DISC0 AWARDS

9/25/25

$78,862,290 GWG RECOMMENDED

$73,327,567 CIRM TEAM RECOMMENDED

$74,200,000 AMOUNT AVAILABLE

$0 BOARD APPROVED

	APP #	TITLE	BUDGET REQ	GWG Recmd	CIRM Recmd	SCORE (MEDIAN)	Mean	SD	Score Range Low	Score Range High	Number of GWG Votes Y	Number of GWG Votes N	Resubmission	Previous CIRM Funding	TEAM TRACK
	DISC0-17363	Allele Prospector: Leveraging human genetic variation to enable therapeutic genome editing in hundreds of disease genes	**$5,112,209**	**Y**	**Y**	**98**	96	5	80	98	13	1	N	Y	Y
	DISC0-17394	Harnessing developmental biology to achieve safe and efficient in vivo genome editing of HSCs	**$2,316,683**	**Y**	**Y**	**95**	93	2	90	95	14	0	N	Y	
	DISC0-17652	Next generation stem cell transplantation approaches for pediatric neurodegenerative disorders	**$4,628,762**	**Y**	**Y**	**93**	92	3	88	95	15	0	N	N	Y
	DISC0-17515	Hearing the Silence: Genome-wide Mapping of Cell-Type-Specific Silencers in the Developing Human Brain	**$4,244,432**	**Y**	**Y**	**90**	91	2	87	95	14	0	N	N	Y
	DISC0-17608	Interrogation of tandem repeat variants contributing to neurodevelopmental and psychiatric traits using stem cell models	**$2,405,997**	**Y**	**Y**	**90**	90	1	88	91	14	0	Y	N	
	DISC0-17610	Unraveling the developmental path from altered hematopoietic stem cells to leukemia in Down syndrome	**$2,337,847**	**Y**	**Y**	**90**	90	3	80	95	14	1	Y	Y	
	DISC0-17674	Identifying and Overcoming Roadblocks to Hearing Restoration Using Human Induced Pluripotent Stem Cells	**$4,608,000**	**Y**	**Y**	**90**	90	3	82	93	14	1	N	N	Y
	DISC0-17626	Development of in vitro and in vivo functional human synthetic kidney organoid (hSKO) model as a platform technology for kidney research	**$2,287,926**	**Y**	**Y**	**90**	90	4	84	96	12	1	N	N	
	DISC0-17364	Mechanisms of Transcription Factor Haploinsufficiency in Human Congenital Heart Disease	**$2,444,376**	**Y**	**Y**	**90**	89	1	85	90	15	0	N	Y	
	DISC0-17635	Genetic and Epigenetic Regulation of XIST and X-chromosome silencing in hiPSCs: Overcoming Barriers in Stem Cell-Based Therapies for Women's Health	**$2,358,742**	**Y**	**Y**	**90**	89	3	85	95	15	0	N	N	
	DISC0-17421	Developing replacement islet cells for diabetes using human stem cells	**$3,943,364**	**Y**	**Y**	**90**	89	5	75	95	13	2	N	N	Y
	DISC0-17487	Mechanisms underlying dosage sensitivity in developmental disorders	**$2,304,000**	**Y**	**Y**	**90**	89	3	80	92	13	1	N	N	
	DISC0-17685	Dissecting the cellular and molecular interactions between embryo and endometrium during human implantation.	**$2,290,157**	**Y**	**Y**	**90**	89	2	85	90	13	0	Y	N	
	DISC0-17946	High-Throughput Discovery of Embryo Formation Factors Using Stem Cell-Based Human Embryo Models	**$2,872,697**	**Y**	**Y**	**88**	87	2	85	90	13	0	N	N	Y

	APP #	TITLE	BUDGET REQ	GWG Recmd	CIRM Recmd	SCORE (MEDIAN)	Mean	SD	Low	High	Y	N	Resubmission	Previous CIRM Funding	TEAM TRACK
	DISC0-17976	Enhancing clinical predictability with novel models of iPSC-derived nociceptor for chronic pain	**$1,498,623**	**Y**	**Y**	**88**	87	3	80	90	12	1	N	Y	
	DISC0-18130	Unlocking the regenerative potential of hepatocyte plasticity for diseases of the biliary system	**$4,871,000**	**Y**	**Y**	**86**	87	3	80	90	13	2	N	N	Y
	DISC0-17315	IFN-γ suppresses AT2 cell regeneration to promote lung fibrosis	**$4,525,190**	**Y**	**Y**	**86**	86	3	80	90	11	4	N	N	Y
	DISC0-17998	Global profiling of miRNA-based gene activation to enable a new category of genetic medicine	**$2,997,574**	**Y**	**Y**	**86**	86	2	80	90	10	2	N	N	Y
	DISC0-17566	In neurons and beyond: how protein interactions shape the cellular response to Huntington's Disease	**$2,056,195**	**Y**	**Y**	**86**	85	2	80	90	11	3	N	N	
	DISC0-17276	Modeling Rett syndrome neurological disorder with human pluripotent stem cells to develop in cellulo screening platforms.	**$2,393,281**	**Y**	**Y**	**85**	86	1	85	90	14	0	Y	Y	
	DISC0-17488	A novel platform to rescue neurodevelopmental disorders caused by haploinsufficiency	**$4,086,486**	**Y**	**Y**	**85**	86	2	84	90	13	1	N	Y	Y
	DISC0-18038	Base Editing, Single-Cell Multiomics, and Cardiac Organoids to Decode Genetic Variants	**$4,606,248**	**Y**	**Y**	**85**	85	2	83	89	10	3	N	N	Y
	DISC0-17513	COPA Syndrome as a paradigm to define mechanisms of epithelial progenitor cell dysfunction in autoimmune lung disease	**$4,034,724**	**Y**	**N**	**85**	83	2	79	86	8	7	N	N	Y
	DISC0-17507	Unraveling nuclear Tau functions using age-equivalent human induced neurons from healthy aging donors and tauopathy patients	**$2,137,778**	**Y**	**Y**	**85**	83	3	80	86	7	7	N	N	
	DISC0-17954	APOE4 compromises BBB integrity through pericyte-microglia cross talk	**$1,499,999**	**Y**	**N**	**85**	83	3	80	90	7	7	N	N	
	DISC0-17263	Neuroprotective discovery for Parkinson's disease using human iPSC models	**$2,244,452**	**N**	**N**	**84**	82	2	80	86	1	13	Y	N	
	DISC0-18123	Maternal/embryonic/fetal communication, from implantation through the first trimester: Development of physiologic placentation models from stem cells.	**$2,304,886**	**N**	**N**	**83**	83	3	80	90	4	10	N	N	
MR	**DISC0-17822**	Role of stem-like T cells in autoimmune diseases	**$4,603,793**	**N**	**N**	**83**	82	4	75	85	7	8	N	N	Y
	DISC0-17737	Multi-Ome Profiling in Neurodevelopmental Patient iNeurons to Identify Therapy-Responsive Biomarkers	**$2,349,271**	**N**	**N**	**83**	82	2	80	86	1	12	N	Y	
	DISC0-17690	A function-based screening platform for identifying implantation-competent embryos	**$3,675,887**	**N**	**N**	**80**	81	4	70	85	4	11	N	N	Y

	APP #	TITLE	BUDGET REQ	GWG Recmd	CIRM Recmd	SCORE (MEDIAN)	Mean	SD	Low	High	Y	N	Resubmission	Previous CIRM Funding	TEAM TRACK
	DISC0-17756	De Novo Epigenetic Programming of Human Regulatory T Cells	**$2,609,641**	**N**	**N**	**80**	80	5	70	90	4	11	N	N	
	DISC0-17973	Leveraging Area Specific Cortical Organoid Models to Understand Neurodevelopment Disorders	**$2,342,488**	**N**	**N**	**80**	80	4	75	91	2	12	Y	N	
	DISC0-17580	Developing a scalable platform for ex vivo manufacturing of universal red blood cells	**$4,787,600**	**N**	**N**	**80**	80	2	80	86	1	13	N	N	Y
	DISC0-17322	Profiling the molecular landscape of the injured newborn brain may inform how neural stem cells preserve & may recreate neural circuitry	**$4,586,156**	**N**	**N**	**80**	80	2	78	84	0	13	N	Y	Y
	DISC0-17391	A Multimodal Atlas of Redox Signaling and Organelle Dysfunction in Stem Cell Models of Neurodegeneration and Aging	**$4,354,071**	**N**	**N**	**80**	80	2	75	84	0	14	N	N	Y
	DISC0-17579	Gene-edited CD19 CAR-T cells with superior proliferation, persistence and serial-killing activity	**$2,429,480**	**N**	**N**	**80**	79	3	70	84	0	14	Y	N	
	DISC0-17428	Minimal invasive approach for monitoring stem cell and gene therapy	**$2,030,050**	**N**	**N**	**79**	79	3	75	85	1	11	N	N	
	DISC0-17269	Microglia replacement with non-myeloablative hematopoietic stem cell transplantation for Alzheimer's disease	**$2,303,684**	**N**	**N**	**79**	76	7	60	86	1	13	Y	N	
	DISC0-17603	Cord-to-Cure: Uncovering Genomic Mechanisms of Placental Resilience and Vulnerability for Improved Maternal-Fetal Outcomes	**$4,278,894**	**N**	**N**	**75**	76	2	75	80	0	14	N	N	Y
	DISC0-17367	Generation of Functional Proximal Tubules in Organoids through Gradual Developmental Mimicry for Kidney Injury Modeling	**$2,153,295**	**N**	**N**	**75**	74	3	70	78	0	15	Y	N	
	DISC0-17386	Machine-Guided and Quantitative Solutions to the Unique Challenges in Gene Therapies for Neurogenetic Disorders	**$2,186,981**	**N**	**N**	**75**	74	3	70	80	0	14	N	N	
	DISC0-17609	Exploring Potential Drugs to Enhance Neural Recovery in Combination with Neural Stem Cells after Spinal Cord Injury (SCI)	**$2,403,379**	**N**	**N**	**75**	74	2	70	75	0	14	Y	N	
	DISC0-17688	Regulation of Cardiac Cell Reprogramming by Macrophages	**$4,558,759**	**N**	**N**	**75**	73	5	60	75	0	14	N	N	Y
	DISC0-17614	A humanized organ-chip model to investigate neuroinflammation contributions in Frontotemporal Dementia	**$1,519,882**	**N**	**N**	**70**	73	4	70	80	0	14	N	N	
	DISC0-18051	Reversing Neuroinflammation and Alzheimer's Pathology via Therapeutic Blood Exchange, Regenerative EV Targeting, and Biomarker Discovery	**$4,616,715**	**N**	**N**	**70**	72	10	55	85	2	12	N	N	Y
	DISC0-18026	Nucleoporin regulation of hematopoietic and leukemic stem cells	**$2,304,918**	**N**	**N**	**70**	71	10	50	85	2	13	N	N	

