluate whether the proposed studies will be fruitful. • The applicant has established reporter cell lines and has shown that CME changes upon differentiation. This does not mean that the changes are of significance for maintenance of pluripotency or for differentiation. Reference 4, which supports a role for CME in pluripotency maintenance in mouse, has already demonstrated how this pathway interacts with TGF beta and ECAD. What does the applicant intend to show in human cells? • Any basic understanding of human cell biology has relevance to human disease. • Any results uncovered using iPSC lines might need to be validated with the gold standard - ESC - as reprograming might alter cellular properties. • The rationale is weak and incorporates some basic misunderstanding of human pluripotent cells. For example, the applicant states that iPSCs have stable chromosome, and that they represent a single cell type, etc. • The preliminary data are mostly a review of the Principal Investigator's research program leading to the decision to transit to stem cells. This is not a sufficient rationale for the project. • No preliminary data.
GWG Votes	**Is the project well planned and designed?**
Yes: 5	• In general, the study design is adequate. • Aim 1 is largely descriptive and lacks clear hypotheses concerning the key signaling molecules. Aim 1 is also too wide ranging. How many pathways will be examined, and how many knockdowns will be performed, and what will be the cell biological endpoints? • Aim 2 is also descriptive. Why are these differentiated endpoints chosen? The drugs will almost certainly have effects but how specific will these effects be? Why intestinal organoids, which are much more complex than 2D cultures? • What are the specific endpoints in Aim 3 to test the roles of CME in establishment and maintenance of the differentiated state? The applicants need to be more specific here. • The study seems open ended and it is unclear what urgent outcomes are addressed. • No problems or pitfalls seem to be anticipated.

No: 9	• Most of the grant is written referring to the Principal Investigator in the third person. The style of the proposal is not high-level. Meaningful results might still be attainable. • Pitfalls are addressed to some extent. • The project does not represent an urgency based on CIRM's mission. • The project's budget rationale is appropriately justified. • Poorly written grant.
GWG Votes	**Is the project feasible?**
Yes: 8	• Not entirely, this depends on how many pathways and mechanisms are investigated at sufficient depth to yield meaningful information. • The Principal Investigator is an internationally recognized expert in the CME field, the postdocs are both well qualified. • Required resources are available including cell lines, microscopy, and an outstanding core facility.
No: 6	• The proposed aims and the project outcome can be achieved in the proposed timeline. • The institution is a world class research institution and is well equipped to carry out all of the proposed activities. • The feasibility is unknown due to limited preliminary data and insufficient description of iPSC assays. • The proposal is adequately staffed. • The budget is appropriate.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 10	• This proposal adequately address this and accounts for the influence of race ethnicity sex, gender and age diversity. • This project's outcomes would validate the applicability of regenerative medicine discoveries to underserved population including racial and ethnic communities. • The applicant describes various efforts in outreach, educational activities, and long time support of DEI at the institution. • Cell lines from diverse genetic ancestries will be used only in the third year. • The Principal Investigator has an excellent track record of promoting diversity. • No concerns.
No: 4	• The access to diverse cell lines is a strength, however there is no clear breakdown of the ethnic background of these cells or of the specific lines that the applicant will use.

Application #	DISC0-13923
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 versus 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 human neural precursor cell (hNPC)-induced remyelination and clinical recovery using mouse MS models. • We will characterize a novel molecule expressed in Tregs that may be involved in the 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 versus 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 versus regenerative cellular topology within the CNS of MS mouse models.
Statement of Benefit to California (as written by the applicant)	Multiple sclerosis (MS), a disease that typically presents in early adulthood, afflicts many Californians. 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. A population of anti-inflammatory T cells called Tregs that are induced by transplantation of neural precursor cells (NPCs) and may facilitate the neurological repair and clinical recovery in people with progressive MS. In this project we will characterize the function of these Tregs.
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: 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	3
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: 5	• Strengths: This project is a logical continuation of the Principal Investigator's previous findings that stem cells promote remyelination in animal models of multiple sclerosis (MS). Here, the project will explore potential mechanisms underlying the therapeutic effects of stem cells in MS animals. • To date, the mechanisms remain unknown, thus the envisioned data on the effects of stem cells on increased regulatory T cells (Tregs) and subsequent proliferation and function of oligodendrocytes (OPCs) and microglia via the stem cell secretion of Transglutaminase 2 (Tgm2) will add significant scientific value to the field of stem cell therapy for MS. • Weaknesses: Aims 1 and 3 are geared towards interrogating the effects of Tregs on OPC maturation and the stem cell secretion of Tgm2, allowing elucidation of the crosstalk between Tregs and Tgm2 in modulating OPC cell fate. However, Aim 2 is simply probing the effects of Tregs on microglia. To be consistent in testing the hypothesis of Tregs-Tgm2 interaction, the levels of Tregs and Tgm2 in microglia should be assessed. Otherwise, Aim 3 appears an incomplete study. • Since this is a highly mechanistic set of experiments, we will gain critical insights into how stem cells promote remyelination in MS models. • The proposed MS animal models here are well validated and recapitulate the spinal cord and CNS demyelination pathology reminiscent of MS. Accordingly, the envisioned results are translatable to clinical application, clearly equivalent to world-class science.
No: 10	• The proposed work will investigate the mechanism(s) by which treatment of mouse models of demyelination with transplantation of human neural progenitor cells (hNPCs) improves motor function and myelin levels. • In preliminary studies, the applicant shows that although hNPCs are rejected by the host mice, transplantation of these cells leads to increased frequencies of regulatory T cells (Tregs) that appear to ameliorate disease progression. • Previous research has already established the immunomodulatory role of hNPCs. The applicant's published data have implicated Treg cells as mediators of this and other work (not cited) has established a beneficial role of Treg cells in remyelination. That microglia play an important role in remyelination is also not novel. • This project involves the use of human neural stem/progenitor cell (hNPC) transplantation as a means to treat progressive forms of multiple sclerosis (MS), as modeled in mice. The central premise of this application is that NPC therapy can promote remyelination. The overall goal is to determine the mechanisms by which this occurs. • The proposed work will take advantage of hNPCs generated from human embryonic stem cells. However, hNPCs may not be directly responsible for the increased myelination. As these cells are rejected by the recipient mice, hNPCs may be acting indirectly by modulating the immune environment. • There is the possibility that the proposed work will lead to new insights. However, the focus should be placed on understanding the mechanism for the unique immune rejection response mice have to hNPCs.
GWG Votes	**Is the rationale sound?**
Yes: 7	• Strength: Yes, the rationale to examine Tregs as the main trigger in modulating OPC and microglia fate is well-developed. • Strength: The goal to probe the putative crosstalk between Tregs and Tgm2 (albeit in OPCs) is also well-supported.

	• Weakness: Probing the crosstalk of Tregs and Tgm2 in microglia should also be examined in this proposal in order to reveal the key role of Tgm2 in Treg-induced remyelination in MS. • Preliminary data are compelling and provide feasibility guidance for the proposed experiments. • Yes, if Tregs and Tgm2 crosstalk is demonstrated in OPCs (and in microglia), then such a mechanism can be used to guide the safety and efficacy optimization of a stem cell therapy for MS.
No: 8	• The scientific rationale of localized implantation of NPCs into the MS brain and spinal cord as a global means to treat progressive MS is not a sound rationale and is not supported by the applicant's previously published data. hNPCs are rapidly rejected by the mouse immune system. Thus, there is no rationale for a direct functional contribution of the implanted cells. No evidence for improved remyelination is presented. Rather, the published and preliminary data is consistent with an immunomodulatory mechanism. • The applicant's preliminary data show that hNPC transplant treatment has no effect on clinical scores in one mouse model. The effect of hNPC transplantation in the other mouse model was highly localized. These results limit the potential therapeutic translation of this strategy. • The mouse model may not be representative of the human disease(s). • Xeno-rejection through active processes may not be therapeutic and have a strong clinical impact. • The preliminary data highlight the need to better understand the unique aspects behind the rejection of hNPCs, as opposed to other cells in the intracranial space. Unfortunately, this is not directly addressed, rather the focus is on the increase in Tregs and potential role of Tgm2 expressed by Tregs.
GWG Votes	**Is the project well planned and designed?**

Yes:
1
	• Overall, yes, but I have a few concerns:
	  ◦ The main weakness is the issue of remyelination, including the weak pilot data as well as the objective measures in characterizing remyelination in the present study.