	APP #	TITLE	BUDGET REQ	GWG Recmd	CIRM Recmd	SCORE (MEDIAN)	Mean	SD	Low	High	Y	N	Resubmission	Previous CIRM Funding	TEAM TRACK
	DISC0-17716	Progression or Differentiation? What are the key factors that delineate Hypertensive Disorders of Pregnancy	**$3,406,745**	**N**	**N**	**70**	71	5	65	80	0	13	N	Y	Y
	DISC0-17622	A Comprehensive Framework to Decipher and Leverage Human Skeletal Stem Cell Biology	**$2,328,426**	**N**	**N**	**70**	70	12	50	91	2	13	N	N	
	DISC0-17351	Hematopoietic stem cell response to trained immunity	**$1,994,415**	**N**	**N**	**70**	70	1	70	75	0	15	N	N	
	DISC0-17940	Lnc-ing myelin and oligodendroglial dynamics to autism spectrum disorders	**$1,386,620**	**N**	**N**	**70**	70	3	65	80	0	13	N	N	
	DISC0-17893	Exploring the potential of RNase H1 inhibition and forced accumulation of R-loops as new therapeutic strategies for myeloid malignancies	**$2,727,360**	**N**	**N**	**70**	68	5	55	75	0	15	N	N	
	DISC0-17853	Oncogenic drivers of mixed phenotype acute leukemia and its genetic instability	**$3,152,434**	**N**	**N**	**70**	67	4	60	71	0	15	N	N	Y
	DISC0-17365	Targeting Aberrant Basaloid and Basal Cell Transdifferentiation for Therapeutic Intervention in Pulmonary Fibrosis	**$2,483,451**	**N**	**N**	**65**	65	3	60	70	0	15	N	Y	
	DISC0-17677	Cell-specific effects of APOE4 gene editing in Alzheimer's disease neurodegeneration	**$4,838,321**	**N**	**N**	**65**	64	6	50	75	0	14	N	N	Y
	DISC0-17499	Engineering universal hPSCs for skeletal muscle	**$4,225,803**	**N**	**N**	**60**	60	8	50	80	0	14	N	N	Y
	DISC0-17929	Engineering synthetic gene oscillators to slow hematopoietic stem cell aging	**$2,310,000**	**N**	**N**	**-**	**-**	**-**	**-**	**-**	0	15	N	N	
	DISC0-17442	Generation of a human synthetic limb-inducing cell	**$3,323,200**	**N**	**N**	**-**	**-**	**-**	**-**	**-**	0	15	N	N	Y

Application #	DISC0-17363
Title	Allele Prospector: Leveraging human genetic variation to enable therapeutic genome editing in hundreds of disease genes
Project Objective (as written by the applicant)	We are building a foundational platform for developing genome editing technologies that increase patient coverage by 20-40X and can be applied to over 700 genetic diseases in diverse populations.
Impact (as written by the applicant)	Many genetic diseases have hundreds of different mutations so correcting mutations one-by-one is impractical. We are developing an approach that can reach more patients with curative treatments.
Major Proposed Activities (as written by the applicant)	• Build a genomic software pipeline to identify high-impact allele-specific edits at genomic scale in diverse human genomes. • Publicly disseminate information on high-impact allele-specific edits via human genome browser tracks so anyone can use them. • To make CRISPR editing safer, we are investigating ways to make precise changes in the relevant cell types we are trying to treat such as neurons. • Uncover the rules of gene editing repair to help us cut out all or part of a disease gene in a way that is therapeutic, safe, and effective.
Statement of Benefit to California (as written by the applicant)	This project benefits California by helping accelerate research to find cures for over 700 genetic diseases of the nervous system, the heart, and other tissues. This project will provide foundational knowledge that will make therapeutic editing more efficient, so that a single edit can treat 20-40X more people while also finding ways to make these edits safer and more precise. These efforts will reduce the costs and increase access to curative genetic therapies.
Funds Requested	$5,112,209
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: 98

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	96
Median	98
Standard Deviation	5
Highest	98
Lowest	80
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	13
(1-84): Not recommended for funding	1

KEY QUESTIONS AND COMMENTS

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

Does the project hold the necessary significance and potential for impact?
• Yes - the project could have powerful impacts across the genome editing field. • The studies aim to establish new tools and strategies to address genetic diseases with a predominantly dominant inheritance. The work could result in more durable and curative strategies for these patients. Understanding genomic deletion with paired nucleases is a key knowledge gap in moving genome editing to additional disease targets. • The combination of bioinformatic and experimental capabilities enables the work and broadens the study's impacts. • The proposal is build on a highly innovative premise and focus. The concept of gene editing a a therapeutic approach with potential broader applications, namely the focus on haplosufficient disease in very innovative and has the potential to benefit a larger number of patients compared to patient specific variant editing. • There is clearly a significant knowledge gap related to haplosufficient diseases. The proposed approach is highly innovative. • The team is highly qualified and experienced. • Yes, they want to develop a computational tool to look for haplotypes that would allow them to target dominant mutations in a haplosufficient gene (one copy does the trick). They are also trying to better understand how concurrent DSBs are resolved and how to leverage them to increase efficiency and accuracy of allele specific inactivation. • This is a strong team, bringing together wet and dry lab expertise required for this study.
Is the rationale sound?
• Yes - very strong. • The Color-seq assay is novel and will provide new data and resources for the field. • The proposed studies are highly innovative and have not been addressed in this scale. The use of large datasets to identify haplotype structures is highly innovative. The analysis approach is well described and fully support the proposed downstream experiments. • Yes, sufficient preliminary data are provided. They have shown that they can make targeted deletions at allele specific haplotypes. Little is known about the proteins involved in concurrent DSB repair (inversions/deletions). • Yes, CRISPRi screens can be done, data from global genomes are available, genome editing and computational expertise to do this project are in the team.
Is the project well planned and designed?
• Yes - very strong. • The plan is well considered and draws upon strengths in computation and experimentation. • The consideration of 3D genomic structure is well considered and the approach to analyze it is novel. • The aims are well developed. Aim 1 identifies haplotypes in diverse populations. This builds the foundation for the proposed editing approach. • Beyond the specific focus of the proposal, aim 2 will provide important new insights which will be relevant to the concept of therapeutic gene editing. This is a distinct strength of the proposal. • Aim 3 further expands the concept and exports mechanism to improve editing. The concepts and insights gained from this aim will again have broader implications beyond the proposal. • The investigators proposed two cell types, namely cardiomyocytes and neurons. While the applications proposes to focus on diseases which are influenced by haplosufficiency, the applicants do not discuss for how many disease these cell types are potentially disease relevant. It is quite likely that other cell types are potential therapeutic targets. This limits the direct application to treatment. The general insights and methods could be extended. • Potential pitfalls and limitations are well described.

- Yes, Aim 1 will provide tools that can be used for this and other projects, and they are planning to make these tools publicly available. Aim 2 will help elucidate proteins involved in post mitotic cells, which will be key for many of the diseases that are being considered as targets. Aim 3 will use a iPSC reporter to assess and try to predict deletion and inversion outcomes.
- Yes, Org chart included. Each investigator has domain expertise and associated team.

Is the project feasible?

- Yes - very strong.
- No concerns.
- Resources and infrastructure are excellent.
- Budget and timeline appear appropriate.
- Yes, great preliminary data, great team, well described, and will create tools for the community. Great!

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- Yes - very strong.
- Yes, the work uses iPSC lines from several backgrounds.
- Yes, the work would expand the potential scope of genome editing therapies to more Californians.
- Yes, the team has shared code and worked with patients for several years. Their track record is commendable and would enhance the dissemination of the study results.
- The underlying concept requires genetic variability and the inclusion of diverse populations as it focuses on common targets for gene editing.
- If successful, the results will provide the foundation for gene editing in diverse populations.
- Outreach, and partnerships are well described.
- Yes, not focused on one dHS disease, but looking at a list of over 700 that could benefit from their work in targeting the disease specific allele. They are looking for common haplotypes. They use over 50 different iPSC lines.
- Yes, they host patients on lab tours. PI is on a patient advocacy group and has a letter of support from patient parent. The lab is involved in CIRM Bridges Stem Cell training.

Application #	DISC0-17394
Title	Harnessing developmental biology to achieve safe and efficient in vivo genome editing of HSCs
Project Objective (as written by the applicant)	We seek to develop a more accessible method of genome editing of blood stem cells directly in the body, so that the treatment can be safer and less expensive than the currently available approaches.
Impact (as written by the applicant)	We think our new protocol could transform the care of children with blood diseases like sickle cell disease (SCD), thalassemias, and metabolic conditions.
Major Proposed Activities (as written by the applicant)	• We will develop a model system to test systemic delivery of genome editors to blood stem cells. • We will test an array of lipid nanoparticles to identify those that are most efficient in targeting blood stem cells. • We will test the effects of two different genome editing technologies on blood stem cells. • We will test the effects of genome editing in blood stem cells obtained from patients with alpha thalassemia.
Statement of Benefit to California (as written by the applicant)	Blood diseases like thalassemias and sickle cell disease (SCD) affect many patients in California. We are developing a genome editing treatment protocol that would correct the mutation in the blood stem cells directly in the body. Our strategy would avoid the toxicities and expense of the current therapies, which require taking out the blood stem cells, correcting them in the lab, and transplanting them back into the patient. If successful, our strategy would be safer and less expensive.
Funds Requested	$2,316,683
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: 95

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

Mean	93
Median	95
Standard Deviation	2
Highest	95
Lowest	90
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	14
(1-84): Not recommended for funding	0

KEY QUESTIONS AND COMMENTS

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

Does the project hold the necessary significance and potential for impact?
• This application proposes evaluation of in vivo gene editing of HSCs using lipid nanoparticles (LNPs). The applicant is targeting HSCs in fetal liver and neonatal circulation as they may be more amenable to editing than adult HSCs. Upon successful completion, this proposal will advance several key questions in HSC biology, including optimizing LNPs for in vivo gene editing of HSCs, exploring neonatal HSCs as a potential target for gene editing, and having the opportunity to translate this technique for multiple diseases. • Given that the PI is leading a clinical trial of allogeneic transplantation for [redacted indication]. An autologous gene therapy is a logical next step and the data presented support the rationale. • The plan is to develop LNPs to edit liver resident or circulating HSCs (in mice) at percentages that would have clinical impact for patients with hemoglobinopathies. • There are several innovations that appear to be central to this proposal: 1. Editing of HSCs in fetal circulation. 2. Choice of disease model (alpha thalassemia) which would benefit from in utero correction. 3. Multiple LNP formulations that have been tested for their relative efficacy. • The development of a barcode screen of candidate HSC-tropic LNPs would be highly significant and impactful. • The team is diverse with non-overlapping strengths (barcode screens; novel LNP compositions; gene editing; etc.) • This is an individual PI grant with strong collaborations.
Is the rationale sound?
• The presented preliminary data are sound and support all three of these innovations. • The aims are carefully thought out and sound. Since the PI has focused on basic fundamental questions related to HSC biology, the outputs from these experiments are bound to yield meaningful results. • Yes, in vivo genome editing is a major unmet need. Editing of HSCs in neonates or fetuses would be ideal to 1) reduce cost per treatment and 2) prevent the damage associated with the disease that occurs up until the age of treatment. • The proposal includes a lot of great preliminary data: LNPs edit HSCs in mouse model, editing in human HSPCs, access to and characterization of cord blood HSPCs from thalassemia patients, treatment of human fetal liver and adult CD34+ cells with LNPs to turn them green (~30%), and editing neonatally transplanted HSPCs by systemic injection into WT mice. • Yes, the rationale is sound. Targeting (specifically) of HSC is not well defined. Is the LNP specific for HSC?
Is the project well planned and designed?
• The proposal proceeds in a logical, stepwise fashion and will yield data and proven models that would be useful to the community: 1) cell atlas of HSC responses to different LNPs/genome editing, 2) test of whether underlying pathology in patients impacts in vivo editing, comparison of two mouse models for in vivo editing, and study of LNP composition and associated cellular/genome editing effects. • Yes, the project is well planned. • Aim 1 with barcode screen is exciting but may not work. A lead candidate HSC-tropic LNP has been developed. • Insufficient data are provided on the lead HSC-tropic LNP. • In both human HSC / mouse model studies, mouse and human HSCs will reside in the same mouse. Will these experiments have interference or competition for editing? • The development of multiple HSC-tropic LNPs and the collaboration with the biotech partner are strengths. • The traffic light reported (TLR) mice are a great strength. • The studies outlined in Aim 3 are not well justified or described. • Potential pitfalls are not well described.

• The description of pitfalls is a bit lacking.
Is the project feasible?
• The project is feasible. Both TLR and human xenograft models have already been developed. • The PI is leading a multidisciplinary team of collaborators that will have regular team meetings. • The PI is also leading an allogeneic transplant study. This study is a logical next step and would have applications beyond [redacted indication]. • The team is very diverse, with every expert in the field seemingly contributing. • No issue with budget.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• The project is likely to yield meaningful data on HSC and in vivo genome editing that is likely to advance therapeutics for multiple diseases. • Yes, they have access to biospecimens linked to patient data, so they can connect editing outcomes with demographic information and disease. They will test both male and female specimens. • Yes. Donor match percentages are low for every patient, but especially Black and Asian patients, who have a higher prevalence of alpha thalassemia and SCD. • Yes; the applicant has a really strong history of outreach and is working with several patient groups.

Application #	DISC0-17652
Title	Next generation stem cell transplantation approaches for pediatric neurodegenerative disorders
Project Objective (as written by the applicant)	A complete methodology for high efficiency, minimal toxicity hematopoietic stem cell transplantation for treatment of pediatric neurodegenerative disorders.
Impact (as written by the applicant)	Improve treatment and outcomes for patients with neurodegenerative disorders treatable by myeloid replacement.
Major Proposed Activities (as written by the applicant)	• Delineate the most efficacious and least toxic conditioning regiment for brain engraftment after hematopoietic stem cell transplant. • Identify the optimal hematopoietic source for stem cell transplantation in pediatric neurological disorders. • Identify the optimal gene expression construct for high levels of therapeutic gene delivery in central nervous system myeloid cells.
Statement of Benefit to California (as written by the applicant)	Stem cell transplant is the only treatment that can treat multiple inborn errors of metabolism and has the potential for broad efficacy in neurodegenerative disease. However, variable efficacy, limited brain engraftment, and serious treatment-related toxicities are all hurdles to wide application of this therapy. This project will improve patient outcomes in California and society by advancing the protocols, stem cell types, and gene therapy vectors for transplant in neurodegenerative disease.
Funds Requested	$4,628,762
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: 93

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	93
Standard Deviation	3
Highest	95
Lowest	88
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.

Does the project hold the necessary significance and potential for impact?
• This is an exciting, well conceived, achievable project that will have significant impact on the treatment of children with leukodystrophies and inborn errors of metabolism. • This is a scientifically rigorous and innovative proposal targeting a significant clinical need: the toxicity, inefficiency, and limited accessibility of stem cell transplantation (SCT) for pediatric neurological diseases, particularly inborn errors of metabolism (IEM). A critical unmet need in pediatric neurodegeneration, particularly IEMs (~1 in 1,500 births in California). • The application presents a sophisticated and thoughtful approach that combines central nervous system (CNS)-targeted conditioning, optimization of stem cell ontogeny, and development of brain myeloid-specific gene therapy promoters. It could quickly inform the design of safer clinical SCT protocols for neurological diseases and may help reduce disparities for populations with limited HLA match availability. • Aims to overcome key limitations of SCT: GVHD (Graft-versus-Host Disease) risk, harsh conditioning regimens, and inadequate CNS engraftment. CNS-targeted conditioning and myeloid-specific gene regulation are innovative strategies with wide-ranging potential. • Stem cell transplantation to treat or reverse pediatric neurodegeneration inborn errors of metabolism would represent a major transformative advance and would be highly significant. • The proposal is supported by a highly capable, well-integrated investigative team, and access to extensive institutional resources. • No evidence yet that replacement of host microglia in total or in part would reverse the defect with or without gene therapy would reverse the defect. The combination of transplant and gene therapy in mouse models for disease like MPS has been optimal and of interest both together resulted in optimal effects. • The use of antibody-based conditioning has been emerging as a novel approach to reduce toxicity in both the syngeneic and allogeneic transplant setting. Especially targeting CD117 as planned by this investigative team and secondarily CD45 and c-MPL by other groups. • Collaborations between [redacted PI name] Lab and [redacted PI name] Lab are limited but strong and synergistic. • [redacted PI name] is an expert in neuroimmunology and [redacted PI name], is a trained physician scientist and transplantation and gene therapy expert.
Is the rationale sound?
• The project is ambitious, but the investigators have the ability to accomplish the work proposed. They are logically approaching the main obstacles to treatment with these diseases with HSCT or gene therapy. Solving the problems of microglial depletion and engraftment as well as reducing intensity of cytoreduction is more feasible in animal studies and can guide future clinical trials in humans. • Sound mechanistic rationale based on current literature and the team's own findings. Preliminary data support differential engraftment potential by stem cell source and document transcriptomic consequences. • Using human and mouse models to study microglial niche replacement is appropriate and current. Proposal highlights key SCT limitations: microglial depletion, developmental fate, and gene regulation safety. • The rationale is sound and supported by mouse studies by a number of labs focused on these inherited inborn errors of metabolism. The team is relatively small and seems to lack some depth.
Is the project well planned and designed?
• The project is well designed and the pitfalls adequately anticipated. • Provides highly detailed and methodologically sound experimental plans with appropriate controls. Uses flow cytometry, single-cell and bulk RNA-seq, confocal microscopy, behavioral assays, and solid power calculations. • There is clear progression from conditioning optimization (Aim 1) to cell source characterization and promoter discovery (Aim 2). Proposal considers sex as a biological variable throughout. • The project is well planned and the application is well written and focused. • The use of the [redacted model name] mice will be an important reagent to test this. Again, experiments are very well organized and defined. • Testing of dosing of CD117, PLX3379, and source of HSC are all strengths

- Similar experiments will be repeated in Aim 2 using humanized mice. These studies are likewise well organized and use of iPSC, bone marrow CD34, G-mobilized CD34 are all of interest to see which if any stem cell source better differentiates into CNS microglia.
- Identifying promoters with strong brain myeloid specific expression also represents an exciting approach.
- Excellent comprehensive review in both aims of potential pitfalls and alternatives approaches. A strength.
- One concern is if CNS penetration is necessary for CD117 to be an effective depletion rational of CNS microglia. The experiment and approach are well organized and designed to validate this approach.
- Scope is too ambitious for a 3-year Discovery Award: optimizing conditioning, testing stem cell sources, and performing promoter discovery are each major tasks.
- Founder effects in the lentiviral promoter screen (Aim 2B) may limit the generalizability of the findings. Some studies require large animal cohorts (e.g., 21 mice per group), raising concerns about feasibility and ethics.
- The comparison to busulfan conditioning is important. Also TBI conditioning should be considered as well.
- No ADC-CD117 are being tested which may be a significant weakness.
- There is heavy reliance on fragile [redacted model name] mice, which are susceptible to high attrition and limited throughput.

Is the project feasible?

- This project is feasible as proposed by the investigators.
- Host institution provides strong institutional support, including access to genomics cores, flow cytometry, and high-throughput sequencing. Investigators can utilize over 70 population-representative CD34+ and iPSC lines. Key proof-of-concept experiments, such as CD34+ xenotransplantation and lentiviral delivery, have been successfully conducted.
- Budget justification is insufficiently detailed for the scale of effort and cost (~$2.5M).
- Yes, team has access to all resources.
- No issues with budget or timelines.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- This work has major implications for translation to the clinic after the preclincal questions posed are better understood. The work proposed will make this possible.
- Inclusive use of ethnically and genetically diverse human samples from >70 donors.
- Clear articulation of California-specific relevance (e.g., limited HLA match availability in diverse populations).
- There are plans for community engagement with patient advocacy groups.
- Applicant has demonstrated prior commitment to mentorships in STEM.
- Proposal has open and responsible data sharing through GEO/dbGaP Gene Expression Omnibus/database of Genotypes and Phenotypes (GEO/dbGaP) and public portals.
- Earlier and more sustained community engagement (e.g., in project design or priority setting) would strengthen equity impact. Primary focus on IEMs, while important, may limit near-term generalizability.
- Proposal lacks specific dissemination or implementation strategies for reaching underserved populations beyond sample inclusion.