	  ◦ The proposed experiments are generally well planned and designed, except for Aim 2 which should incorporate Tgm2 in microglia, as in Aims 1 and 3.
	  ◦ Pitfalls and alternative approaches are not well discussed.
	• Project deliverables and timeline are provided, and are feasible and within CIRM's mission.
	• Budget is appropriate.

No: 14	• The proposed work is outlined as three main aims. The first aim is focused on further elucidating the role of hNPC transplant in the remyelination process, including the recruitment and function of endogenous oligodendrocyte progenitor cells (OPCs). This is clearly outlined and makes use of reporter mouse models. • Aim 1, however misses the mark by not focusing on the differences in immune rejection, as the applicant states that other cells transplanted into mice are also rejected, like hNPCs, but their effect is either transient or negligible. Thus, a unique aspect of hNPC rejection is likely at play. Perhaps, characterizing the immune cells in either set of rejection outcomes could be a way to gain insights as to why the hNPCs show a better outcome. • It unlikely that hNPC function is the main mechanism of disease amelioration, as the hNPC cells are rejected. Thus, comparisons to other treatments where hNPC have regenerative effects makes little sense and confuses the issue to be tested. • The expectation that Treg depletion will result in diminished recruitment of proinflammatory effector T cells is counterintuitive and a challenge to the logic behind the experimental approach. • Aim 2 will address the role of hNPC transplants in reducing disease associated molecules, and will examine the role of microglia in mediating these changes. A key aspect of Aim 2, like in Aim 1, is that Tregs will be removed to investigate their role alone or in combination with microglial cells in improving disease outcomes. • Aim 3 will directly test the role of Transglutaminase 2 (Tgm2), which is shown to be expressed by Tregs, and may act on OPCs, as an important mediator of the improvements in disease outcomes.

	• There are several issues that need to be addressed. Group sizes are not mentioned. What time point(s) will be analyzed? • Aims are largely descriptive and incremental in nature. • The choice of mouse models needs to be reconsidered.
GWG Votes	**Is the project feasible?**
Yes: 10	• The proposed experiments represent the core expertise of the Principal Investigator and a co-investigator - namely their solid track record in animal models of demyelinating disease. • The Principal Investigator and assembled team are well-qualified to conduct the studies. • The team has access to the personnel and equipment necessary to carry out this project. • Yes, the proposed aims are well structured and likely feasible within the outlined timeframe. • Yes. The project is feasible. The investigators have demonstrated expertise in both animal models and with the use of human neural progenitor cells. • The budget is appropriate. • No concerns.
No: 5	*none*
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14	• The applicant provides sufficient discussion on diversity, with age and gender of the proposed subjects capturing the clinical scenario. • The envisioned mechanism-driven results can be used to extend and optimize the safety and efficacy of stem cell therapy for MS, directly impacting underserved populations. • Yes. Both sexes will be used in animal studies, though how this will affect power calculations and group sizes is not discussed. • The applicant has outlined outreach, partnership, and educational activities. • Yes, the applicant points out and addresses these factors in the proposal. • No concerns.
No: 1	*none*

Application #	DISC0-13805
Title (as written by the applicant)	Evaluating the role of ancestry in chronic liver disease using human induced pluripotent stem cells
Research Objective (as written by the applicant)	Our approach of using stem cells as tissue models, will allow us to uncover new insights into the roles of mechanical stimulation during development and mechanisms that drive liver dysfunction.
Impact (as written by the applicant)	With the ability to modify individual DNA base pairs and derive liver tissue from patient specific stem cells, we will be able to uncover the role of ancestry in liver disease predisposition.
Major Proposed Activities (as written by the applicant)	• Create a multiethnic biobank of stem cell derived liver cells • Create functional liver cells from stem cells using mechanical cues • Recreate liver disease in a dish • Understand the role of ancestry in susceptibility to metabolic liver dysfunction • Modify individual DNA base pairs to understand the role of gene to environment interactions in liver disease onset
Statement of Benefit to California (as written by the applicant)	California contains the highest population of Hispanic Americans, who are disproportionately diagnosed with nonalcoholic fatty liver disease (NAFLD). The link between ancestry and disease predisposition is not fully understood. By using patient-specific stem cells, we propose that we can unveil why Hispanic Americans are most susceptible to NAFLD and postulate that a combination of drug screening and gene editing techniques can provide a new solution to this untreatable and growing disease.