Application #	DISC0-17515
Title	Hearing the Silence: Genome-wide Mapping of Cell-Type-Specific Silencers in the Developing Human Brain
Project Objective (as written by the applicant)	We will develop genome editing tools to identify silencers that regulate neural stem cell fate, uncovering key DNA elements that guide neurodevelopment and are disrupted in neurodevelopmental diseases
Impact (as written by the applicant)	The developed technology will functionally resolve the noncoding genome at unprecedented scale. The identified silencers will reveal the repressive genome architecture that governs brain development.
Major Proposed Activities (as written by the applicant)	• Technological Innovation: advance our dual-CRISPR platform to enable single-cell genome editing and profiling at million single cell scale. • Massive Parallel Profiling: generate the first map of genomic silencers across diverse cell types, revealing cell-type-specific repression programs. • Functional Characterization: conduct neurobiological experiments to define the cellular roles of identified silencers. • Resolving CHD8's Regulatory Complexity: knocking out single CHD8 binding sites across cell types to expose the core component in autism pathogenesis. • Learn DNA Gramma for Genomic Silencers: develop a deep learning model to predict cell-type-specific silences. • Uncover a New Class of Pathogenic Regulatory Mutations: leverage deep learning to scan autism genomes and identify deleterious silencer mutations.
Statement of Benefit to California (as written by the applicant)	Autism rates in California are double the national average, highlighting the need for innovation. This project will advance genomic and stem cell research, providing insights into brain development and autism. It will position California as a leader in noncoding genome science, support precision therapies, and deliver scalable technologies. Additionally, it will train future scientists, foster innovation, and strengthen the state's leadership in regenerative and neurodevelopmental research.
Funds Requested	$4,244,432
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	91
Median	90
Standard Deviation	2
Highest	95
Lowest	87
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.

Does the project hold the necessary significance and potential for impact?

- CHD8 is a chromatin remodeler frequently mutated in individuals with ASD that regulates thousands of genes as both an activator and repressor in neural stem cells or neurons. The hypothesis is that target genes of CHD8 represent novel ASD risk genes and/or points of therapeutic intervention.
- The proposal will functionally dissect the CHD8 regulatory network in the developing human brain, revealing ASD-associated components and targets for future therapeutic development.
- The genome mapping technologies central to this project were originally developed by one of the collaborators and the Co-Investigator (Co-I). The PI collaborated with the methods collaborator to adapt and implement parallel genome editing in single primary human brain cells.
- *[Investigators' names redacted]* will co-lead assay development. *[Investigator's name redacted]* will further lead single-cell RNA-seq experiments and functional characterization of regulatory element deletions. *[Investigator's name redacted]* will direct the computational biology efforts, including data analysis, integration, and data sharing.
- *[Investigator's name redacted]* has deep expertise in computational biology and will lead data management and analysis for this project. *[Investigator's name redacted]* is their institution's director of a center for genomics and personalized medicine and a pioneer in functional genomics and systems biology. *[Investigator's name redacted]* is a clinical scientist and neurologist with long-standing track record in single cell genomic approaches to classify cell types in the developing brain.
- A unique mechanism is used to screen for silencers.
- Outstanding proposal to identify repressor DNA elements in human fetal tissue generally, and to focus on a well established neurodevelopmental disorder causing gene.

Is the rationale sound?

- Silencers generally lack epigenetic signatures and are difficult to identify, but preliminary data indicates that genomic silencers are widespread across the genome.
- The team previously found and published that silencers often occupy open chromatin regions as binding sites for repressive transcription factors.
- The team developed and published novel genome editing system for parallel screening of noncoding regulatory elements in primary cells from the developing human brain at single-cell resolution of the genome.
- The hypothesis is that noncoding variants disrupting cell-type-specific silencers represent a major, previously unrecognized contributor to neurodevelopmental disorders.
- The rationale builds on previous results.

Is the project well planned and designed?

- The bottom-up approach will generate a comprehensive atlas of cell-type-specific silencers, while the top-down strategy will focus on CHD8, a central chromatin remodeler frequently mutated in autism.
- Drawing from existing CHD8 ChIP-Seq data from human cortical neurons and neural progenitors, proposal will apply team's novel genome editing approach to systematically ablate individual CHD8 binding sites across cell types in the developing human brain.
- Genome-wide mapping of cell-type-specific silencers in the developing human brain, targeting 10,057 sites for dual-CRISPR deletion. Unlike perturb-seq, that introduces short indels, the dual-CRISPR system excises entire regulatory elements at single-cell. Unlike 10x methods, which require ~100 cells per knockout so limited to ~100 perturbations (10,000 cells), Parse system to profiles up to 5 million cells.
- Genome editing on primary cells from anonymous donated prenatal fetal brain samples; maternal age and sample sex included as covariates and regressed out in downstream analyses.

- Develop a deep-learning-based mutation mapping tool to identify disruptive genomic variants in silencers and screen large-scale autism genomes. This tool will be applied to scan large-scale autism genomes to uncover a previously unrecognized class of pathogenic mutations affecting genomic silencers.
- Strong preliminary data in all aims.
- Screening in primary cells will be extended to human cortical organoids (weeks) and mouse xenografts (months) to assess the long-term impacts of site deletions.
- Bottom-up approach will generate a comprehensive atlas of cell-type-specific silencers, while the top-down strategy will focus on CHD8, a central chromatin remodeler frequently mutated in autism.
- Drawing from existing CHD8 ChIP-Seq data from human cortical neurons and neural progenitors, proposal will apply team's novel genome editing approach to systematically ablate individual CHD8 binding sites across cell types in the developing human brain.
- Genome-wide mapping of cell-type-specific silencers in the developing human brain, targeting 10,057 sites for dual-CRISPR deletion. Unlike perturb-seq, that introduces short indels, the dual-CRISPR system excises entire regulatory elements at single-cell. Unlike 10x methods, which require ~100 cells per knockout so limitied to ~100 perturbations (10,000 cells), Parse system to profiles up to 5 million cells.
- Genome editing on primary cells from anonymous donated prenatal fetal brain samples; maternal age and sample sex included as covariates and regressed out in downstream analyses.
- Develop a deep-learning-based mutation mapping tool to identify disruptive genomic variants in silencers and screen large-scale autism genomes. This tool will be applied to scan large-scale autism genomes to uncover a previously unrecognized class of pathogenic mutations affecting genomic silencers.
- Strong preliminary data in all Aims.
- Screening in primary cells will be extended to human cortical organoids (weeks) and mouse xenografts (months) to assess the long-term impacts of site deletions.
- A vast amount of data will be generated.
- Builds on the applicant's published work in which 1% of the genome was screened and 5,000 silencers were identified.
- This is a highly innovative, but for this team feasible, proposal.

Is the project feasible?

- Yes.
- Reasonable.
- A well developed and clear team communication and management plan is in place.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- The proposal describes plans to work with ASD patient groups and local high school and college students.
- The project should benefit all people affected with ASD that have genetic testing information.
- The team has a strong track record in this regard, especially the Co-I.
- The PI has led a community engagement program, which connects women experiencing pregnancy complications with clinicians at academic medical centers.
- One of the affiliated hospitals serves a diverse population.

Application #	DISC0-17608
Title	Interrogation of tandem repeat variants contributing to neurodevelopmental and psychiatric traits using stem cell models
Project Objective (as written by the applicant)	Our project will identify molecular and cellular changes induced by specific genetic variants implicated in schizophrenia and autism spectrum disorder in stem cells, neuroprogenitor cells and neurons.
Impact (as written by the applicant)	Schizophrenia and autism spectrum disorder
Major Proposed Activities (as written by the applicant)	• Perform multi-ancestry GWAS and fine-mapping to identify TRs associated with schizophrenia risk • Identify high-impact de novo mutations at TRs associated with autism spectrum disorder • Prioritize autism spectrum disorder associated and other TRs for genome editing experiments • Perform genome-editing of target TRs in human iPSCs • Differentiate edited iPSCs into neuroprogenitor cells and neurons • Perform detailed molecular and cellular characterization of edited cells and their derivatives
Statement of Benefit to California (as written by the applicant)	Our project has the potential to result in novel therapeutic targets for schizophrenia and autism spectrum disorder. We specifically leverage data from multiple ancestry groups, which can therefore benefit the State of California and its highly ethnically diverse citizens. Additionally, we focus on induced pluripotent stem cells (iPSCs), which has the potential to advance the understanding of physiology and disease by using samples obtained from individuals from various genetic backgrounds.
Funds Requested	$2,405,997
GWG Recommendation	**(85-100): Exceptional merit and warrants funding, if funds are available**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 90

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

Mean	90
Median	90
Standard Deviation	1
Highest	91
Lowest	88
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.

Does the project hold the necessary significance and potential for impact?
• Schizophrenia (SCZ) and autism spectrum disorder (ASD) are complex disorders and new mechanistic insights would be clearly of high impact. • Tandem repeats (TRs) may play a role and are the central focus of the proposal. Aim 1 will offer a comprehensive assessment of TRs in patient cohorts and Aim 2 will functionally explore this with genome editing in iPSCs and neural derivates. • Small team of adjacent labs but with relevant expertise. • A very well revised application addressing a relatively understudied potential contributor to genetic risk for neurodevelopmental disorders. • Yes. Prior studies focus on single-nucleotide polymorphisms (SNPs) and this proposal extends to other variant types (tandem repeats).
Is the rationale sound?
• The case for a possible involvement of TR is strong and the study will comprehensively identify and then experimentally validate/explore the consequences of TRs. • Yes. The proposal cites recent work from the PI's lab showing strong SNPs associations might be markers for the real causal variants (TRs).
Is the project well planned and designed?
• Both aims are well structured and designed. • Aim 1 will identify candidate TRs for SCZ and ASD. Aim 2 will complement that with a set of edited/engineered cell lines that are used by the applicant and can have further utility in the field. • Yes. The project combines computational approaches for TR identification (Aim 1, GWAS & rare variants) with experimental validation (Aim 2 using gene editing).
Is the project feasible?
• Yes, the team has key and complementary expertise.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• The proposed computational analysis will depend on the available cohorts. • Yes. The proposal includes a thoughtful analysis of multiancestry data.

Application #	DISC0-17610
Title	Unraveling the developmental path from altered hematopoietic stem cells to leukemia in Down syndrome.
Project Objective (as written by the applicant)	Our goal is to map altered Trisomy 21 developmental hematopoiesis to understand the first steps of leukemia initiation in myeloid leukemia of Down syndrome
Impact (as written by the applicant)	We will provide critical knowledge of how Trisomy 21 hematopoietic stem cell development is altered and the factors that predispose to leukemic transformation before birth
Major Proposed Activities (as written by the applicant)	• Generate a single cell and spatial map of T21 HSC development • Identify dysregulated processes in T21 HSC development and differentiation • Dissect the developmental tissues, stages and cell types where first transforming mutations occur in T21 • Identify mechanisms that predispose to transformation in T21 developmental tissues • Create a pluripotent stem cell model to dissect T21 HSC development and differentiation in culture • Determine cell intrinsic and environmental factors that predispose to leukemia initiation using T21 pluripotent stem cell model
Statement of Benefit to California (as written by the applicant)	The research will help understand the development of hematopoietic cells that can give rise to childhood leukemia in Down syndrome (DS). This work will also help understand dysregulated immune cell development in DS. Our T21 iPSC HSC differentiation in vitro model will create a framework how to study factors affecting the development of leukemia in utero. This research will not only help families with children with DS, but it will also help study other blood disease that originate in utero.
Funds Requested	$2,337,847
GWG Recommendation	**(85-100): Exceptional merit and warrants funding, if funds are available**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 90

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

Mean	90
Median	90
Standard Deviation	3
Highest	95
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.

Does the project hold the necessary significance and potential for impact?
• The project will provide important and difficult to obtain experimentally insights into the cell of origin and course of events underlying the pathogenesis of leukemia in Down syndrome (ML-DS) patients, with broader implications for fundamental questions in stem cell and cancer biology, namely the interplay between cell autonomous and environmental factors in malignant transformation in cases of neoplasms with prominent developmental components, such as ML-DS. • This is a dense application. The application focuses on early development of myeloid leukemia of Down syndrome. T21 patients develop truncations of GATA1 and then develop transient abnormal myelopoiesis (TAM) then go on (several months later after birth and after acquiring other mutations) to AML (ML-DS). Understanding the mechanisms of this transition may provide insights into therapeutic interventions and thus be of clinical significance and impact. • Yes. Could provide a understanding for T21 leukemia and other implications to leukemias and normal hematopoietic regulation although this is not detailed beyond the exact experiments proposed for T21 ML. • Yes. This is the most positive output, as the molecular data is both unique and rare, and will be made available. The applicant has a track record of doing this in the past, and the data has had major impact to other fields and investigators. • This is not a Team Track project, but collaborators bring complementary expertise in several important aspects of the proposed work, namely providing rare samples from patients with T21, TAM and ML-DS; techniques for integrating multi-omics with genotyping for GATA1s isoform, xenograft assays, etc... • The PI will identify unique vulnerabilities in T21 HSCs (Aim 1), cellular origins of TAM in T21 (Aim 2) and establish iPSC models to dissect altered T21 HSC development. • Significant strength with team and the development of the iPSC differentiation system for normal and T21, iPSC HSC. Generation of similar expression signatures and immunophenotype as T21 HSCs. Many elegant studies have been done using, spatial transcriptomics, scRNA Seq, FACs, niche biology, mutations. • Unclear, and is focused on a very rare disease with good outcomes in T21 ML.
Is the rationale sound?
• Grant is complex, and included all potential OMIC methods the applicants are capable of in nearly every aim proposed, but unclear how and when this will be applied. No causal experiments other than overexpression candidates in Aim 3 using IPSC are proposed. • Given the number of molecular and in vitro cellular behavior experiments proposed, e.g., cell cycle, transplant etc., it is unclear if the amount of cells or tissue is feasible for this project, and how it will prioritized or used in other tissue dependent grants the applicants have committed. • The experimental vs. biological replicates designated to each experiment is unclear and repeated throughout proposed plan, raising questions as to the feasibility and how comparisons and statistics will done to generate conclusions from subaims 1 and 2. How will these be functionally validated? • How will data be compared between experimental results? For example, Aim 2 subaim A of TAM precursors to GATA mut TAM precursors in subaim B and then compared to ML from DS patients. This is a unresolved concern, and description is lacking to better understand how results will relate to questions and hypothesis posed. • What is the source or assay system of the niche cells described? How are these selected or compared? Raises an issue of feasibility? • Are other leukemias going to be compared? How will the results be deemed unique to T21 and/or T21 ML vs. any leukemic cells? Cell lines, non-T21 ML? Lymphoid leukemias? • Capability in OMICs, e.g., spatial, scRNA, ATAC and unified bioinformatics is unparalleled and demonstrated, and use of unique samples, several dozen donors between 9-20 weeks and > 5 T21 MLs. • Preliminary data is largely based on multiomic analysis from fetal hematopoiesis mined from published works and data sets that supplies a strong foundation for analysis of T21 TAM and ML development.

- Specifically, iPSC work up shown in Fig 5 demonstrate the T21 iPSC are unique for HSC and TAM precursor acquisition, forming the basis of Aim 3 and creation of the T21 ML model.
- Relevance of the overall preliminary data is difficult to determine as most of this is descriptive, with molecular phenotypes based on gene mapping, Lots of information but other than demonstrating expertise and skills, it is difficult to know how this this will be used in Aims 1 and 2.
- There is strong rationale that a strong developmental component, pertaining to the cell of origin and niche factors are key to the pathogenesis of ML-DS and, likely to other infant and pediatric leukemias.
- Rationale is sound but experiments are many and not organized well. Specific hypotheses are not being tested.

Is the project well planned and designed?

- Yes, the project is appropriately planned and designed to give meaningful results.
- Yes, there is a well-constructed plan for team communications and management of all aspects of the project, but it is sometimes unclear.
- The project is well-planned, and the anticipated outcomes discussed with detail and thoughtfully. The project balances nicely single-cell multi-omics analyses of rare patient samples with in vitro modeling and functional studies using exciting and cutting edge PSC models, including a groundbreaking new protocol to derive transplantable HSCs from hPSCs.
- Potential problems and solutions are appropriately discussed in detail.
- Dense application looking at fundamental issues of HSC development in T21 models. Major issue is that specific hypotheses are not proposed or tested weakening the proposal.

Is the project feasible?

- This group are experts in this area, and it is highly unlikely another group would be able to do this work given the unique skill sets and material in fetal tissues and assays proposed.
- Yes, the team has access to all the resources and staff necessary to conduct the proposed activities.
- Yes, likely funds requested will allow all the experiments to be completed, but it unclear what will be done vs. not completed given the experimental plan provided. E.g., replicates from experimental or biological standpoint. Staff is well justified.
- Critically, fetal liver samples from patients with T21, as well as TAM and ML-DS samples, hard to obtain, are available to the investigators and have already been used for the preliminary studies. All technologies, including computational expertise, and expertise in interpretation and integration of such datasets, are outstanding.
- Budget is appropriate and justified.
- No issue with budget.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- Yes, the project plan and design adequately address and account for the influence of genetic, environmental and/or other external factors that may impact research findings.
- Yes, project outcomes extend or validate the applicability of regenerative medicine discoveries to additional affected populations, patients or communities, well described.
- Yes, the applicant is heavily involved in many aspects of outreach to diverse groups and has lead and participated at all levels, including CIRM hosted events. Excellent.
- The plan adequately addresses genetic and environmental factors.
- The outcomes have far-reaching implications for patients with leukemia and other hematologic disorders.
- The PI has participated in numerous outreach and educational activities pertaining to stem cell research.

Application #	DISC0-17674
Title	Identifying and Overcoming Roadblocks to Hearing Restoration Using Human Induced Pluripotent Stem Cells
Project Objective (as written by the applicant)	To utilize human iPSCs as an in vitro model of hearing loss and overcome obstacles to hair cell regeneration by prompting post-mitotic supporting cells to re-enter the cell cycle.
Impact (as written by the applicant)	This research will help determine and overcome the molecular roadblocks to cochlear hair cell regeneration in humans, potentially informing new approaches to treat hearing loss.
Major Proposed Activities (as written by the applicant)	• Generation of human inner ear cells from induce pluripotent stem cells to establish an in vitro model of hearing and hearing loss • Temporal mapping of cell cycle exit and omic analysis of inner ear cells from human iPSC-derived inner ear organoids • Developing a conditional hair cell ablation model in human inner ear organoids • Characterizing the maturity and function of the sensory cell types derived from human inner ear organoids • Analysis and timing of signaling pathways preventing supporting cell mitosis after hair cell ablation in human inner ear organoids • Targeted and high-throughput screening of drugs and small molecules that can prompt supporting cell mitosis in human inner ear organoids
Statement of Benefit to California (as written by the applicant)	Hearing loss affects >44 million people in the US, with 12.3% of California adults impacted. It diminishes quality of life and imposes nearly $200 billion in costs to the US annually. This research will utilize human iPSCs to develop inner ear organoids towards identifying and addressing barriers to hair cell regeneration. Successful outcomes could lead to new hearing loss therapies, greatly benefiting California's diverse population by improving public health, productivity, and patients' lives.
Funds Requested	$4,608,000
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: 90

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

Mean	90
Median	90
Standard Deviation	3
Highest	93
Lowest	82
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.

Does the project hold the necessary significance and potential for impact?
• Unlike birds, human sensory hair cells lack the ability to regenerate from neighboring supporting cells following injury or loss. Understanding the barriers to cell cycle re-entry and sensory cell fate specification in humans could unlock therapeutic strategies to prevent or reverse hearing loss. • This project promises to resolve a key knowledge gap in characterizing iPSC-based models of hearing loss, and identifying strategies to overcome mitotic molecular roadblocks following hair cell loss for therapeutic intervention. • RMAs represent a key advance in monitoring therapeutic activity.
Is the rationale sound?
• The scientific premise is strong. The existing literature, history of failed clinical trials, and well described species difference in cell biology in the cochlea support the need for human preclinical models for HC regeneration. • Yes, the project is based on a sound rationale, motivated by a critical need for human models of hearing loss and a lack of available therapies. • Well planned, although Aims 2b and 3b are therapeutic strategies that are unlikely to be developed into actual human therapeutics without significant revision.
Is the project well planned and designed?
• Both PIs are at the same institution and have a history of collaborative science. Weekly Zoom meetings between PIs and monthly joint lab meetings will occur alongside more informal meetings between the PIs and across labs. There is a clear plan for communication as well as conflict resolution and experimental oversight. • Logical design of experiments. Well described endpoints and alternative approaches. • The project is very well planned, and is highly likely to yield informative results. One weakness is the use of only 2 iPSC lines, and the known variability in organoid approaches across lines. While the extensive amount of work and characterization proposed would be unrealistic to replicate across multiple cell lines, the team might consider the validation of key findings (timing of exit from mitosis, possible successful treatment strategies) in additional lines of varied genetic backgrounds. • The issue of immunogenicity needs to be addressed.
Is the project feasible?
• The team is well positioned to achieve the stated goals and aims of this project. No concerns noted. • Yes the team has the appropriate expertise and leadership, spanning clinical, animal model and iPSC based studies of hearing loss, in addition to molecular and cellular biology expertise. • Preliminary data support feasibility.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• The applicants acknowledge the potential weakness in studying only two patient derived cell lines in these database-generating experiments. They propose that these databases can be interrogate and identify key markers which could then be more practically examined across wider genetic diversity. • The applicant plans to recapitulate hearing loss that usually occurs due to aging or damage. However, they do not necessarily consider sex or genetic background. The sex and genetic background of the two lines are not described in the proposal.