Funds Requested	$1,521,473
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	61
Median	60
Standard Deviation	4
Highest	65
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: 10	• This grant aims to greatly improve the ability to produce mature hepatocytes from pluripotent cells and would address this roadblock, and if this can be achieved, would then use this improved model to study NAFLD. • If successful, this work could shed light on why NAFLD is prevalent in Hispanic populations, by developing and using improved organoid models. • The work proposed here would constitute a major advance if successful. However, there is a high risk of failure, as organoid methods have not yet been tried or tested by this group. • Very unique in the field. Would provide a potential model for NAFLD if phenocopy is possible. • Yes, the genetics of NAFLD could be uniquely understood using iPSC models that have not been applied previously. This should be compared however to existing models of NAFLD. • The gap is in understanding NAFLD, but the stem cell application is not clear other than use of iPSCs as a model.
No: 5	*none*
GWG Votes	**Is the rationale sound?**
Yes: 6	• Genome-wide association studies (GWAS) studies show that Hispanic populations have higher rates of NAFLD. In addition, ethnicity-influenced variation in NOTCH signaling may be involved. • A key hypothesis is that mechanical signaling improvements will lead to better liver organoids, but should this hypothesis not yield improved organoids, the goals of the grant may be jeopardized. • The approach to make organoids is to make better hepatocytes, then assemble the differentiated cells into organoids. The organoid assembly phase is not yet in hand, and therefore, there is some risk that this approach may not make the desired high-quality organoids.
No: 9	• No treatment has been approved, and NAFLD is highly prevalent so models are critical and much needed. • NAFLD is highly prevalent and higher in Hispanic populations. • Very strong genetic rationale, but use of iPSCs as proposed is unfocused and unclear. • The foundation of this proposal is use of the established patient derived iPSCs. However, details into this bank would have better supported the understanding and description of the proposed experiments to avoid these being under-appreciated. • Unclear how the SNPs will be identified/validated. If from the iPSC lines already established, then it will have to be determined that these exist in the original patient samples, and not generated during reprogramming. • Unclear why introducing known SNP and genetics into any iPSCs or ESC would not provide the proof of principle required, similar to that outlined in Aim 2.4. Seems the NAFLD is complicating the preliminary needs and goals of the proposal for an unclear reason. • Methods shown in Figure 2 are not unique, and it is unclear how these are going to be used in the mechanical approaches suggested. This could be tested and done on any iPSCs lines, and need not be specific to NAFLD. • Are cohort iPSC lines large enough for statistical power in the comparison and experiments proposed? Based on Table 1 there are three lines, which is not considered a bank, and it is not clear if these are clones or bulk iPSC lines. • Addition of Notch evaluation seems tangential and prevents focus on to understanding the genetics of NAFLD as indicated by applicant.
GWG Votes	**Is the project well planned and designed?**
Yes: 1	*none*
No: 14	• The plan will assess fat metabolism in liver organoids from iPSCs of Hispanic White (HW), non-Hispanic White (W) and African American (AA) populations. Since NAFLD is known to be highly prevalent in HW, prevalent in W, and less in AA populations, this diversity panel of iPSCs may form the basis of determining cellular mechanisms that are predisposed to NAFLD.

	• A risk is that the design will only assess cell-autonomous aspects of NAFLD. Should extracellular factors be of major importance, these models will miss this. This design problem is recognized however, and they will test conditioned medium from organoids to see if there are effects on stellate cell cultures, though surprisingly, they do not propose to test stellate cell conditioned medium upon organoids. • Consideration of non-cell autonomous phenotypes are needed. • Gene editing and inhibitors will be explored to see if these can mitigate severity of NAFLD features in this model. If successful, these finding may suggest treatment avenues. • The ability to perform Aim 2 (ethnicity assessment in vitro) is entirely dependent upon the success of Aim 1 (production of high quality liver organoids). Since such organoids are not in hand, if they cannot be made, then no relevant data on the role of ethnicity for NAFLD would be forthcoming. • The proposal suggests the use of 3D organoids and xeno-free directed differentiation of hepatocytes. Unclear if this is required or not, and should be compared to assure the most efficient method is used given the power of this study comes from the cohort of established iPSCs. • How will iPSC lines be compared for the various tests and how will this be related back to the patient sample? • Aims are not focused on the goal of understanding the genetics of NAFLD and creating a model via iPSCs. Several experiments proposed are unrelated to this goal.