Application #	DISC0-17626
Title	Development of in vitro and in vivo functional human synthetic kidney organoid (hSKO) model as a platform technology for kidney research
Project Objective (as written by the applicant)	Development of a human stem cell-derived, spatially-patterned, mature and functional human synthetic kidney organoid (hSKO) model as a platform technology for basic and translational kidney research.
Impact (as written by the applicant)	Lack of a reliable human kidney model is a major bottleneck in kidney research. This project will establish the most physiologically relevant model for studying human kidney development and disease.
Major Proposed Activities (as written by the applicant)	• Establish human synthetic kidney organoid (hSKO) model in vitro and in vivo • Characterize the hSKO model at the molecular, cellular, and functional levels in vitro and in vivo • Develop hSKO-based model for autosomal-dominant polycystic kidney disease (ADPKD) • Characterize the hSKO-based ADPKD model and benchmark it to human patient samples • Validate the hSKO technology with different iPSC lines representing diverse race/ethnicity/sex populations
Statement of Benefit to California (as written by the applicant)	Kidney diseases are highly prevalent in the population (1 in 7 adults) and affect all ethnicities and genders. Success of this project will provide an unprecedented human synthetic kidney organoid model that can faithfully recapitulate human kidney anatomy, physiology and pathophysiology. This platform technology will advance our understanding of kidney diseases and accelerate drug discovery, benefiting all patients with kidney diseases, including citizens in California.
Funds Requested	$2,287,926
GWG Recommendation	**(85-100): Exceptional merit and warrants funding, if funds are available**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 90

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

Mean	90
Median	90
Standard Deviation	4
Highest	96
Lowest	84
Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	12
(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.

Does the project hold the necessary significance and potential for impact?

- This proposal aims to further mature and characterize a human synthetic kidney organoid (hSKO) model pioneered by the applicant. This is a very important quest and the potential for impact is very high, as this work is needed for advancement of our understanding of human kidney diseases, development of a novel kidney in vitro assay, and development of replacement therapies.
- Yes, this proposal addresses a key knowledge gap, as the kidney cells generated under current methods lack functional maturity, are highly heterogenous, and suffer from poor scalability and inefficient differentiation.
- The Principal Investigator (PI) is uniquely positioned for this project and has assembled an exceptional team with the expertise needed for success. Additionally, the proposal includes very detailed information on the role of each team member, which was very useful.
- The potential for impact is immense. This is a very important area and direction.
- The project holds great potential for using hiPSCs to enhance our understanding of how the adult human kidney develops into its mature spatially patterned and fully functional form.
- The project furthermore offers the exciting possibility of developing stem cell-derived human synthetic kidney organoids (hSKO) as a model for the human kidney that would allow testing drugs to treat various renal disorders, modeling genetic renal diseases such as autosomal dominant polycystic disease (ADPKD), and possibly even providing a source of functional kidney as an alternative to current live donor and cadaver-derived kidneys for transplant.
- The potential impact of this project is highlighted in the application by illuminating the tremendous burden of kidney disease in the U.S. (37 million people with chronic renal disease (CRD); 750K with end stage renal disease (ESRD)).
- Developing a human stem cell-based model of the kidney to explore the physiology and pathophysiology of kidney disease is important as most models are currently based on animal models.

Is the rationale sound?

- The project is based on a strong scientific rationale: that improved characterization of the human synthetic kidney organoids will enable the development of novel in vitro and in vivo platforms for kidney research.
- The approach builds on innovative technology developed by the PI, that allows the team to recapitulate in vitro the early stages of kidney development by self-assembling of kidney progenitor cells.
- The PI plans to investigate the maturation of these organoids both in vitro and in vivo, with a comprehensive phenotypic, molecular, and functional characterization of the hSKOs.
- Overall, this project has the potential to significantly advance our understanding of human kidney development and lay the groundwork for translational applications in disease modeling, drug screening, and regenerative medicine.
- The rationale is supported by very strong published and preliminary data on the development of the hSKO. Importantly the team of collaborators has performed preliminary functional studies that support feasibility.
- Comparison to mouse SKO or human control for the functional studies depicted in fig 11 and 12 would have been helpful; as currently presented it is difficult to know what the gold standard would look like.
- The application extensively documents the scientific rationale for the project. The PI's previous work demonstrating that the structure and function of the mature kidney derives from self-organizing kidney precursor cells forms the basis for the hSKO approach.
- The ability to generate "nephron progenitor cells (NPCs)" and "ureter progenitor cells (UPCs)" from hiPSCs is clearly documented, as is the ability of the NPCs and UPCs to interact to form the SKO which has molecular, morphologic patterning, and functional characteristics of the developing mature kidney.
- A kidney organoid would be a major advancement for the field. The applicants show their ability to develop an organoid. To establish the usability of the model, further functional and molecular characterizations are the next critical step.

Is the project well planned and designed?
• The project is well-planned and designed, with clearly defined and independent aims. The central hypothesis is that functional maturation of the organoids will occur over time, and that specific cell types and functions—currently absent in existing models—will emerge either in vitro or in vivo. • In Aim 1, the team will perform a comprehensive characterization of the hSKOs both in vitro and in vivo. This includes longitudinal monitoring of hSKO development, using single-cell sequencing to compare their cellular composition and maturation status to that of human fetal and adult kidneys. • Organoid functionality will be assessed using a range of techniques, including substrate transport assays, patch clamp electrophysiology, tubule microperfusion, intravital multiphoton microscopy, and endocrine function assays. However, the proposal would benefit from more clarity regarding the appropriate controls for these functional experiments. • In Aim 2, the team will evaluate the utility of hSKOs as a disease modeling platform by testing their ability to replicate the pathophysiology of autosomal dominant polycystic kidney disease (ADPKD). • In Aim 3, the robustness and generalizability of the hSKO technology will be validated across multiple induced pluripotent stem cell (iPSC) lines. This will demonstrate the reproducibility of the approach and its applicability to diverse human pluripotent stem cell (hPSC) sources, ensuring broader relevance and potential benefits across patient populations. • Project design is appropriate and well described in the application. The plan is divided into three aims each of which follows the others in logical order. Use of mice for in vivo studies of hSKO development is justified. • The applicants propose to further functionally and histologically characterize the organoid model. The proposed experiments might describe certain functional characteristics which the model will serve to capture. This can characterize the organoids as a platform for future disease modeling. • Aim 3 proposes to develop organoids from a number of different stem cell lines. The value of these experiments is not described and therefore this aim appears underdeveloped. The data analysis and statistical approach for this aim is underdeveloped.
Is the project feasible?
• This project is highly feasible and the team comprises experts in stem cell biology, kidney organogenesis and physiology, single-cell sequencing, and bioinformatics ensuring the necessary expertise to execute the project. • The project leverages state-of-the-art facilities and technologies, ensuring access to the necessary resources for research and development. • The PI is a highly experienced and accomplished investigator with an excellent track record of publications in the field. • Excellent resources (lab space, core facilities, and scientific environment) for the PI are well described in the application. Letters from the PI's two department chairs attest to the adequacy of the resources available to the PI. Resources for proposed collaborators are also suitable. • The team is qualified to perform the proposed experiments
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• Yes, aim 3 is designed specifically to validate their platform using hiPSC from donors of different backgrounds. • The project plan, specifically aim 3, explicitly addresses use of diverse iPSC lines to demonstrate the broader applicability of the SKO approach. • If successful, the model can serve as a platform to model kidney disease across affected populations.

Application #	DISC0-17364
Title	Mechanisms of Transcription Factor Haploinsufficiency in Human Congenital Heart Disease
Project Objective (as written by the applicant)	We aim to solve a 30 year old problem, which is to understand using human stem cells how certain genetic mutations cause human disease.
Impact (as written by the applicant)	We currently don't know how mutations that affect master regulators of cell fate cause disease. Knowing molecular events that to date have not been understood will open up new treatment possibilities.
Major Proposed Activities (as written by the applicant)	• We will use a unique set of genetically engineered human stem cells to create heart-like structures that will model congenital heart disease. • Using these cells, we will use new genetic techniques to map how genes are abnormally turned on or off in disease. • We will mine the data that we will obtain in our experiments with new artificial intelligence algorithms that will help unveil new genetic networks. • With a new set of stem cell models, we will for the first time know how disease-related master regulators function in normal and diseased heart cells.
Statement of Benefit to California (as written by the applicant)	The knowledge that we will obtain from our studies will be of great value to the State of California and its citizens. We will finally understand how a broad class of genetic mutations affect fundamental cellular processes that guide the formation of organs, such as the heart, the brain, and the eye. This knowledge will inform innovative strategies that scientists in academia and industry can use to devise therapeutics that could include organ regeneration or gene therapy.
Funds Requested	$2,444,376
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.

Does the project hold the necessary significance and potential for impact?
• The proposal aims to study T-box transcription factor 5 (TBX5), a dosage-sensitive transcription factor (TF) for a congenital heart disease (CHD), in 3D cardiac organoids. The proposal will use single cell multiomics and machine learning approaches to decipher the dose-dependent gene regulatory networks (GRNs) controlled by TBX5. • The proposal aims to resolve a key knowledge gap that the mechanisms underlying transcription factor (TF) dosage sensitivity in human disease. • The collaborations that offer a unique synergy. The collaborator [redacted PI name] has pioneered the use of iPS cells and genome engineering to model human congenital heart disease. The collaborator [redacted PI name] is a pioneer in machine learning, and developed algorithms to deeply understand disease-causing GRNs. • Yes to all, TBX5 play a pivotal role in the development of CHD and its role in the haploinsufficient setting not understood. It's role in human heart is critical to understand the biology of human heart development and associated clinical implications. There is a lack a suitable human models particular complex 3D models to study gene dosage which makes this application very relevant beyond the understanding of TBX5 function. • This a single PI proposal and usage of the host institution's proteomics facility and collaboration with [redacted PI name] are synergistic to the success of the proposal. Particularly [redacted PI name] team's experience on genome engineering and iPSC technology are critical.
Is the rationale sound?
• TBX5 plays an pivotal role in CHD. Complex 3 dimensional cardiac organoid allows the study of anatomic specific impact of TBX5 functions, which can not be studies in 2 dimensional human cardiac models. • 3 dimensional organoid models provide a better model for organ specific physiological conditions to study TF function which includes specific distingued functional cardiac unites and cell types. • Previous work using TBX5 haploinsufficient iPSC lines successfully identified disease-relevant pathways but was limited using 2D cultures that produced only ventricular cardiomyocytes. In the current proposal, applicant will leverage TBX5 edited iPSC lines to model TBX5 haploinsufficiency in chamber-specific 3D cardiac organoids, and employ multi-omics approaches to assess gene expression, chromatin states, and TF occupancy. • Preliminary data is sufficient. The team use TBX5 mono, bi-allelic KO and degron system to studies of gene dosage and timing in cardiac organiods. Preliminary data indicates that TBX5 dose-dependent changes in gene expression that vary over time and are specific to atrial or ventricular cardioids.
Is the project well planned and designed?
• The three aims: modeling TBX5 haploinsufficiency in 3D heart organoids, investigating how reduced TBX5 levels alter DNA binding, and interacting associated chromatin proteins are appropriately planned and designed to give meaningful results. • Yes, engineering of the iPSC lines is straight forward and sufficient experience available. The cardiac organoid model is established. Biotin-tagged TBX5 to study TBX genome occupancy seems straight forward and aligned with the lab experience. The utilization of the CAPTURE tool kit in collaboration with the proteomics core aligns well with the overall goal and is poised to provide important new information on TBX5 in a haplo-insufficient setting. • Communication and management is well laid out in the project organization and management plan. • Potential pitfalls are identified in each aim and reasonable and convincing alternatives provided.
Is the project feasible?
• The team has a TBX5 mono, bi-allelic KO and degron iPSC lines available. The team has the 3D heart organoids differentiation platform established. They have expertise with single cell multiomics and machine learning approaches to decipher the dose-dependent gene regulatory networks (GRNs).

- The PI has expertise in heart development and transcription factor biology. His lab has discovered the cardiac-specific roles played by chromatin remodeling complexes, and pioneered the study of epigenomic landscapes in heart development.
- The PI is a world expert in cardiac development and congenital cardiac diseases. His team and collaborators are very well positions to carry out all aspects of the proposal.
- Host institution is top tier research facility with all required resources available.
- Timeline and budget seems appropriate.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- The project primarily uses the WTC iPSC line, which is a widely used iPSC line. The proposal mentioned they may confirm certain findings in a female line. The scientific findings will be broadly applicable to heart disease cohorts, and also to many other disease related to TF haploinsufficiency.
- Findings from this application are important for the studies of other haploinsufficient models and complex 3D model utilized to model other genes and related patient population - It is not clear what that means or how this affect the review of this application.

Application #	DISC0-17635
Title	Genetic and Epigenetic Regulation of XIST and X-chromosome silencing in hiPSCs: Overcoming Barriers in Stem Cell-Based Therapies for Women's Health
Project Objective (as written by the applicant)	We study how the inactive X chromosome aberrantly reactivates in female pluripotent stem cells and develop ways to prevent it, enabling accurate modeling of female biology for research and therapies.
Impact (as written by the applicant)	The use of female pluripotent stem cells in disease modeling, drug discovery, and regenerative therapies is currently limited by the aberrant reactivation of the inactive X chromosome in these cells.
Major Proposed Activities (as written by the applicant)	• Investigate the control of XIST expression in female hPSCs • Define molecular mechanisms underlying Xi gene reactivation. • Assess the stability and quality of female XIST-positive hiPSCs.
Statement of Benefit to California (as written by the applicant)	This research will enhance the quality and reliability of female human pluripotent stem cells, which are widely used in biomedical research and regenerative medicine across CA. By developing tools to prevent the reactivation of the inactive X chromosome, the project will support more accurate disease modeling and stem cell–based therapies for female patients and improve health outcomes for CA's diverse population. It will also employ 7 individuals to perform the work, creating jobs within CA.
Funds Requested	$2,358,742
GWG Recommendation	**(85-100): Exceptional merit and warrants funding, if funds are available**
Process Vote	All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG." Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."

SCORING DATA

Final Score: 90

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

Mean	89
Median	90
Standard Deviation	3
Highest	95
Lowest	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.

Does the project hold the necessary significance and potential for impact?
• This project addresses a fundamental gap in the human stem cell biology and regenerative medicine field: the mechanistic underpinnings of the erosion of X chromosome inactivation in female hPSC (human pluripotent stem cells) lines, and how to circumvent this in hPSC-based studies. As such, it is highly relevant for all listed criteria. • Human pluripotent cells are a key model and tool. Female lines show variation in X inactivation (erosion) which is a challenge that needs to be addressed. The proposal will explore the underlying molecular cause and aims to identify/generate high-fidelity hPSC lines. • X inactivation is important for a number of developmental mechanisms and also for disease onset. This is important regulation in loss in XX hPSCs. The consequence of this epigenetic erosion is not well understood neither the underlying mechanisms. However, the consequence could be extremely damaging especially in the context of cell based therapy requesting for life engraftment. • This application aims to address this issue. Importantly, the problem is known for a long time but has remained a blind stop in the biology of hPSCs. However, it is likely that such abnormal regulation explains in part why the use of female hPSCs remains marginal. • This project is thus very timely and important. It could address one of the major risk in hPSs therapy. • This is single track PI application. However, it relies on collaborative effort for the single cell multi-omics analyses. This collaboration is synergistic and will increase the impact of the project.
Is the rationale sound?
• The rationale is very compelling and well justified. The erosion of X chromosome inactivation in XX genotype hPSC lines is well established (and accepted) and can compromise the accuracy of findings from XX hPSC lines in both basic and translational studies, along with their use in therapeutic experiments. The rationale is supported by a wealth of preliminary data, both published and unpublished. • Investigating or identifying new cis elements is reasonable. The subsequent extensive mapping is less convincing. The MZT (monozygotic twins) is an interesting idea but with additional confounders not as clean as presented (an engineered isogenic line seems more appropriate as its not clear that any variants exist in the two pairs). Overall the proposal is very extensive on mapping and often only appears descriptive rather than provide actionable new insights. • The rational of this proposal is very strong and well established. X inactivation erosion has been described multiple times and understanding the underlying mechanisms is important.
Is the project well planned and designed?
• The project will generate a number of important results concerning X inactivation in human and also the mechanisms beyond its erosion in hPSC lines. • Back up plans are included but some aspect could be perceived as optimistic. This very high tech application is high risk in some aspects, such as the CRISPRi screen or over-expression of KLF-4 using sendai virus. However, there is no doubt that the applicant will be able to pivot and find appropriate solutions. • The three aims will generate a massive amount of data that will likely provide some new molecular depth of X inactivation. Whether it will translate to truly high-fidelity female human PSC is less clear or at least its not obvious how the bulk of the proposed work contributes towards that goal. • The project is very well planned and thought out, with rigorous plans to identify, in Aim 1, how XIST expression is regulated in XX hPSC lines via a CRISPRi screen, the characterization of cis-regulatory elements and the influence of genetic variation, and in Aim 2 understand the mechanisms underlying underlying Xi gene reactivation including at single cell resolution, and finally in Aim 3 establishing and validating lines with stable XCI. However, the analysis plan for Aims 1and 2 is not clear. • There is some lack of clarity in the project plan, and it appears largely collective rather than strategic.
Is the project feasible?
• The project is feasible and the PI has the all the necessary expertise to carry it out. The PI has the all the necessary resources, including through collaborations and access to core facilities. • Yes, the team has key and complementary expertise. • The lead applicant is world wide recognized leader in the field of X inactivation. There is no doubt that the applicants have all the resources necessary to achieve this program. • The project is very ambitious and based on a large number of hPSCs combined with omics analyses. The timeline is tight while the budget is underestimated.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- This project directly addresses the influence of genetic variability by including cell lines of diverse ancestry, and its main goal is to address the noise introduced by erosion of XCI in XX hPSC lines, therefore directly considering sex as a biological variable. if successful, it would de-risk the use of XX hPSC lines (from diverse genetic background) and render them more broadly accessible.
- Within the means of the collected or used cell lines.
- This is the core of this application. Indeed, erosion of X inactivation could preclude the use of female hPSCs for disease modeling and cell based therapy. Therefore, this proposal will incorporate sex difference into a number of studies. Furthermore, the applicant intends to include hPSCs from Black American ancestry to cover the genetic diversity aspect.
- Most hIPSC lines currently used are male and X inactivation erosion could explain this situation. Thus understanding this problematic will be essential for personalized medicine.
- The proposal includes outreach activities including initiatives with a major stem cell organization on the importance of XCI in hPSCs.

Application #	DISC0-17421
Title	Developing replacement islet cells for diabetes using human stem cells
Project Objective (as written by the applicant)	We will identify genetic regulators and intercellular signals that promote islet cell function, and apply our work to advance stem cell biology for generating replacement islets to reverse diabetes.
Impact (as written by the applicant)	Our efforts could generate improved, safer replacement islet cells for type 1 diabetes, and islets matured in vitro to model type 1 or 2 diabetes pathogenesis, and develop personalized therapeutics.
Major Proposed Activities (as written by the applicant)	• Identify new regulators of pancreatic islet function using genetics and stem cells. • Test if genetic regulators of islet cell maturation can stimulate function of stem cell-derived islet cells. • Test how cell-to-cell signaling regulates maturation of stem cell-derived islet cells. • Identify cell signals that regulate maturation of pancreatic islets and stem cell-derived islet cells. • Combine genetic and signaling regulators to stimulate maturation of stem cell-derived islet cells.
Statement of Benefit to California (as written by the applicant)	Multiple benefits to California and its citizens would ensue from successful conclusion of studies proposed here. This includes (1) improvements in patient care, especially for those with type 1 or type 2 diabetes, (2) emergence of islet transplantation programs for diabetes that would foster increased consultation and use of California health care systems by citizens and outside clients, (3) enhanced support for academic training and research in stem cell biology and islet transplantation.
Funds Requested	$3,943,364
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	5
Highest	95
Lowest	75
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	13
(1-84): Not recommended for funding	2

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.