GWG Votes	**Is the project feasible?**
Yes: 7	• The project is feasible, but there is limited organoid experience. • PI has good experience with stem cell research and differentiation, especially with ECM influences upon differentiation, but does not appear to have extensive experience with stem cell biology leading to the production of hepatocyte organoids.
No: 8	• Experts in the field and everything seems in place. • No, aims not likely to be achieved within the timeline but tools in place, equipment and most assay based methods.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• This grant is specifically centered on the question of why individuals of Hispanic ethnicity are more prone to NAFLD. • Strong impacts in this area. • This is especially an issue with the Hispanic population and genetically targeted.
No: 2	*none*

Application #	DISC0-13852
Title (as written by the applicant)	Therapeutically exploring transplanted stem cell death
Research Objective (as written by the applicant)	This study will gain new insights into the therapeutic implications of stem cell death following transplantation and harness this knowledge to accelerate more robust clinical translation.
Impact (as written by the applicant)	This project can potentially make an immediate impact in treating inflammatory lung injuries including acute respiratory distress syndrome and lung fibrosis, which are unmet medical needs.
Major Proposed Activities (as written by the applicant)	• Prepare and characterize stem cell-particle-drug formulations with different drug doses and releasing profiles. • Develop in vitro assays to characterize stem cell-particle-drug formulations for potency. • Characterize the biodistribution of stem cell-particle-drug formulations and immune cell uptake in vivo. • Conduct pharmacokinetics (PK) analysis of delivered drugs in vivo to demonstrate specific targeting to diseased tissues. • Evaluate reproducible disease-modifying efficacy in an animal model that mimics hyperinflammation and fibrosis processes in the lungs. • Investigate the mechanism of action (MOA) of stem cell-particle-drug formulations in restoring the immune balance to suppress hyperinflammation and prevent fibrosis processes.
Statement of Benefit to California (as written by the applicant)	Our proposed therapeutic approach has the potential to treat a broad range of diseases including inflammation, fibrosis, autoimmune disorders, and cancer. Many of these conditions have disproportionately affected patients from vulnerable ethnic, socioeconomic, and geographic backgrounds in California. For our first intended indication, acute respiratory distress syndrome (ARDS) alone, the proposed therapy, if it works, can save 10,000s of lives in California.
Funds Requested	$1,357,039
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	61
Median	60
Standard Deviation	6
Highest	70
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: 8	• The project is significant, because it has a potential to define a mechanism of action of a type of stem cell. • The applicants question traditional explanations for mode of action of stem cells as lacking evidence. Engraftment and differentiation does not seem to occur significantly and trophic repair is likely to be limited due to trapping of the stem cells in the microvasculature of filter organs, predominantly in the lungs. Instead, the authors propose a new mode of action leading to enhanced anti-inflammatory, tolerogenic and regenerative capacity of the endogenous cells. • Therefore the applicants now plan to engineer the stem cells as a means of driving regeneration. The COVID-induced acute respiratory distress model would be used to explore the effectiveness of this new approach. • It is debatable whether the proposal addresses a key knowledge gap as that would depend on accepting that existing theories of mechanism of action of the stem cells (engraftment and differentiation or trophic repair) are not correct. On the other hand, it remains possible that proposed alternative mechanism via cell death and clearance could play a role.
No: 7	• At the moment there are strong risks that such modification of stem cells will not produce the desirable results or effect will be incremental compared to cells and drug alone or in combination. • If successful the outcome will only moderately advance world class science
GWG Votes	**Is the rationale sound?**
Yes: 7	• The hypothesis is based on a sound scientific rationale. • The rationale is reasonable but not backed by preliminary data. • The applicants have made an interesting case for testing the idea that the stem cells drive regeneration indirectly. However this remains an unproven hypothesis and robust analysis of even this mechanism may still be lacking by the end of the proposed project. • The treatment is expected to induce anti-inflammatory, tolerogenic and regenerative pathways. The overall rationale is theoretically sound but lacks a strong evidence base in terms of the underlying proposed mechanism of action. • The therapeutic aspect of the proposal is based on the well described observation that pulmonary fibrosis (accumulation of extracellular matrix) is the major pathogenic mechanism leading to pulmonary insufficiency and the need for ventilation in patients with COVID and other viral infections of the airways. • Macrophages are central to the process of fibrosis in the lung. They accumulate in lung tissue as a result of virally induced inflammation and drive a cytokine storm as well as fibroblast proliferation. They are therefore the ideal target for new therapeutic approaches.
No: 8	• Preliminary data are not sufficient to support the viability of the proposed approach. Authors should demonstrate that such modification of stem cells will be beneficial compared to cells alone, drug alone and in combination as the drug is already a standard of care in COVID-19-induced ARDS. • It is not clear why in the preliminary data applicants show the feasibility of the product in a LPS-induced lung injury model while the studies are proposed to be done in the bleomycin model. The application goes back and forth between COVID-19, ARDS and pulmonary fibrosis. These are 3 very different clinical conditions, what is the focus? • Drug loading into stem cells may result in the modification of stem cell properties and suppression of cell production of mediators. Although the authors state that stem cell viability status is not important for their mechanism of action, the intrinsic ability of stem cells for immunomodulation should not be completely discarded. • The hypothesis is not tested directly.

GWG Votes	Is the project well planned and designed?
Yes: 1	*none*
No: 14	• It is not clear what the main research question of this application is. If it is to study mechanism of action of stem cells through their engulfment by macrophages after their apoptosis then the experiments proposed here are not sufficient/mechanistic enough to answer this. If the goal is to test the approach of using stem cells as vehicles to deliver drugs to the lungs as a treatment of ARDS, why choose the model of fibrosis? • The experimental plan seems to fall between two projects. On the one hand it sets out to prove a newly proposed mechanism of action of stem cells and on the other it forges ahead with testing in a disease model without fully exploring the underpinning biology. • The project is not properly designed to address the hypothesis being tested. • The logic of the project is very confusing. • The second stage of work will be to test the chosen formulation in a bleomycin-induced lung injury model. Bleomycin induces epithelial damage leading to inflammation and fibrosis. Other models, such as LPS-induced inflammation, are less likely to drive fibrosis and so would be sub-optimal. • As the drug has strong anti-inflammatory effects it is important to test in more relevant models such as live bacteria pneumonia induced ARDS and sepsis induced ARDS.
GWG Votes	**Is the project feasible?**
Yes: 6	• The proposed experiments are very standard; the project can be accomplished in 3 years time. • The experimental plan is feasible. • The project is feasible, but it will not address the hypothesis to be tested.
No: 9	• Lack of expertise in the respiratory field.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 12	• The project will test the effect of the bleomycin lung injury in both male and female mice. • Underserved communities are particularly at risk from COVID/ARDS and therefore this disease target is highly relevant to underserved populations.
No: 3	• The statement of DEI is vague. Although applicants are proposing to use male and female mice in the experiments, no power calculations were included.

Application #	DISC0-13779
Title (as written by the applicant)	Immune Tolerization to AAV Capsid for Gene Therapy
Research Objective (as written by the applicant)	We have constructed DNA plasmids to reduce the immune response to the AAV viral vectors used in gene therapy. The human immune response to AAV weakens the efficacy of gene therapy.
Impact (as written by the applicant)	This research will help overcome the bottleneck imposed by the body's immune response to the virus that contains the gene to be transferred. We intend to reduce immunogenicity of the AAV vector.
Major Proposed Activities (as written by the applicant)	• We shall build on our preliminary data published previously to design and optimize constructs for AAV vectors in gene therapy. • We shall build on our preliminary data to design and optimize DNA constructs for various AAV vectors in gene therapy. We shall test therapy with an adjunctive GpG oligonucleotide constructs. • We shall test cross-specificity of AAV constructs in tolerization, by testing specificity of constructs specific for AAV6 versus constructs specific for AAV9. • We shall continue to optimize and add content to the autoantibody arrays, specifically focusing on AAV immunogenicity.
Statement of Benefit to California (as written by the applicant)	Tolerizing to the viruses used in gene therapy will make gene therapy available to more Californians, including those who are ineligible for gene therapy because of pre-existing immunity to the adenoviruses used in some gene therapies.
Funds Requested	$1,578,000
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	58
Median	60
Standard Deviation	6
Highest	70
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: 8	• The approach is interesting in principle. If successful, it could address a significant issue of current gene therapy approaches. • The immunogenicity of viral vectors and its potential impact on genetic therapies has been a major concern of the field in several areas. Success here could be quite helpful in informing much needed solutions. Potential impact could be high. • However, many other groups have been investigating this issue, and there has been little follow up on this particular approach since the prior publication.
No: 7	• Immune responses to AAV vector components are a significant impediment to the use of this delivery system in gene therapy. • If successful, the project could extend the use of AAV vectors in delivery of gene therapy. This is a widely used vector system and the impact could be considerable. The immunogenicity is well acknowledged and other groups are working on similar strategies in AAV vector design. • The scientific proposal consists largely of refinements to the applicant's previous work. • Applicant does not acknowledge that other groups are working on similar modifications of AAV vectors.
GWG Votes	**Is the rationale sound?**
Yes: 6	• The proposal looks to build on previously published work, which is a strength. • However, the preliminary data does not include any additional work or follow up done in the intervening years, which does not suggest the likelihood of success with continued investigation.
No: 9	• The concept of tolerization to AAV constructs and dystrophin itself is certainly valid. It is less clear how this would help patients who already have high titers of anti-AAV antibodies. The preliminary preclinical mouse data were obtained with naive animals. • The preliminary data for this study appear promising, but these data were published four years ago by the PI and there appears to have been little follow up since, apart from a patent filing. Preliminary findings reported are restricted to this one publication. • A major concern is that the applicant has not done any work on this program since the publication of the original paper. There seems to have been very little follow up by other investigators. While the approach proposed is still interesting in general, the absence of follow-on research during the last few years reduces the probability of success for the proposed studies.
GWG Votes	**Is the project well planned and designed?**
Yes: 2	*none*
No: 13	• The project is designed to give meaningful and cohesive results. • There is concern about the robustness of the effects described in the published paper. The positive effects on muscle performance were observed with the actual DNA construct coding for the gene, but the control plasmid lacking the sequence was also effective, although to a smaller extent. The finding raises the possibility that the procedure rather than the specific immunization plays a major role in the positive effects. • Not clear that these studies will add much to other work ongoing in this field. • No preliminary data to indicate whether the quantity of sequences influences outcome. No proposal to look at the activity of receptors. • No in-depth investigation of immune response. • The applicants indicate that pitfalls are not expected and, as such, outline few alternate approaches. While some confidence is expected given the previous work, no study is without the potential for failure or the unexpected, and the absence of alternate plans is concerning. • There are no alternative approaches for potential pitfalls.
GWG Votes	**Is the project feasible?**
Yes: 8	• Aims and outcomes seem likely to be achieved in the timeline proposed. However, the outlined amount is above what CIRM will cover, and aim 4 includes a timeline well beyond the scope of the grant.
No: 7	• The PI is a highly regarded neuroimmunologist whose main achievements lie in therapy for multiple sclerosis.

	• Yes, the laboratory is set up to perform this work. Costs appear relatively high given 50% time postdoc and lab manager posts. • While the applicant has an outstanding track record of achievement over several decades, the current research group is very small and the productivity (regarding primary basic science publications) has been limited in recent years, raising the concern that it may be difficult to execute the proposed studies. • For the most part, the applicant points to previous results to indicate the experiments will be feasible. • Applicants do not consider potential adverse effects of removal of CpG sequences on vector performance.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13	• Acceptable.
No: 2	• The DEI statement notes that underrepresented patients may benefit from the treatment. • The DEI statement is vague and lacks specific details relating to DEI, as it speaks only about engagement with rare disease foundations. • The project plan and design do not consider or mention race, ethnicity, sex, gender, age or any other aspect of diversity, and applicants may want to revisit their understanding of this aspect of the grant.