Does the project hold the necessary significance and potential for impact?
• This is an important and timely project with the potential to significantly impact regenerative medicine for type 1 diabetes. Early reports from clinical trials have shown that stem cell–derived islets can effectively eliminate the need for insulin injections. However, for this therapy to be widely adopted and equitably accessible to people living with type 1 diabetes, further work is needed to improve the cellular product in terms of functionality and efficacy. • This proposal aims to address this challenge by investigating the effect of over-expressing putative maturation genes—identified through their scRNA-seq analysis of the human pancreas—to accelerate in vitro maturation. The findings from this research could advance the safe use of stem cell–derived islets. • Yes, this proposal addresses a key knowledge gap, as the cells currently generated lack functional maturity. The ability to produce a cellular product with functionality comparable to that of deceased donor islets could enable transplantation with fewer cells and reduce the overall cost of the therapy. • This project, which will develop approaches for generation of developmentally - mature pancreatic β (scβ) -cells from pluripotent stem cells in vitro, will address the current knowledge gaps in using these cells for treatment of diabetes. This is a highly significant goal. • The project has significant potential for increasing our understanding of how to optimally promote differentiation of pluripotent stem cells (PSC's) into mature "adult-like" cells capable of carrying out a vital biologic function. • Specifically, the project focuses on differentiating PSC's into insulin-secreting islet beta cells that are regulated by glucose in a manner that more faithfully replicates mature beta cells than the current version of such cells that are already being used in human transplantation trials for attempted cure of diabetes.
Is the rationale sound?
• The project is based on a strong scientific rationale. The underlying hypothesis is that scβ cells lack (1) the expression of genetic regulators that normally drive β cell maturation, and (2) the regulatory intercellular signals from non-β cells that could drive scβ cell functional maturation. • Conceptually, this makes perfect sense, and the applicants plan to combine the two approaches to identify the key genes and factors that could accelerate maturation. While it is possible that β cells may require the activation of multiple genes (and this study will be limited to a certain number of genes that could be over-expressed), this does not diminish my enthusiasm for the proposal. • The project is based on a strong rationale. While several reliable vitro protocols for generation of scβ cells from pluripotent stem cell sources have been developed, the resultant cells do not fully approximate native β cells, as they produce less insulin, have only modest glucose - regulated insulin secretion, and have more than 2000 differentially expressed genes (DEGs) when comparing scβ cells and native β-cells. • The application documents the sound scientific rationale for the project, showing that current "state of the art" differentiation of PSC's to beta cells yields only a fetal-like beta cell that is deficient in insulin content and in physiologic glucose regulation of insulin secretion.
Is the project well planned and designed?
• This hypothesis is supported by published work and strong preliminary data presented by the applicants. The PI has generated a unique set of islet cell transcriptomes from 31 human subjects spanning fetal, neonatal, and juvenile stages. The PI has identified genes associated with neonatal and juvenile maturation that are very likely linked to functional maturation. • Among the genes identified to correlate with maturation, there are known transcription factors (TFs) as well as unknown players. The team uses these factors in over-expression (O/E) experiments to demonstrate how a single factor can promote increased gene expression of maturation markers in sc-β cells. • The team also presents data supporting the role of a transcription factor as a driver of maturation, showing that transcription factor over-expressing SC-islets secrete higher levels of C-peptide compared to controls. (This might still be lower than in deceased donor islets, and there could be concerns about transgene silencing as the cells differentiate.)

- Additionally, the team has shown in co-culture experiments with deceased donor islets that the presence of primary cells can increase glucose-dependent exocytosis in sc-derived β cells. This suggests that paracrine signals and/or physical interactions with primary cells induces changes in the sc-derived beta cells that may be linked to increased functionality.
- This is a well-designed project that will capitalize on the extensive preliminary results and on the expertise of the investigative team.
- The project is appropriately designed to give meaningful results. The plan proceeds logically from the applicants' prior work such as creation of a human islet developmental "atlas" of gene expression. The project plan exploits the applicants' identification of several genes key to mature islet beta cell development which will be tested sequentially in efforts to create mature beta cell phenotype.
- The project also includes use of gene editing and conditional gene expression techniques (Aim 1), as well as coculture of stem cell-derived beta cells with mature islet clusters (Aim 2) to optimize beta cell maturation. The applicants propose to use beta cell transplantation under the kidney capsule of immunocompromised mice as a method for testing PSC-derived beta cells in vivo.

Is the project feasible?

- The project is well-planned and designed. The most challenging aspect of this proposal will likely be dealing with potential silencing that may occur at the later stages of differentiation. For instance, in the experiment where the team over-expresses a transcription factor, insulin secretion is only improved when the transcription factor is over-expressed in endocrine progenitors, but not in β cells. Could this be due to silencing?
- The inclusion of *[name redacted]'s* team, with their expertise in gene editing and *[name redacted]* with their expertise in directed differentiation will be instrumental in addressing this potential issue.
- Another small issue is that the team will work with one hESC line that has only one functional INS allele. While this is an asset for sorting and imaging, it may limit the functionality of the resulting β cells.
- The aims and assays designed to measure outcomes are straightforward. In Aim 1, the team will initially over-express individual genes from safe harbor loci and test functionality, followed by simultaneous over-expression of multiple genes. If they observe silencing from the safe harbor loci, they may also include additional genes.
- In Aim 2, the team will explore the role of mature cells in promoting β cell functionality. Here, the assay becomes more complex, as the team would need to sort the sc-β cells prior to mixing them to primary islets and after the mixing to assess the expression of maturation genes, as well as re-aggregate them to measure glucose-stimulated insulin secretion.
- It is not clear if they will test different ratios of β cells to unfractionated islets, or what the cutoff would be for the different islet preparations, as each preparation will vary in terms of viability, purity, and insulin content.
- If successful in Aim 2b, the team plans to repeat the experiment using isolated α, β, or δ cells, or dual combinations, by sorting the individual populations. While this is feasible, it remains unclear whether the applicants will have enough islets to isolate the number of cells required for these experiments.
- In Aim 2c, they will combine the approach with sc-β cells over-expressing specific factors to harness potential synergy between intrinsic and extrinsic cues.
- The project organization and management plan are well-defined. The team will have bi-weekly meetings, or more frequently if needed. The division of work has been clearly delineated. The team has a history of collaboration and is located within the same institution, which facilitates logistics.
- The proposal identifies potential risks, such as challenges with gene silencing, and outlines strategies to mitigate them. Alternative approaches, including different gene editing techniques and optimization strategies, are clearly presented to ensure the continuity of the project in case of unforeseen issues.
- The team has no apparent concerns regarding the feasibility of obtaining sufficient cells for their experiments (either primary or sc-islets) before or after mixing, but limitations in cell accessibility could impact feasibility.
- The team comprises experts in stem cell biology, gene editing, and pancreas development, ensuring the necessary expertise to execute the project.
- The project leverages state-of-the-art facilities and technologies, ensuring access to the necessary resources for research and development.
- The team is excellent, with all necessary expertise in place. The leadership plan is well-developed.

  The team has access to all needed resources. The budget timeline is appropriate for the work proposed.
- Project feasibility is well addressed on pages 12-15 of the application. The previous experience and data accumulated by the applicants strongly bolsters the case for appropriate leadership and expertise.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- The project's outcomes will promote the applicability of regenerative medicine to a broad range of patient populations. This goal will be accomplished by employing human stem cell lines from a broad set of HLA haplotypes, and from both sexes. Also, the foundational genomic scale transcriptomics of islets at different stages of human development described in the preliminary results section have been performed from subjects reflecting a broad range of genetic and geographic backgrounds.
- The applicants address this requirement on pg. 6 of the application. They describe their efforts to ensure applicability of their PSC-derived mature beta cells to genetically diverse populations, as well as subjects with both types 1 and 2 diabetes.
- The project plan and design does not adequately address and account for the influence of genetic, environmental and/or other external factors that may impact research findings.

Application #	DISC0-17487
Title	Mechanisms underlying dosage sensitivity in developmental disorders
Project Objective (as written by the applicant)	This proposal leverages pluripotent stem cell models to understand the principles by which changes in gene and protein dosage affect human neural and facial progenitor development and cause disease.
Impact (as written by the applicant)	Proposed research will illuminate mechanisms underlying neurodevelopmental and craniofacial diseases. It will also provide a framework for modeling dosage effects for improved gene and cell therapy.
Major Proposed Activities (as written by the applicant)	• Measure how changes in levels of key dosage-sensitive proteins affect chromatin states and gene expression of neural and facial progenitors • Identify dosage-sensitive cellular processes during specification and differentiation of the progenitors of the brain and face • Build predictive models of dosage sensitivity • Understand how human genetic variation influences disease-associated dosage effects
Statement of Benefit to California (as written by the applicant)	Neurodevelopmental and craniofacial disorders – which are among the most common congenital diseases – are often caused by perturbations in gene dosage. The proposed research will uncover principles underlying dosage sensitivity, and will lay the foundation for interpreting the influence of genetic diversity present in California populations on clinical variability in patients. It will also allow for modeling dosage effects to improve efficacy and decrease toxicity of gene and cell therapy.
Funds Requested	$2,304,000
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: 90

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

Mean	89
Median	90
Standard Deviation	3
Highest	92
Lowest	80
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	13
(1-84): Not recommended for funding	1

KEY QUESTIONS AND COMMENTS

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

Does the project hold the necessary significance and potential for impact?
• Proposal addresses genetic variability-clinical presentation of developmental disorders can be highly variable. • Project addresses a fundamental question in disease genetics-role of gene dosage which is well recognized but inadequately understood. Uses hPSC models, focuses on transcription factors and cofactors and how abnormalities in gene dosage drive developmental disorders. • Proposal also addresses tissue specificity of diseases caused by constitutively expressed transcriptional regulators. • The project will address a major knowledge gap in the understanding of the biology of stem cells relevant to human biology and disease. Also, it will augment the application of human genetic research for meeting the goals of regenerative medicine. • Yes, the project is likely to increase understanding of gene dosage regulation and cell-type specific dosage sensitivity, with possible disease implications in neurodevelopmental and craniofacial disorders.
Is the rationale sound?
• It is well established that many genetic diseases are dosage sensitive, and it is increasingly appreciated that variants for complex traits act by modulating gene expression levels. But even today it is unclear at the molecular level how relatively modest changes in transcript levels of transcription factors or cofactors alter cell fate during development. >3600 human genes show dosage sensitivity. Focus on neurodevelopmental and craniofacial disorders-many are gene dosage sensitive. • Yes, the project is well rationalized, as gene dosage regulation is incompletely understood, and its dysregulation is likely to underlie the majority of human disorders. The approach proposed here and dosage titration, combined with the molecular profiling, is well-suited to address this gap. • PI has extensive experience using neural progenitor cells and neural crest cells as developmental models. PI has established a system to achieve precise temporal control of target protein levels. PI has compelling preliminary data to show that regulatory element sensitivity to protein levels varies considerably and predicts transcriptional outcomes. Response reads out in cellular phenotype. • The rationale for the project is sound. It will take advantage of the tools available in the PI's lab to advance understanding of the impact of human genetic variation on gene dosage responses and disease phenotypes. This will be accomplished by the use well-characterized hPSC models in combination with precise, time-controlled modulation of transcription factors (TFs) and their cofactors levels in disease-relevant cell types. • Some proof of concept for the approach in Aim 3 would strengthen the proposal.
Is the project well planned and designed?
• This is an excellent well-written project. The specific aims are logical, and the proposed research plan that takes advantage of sophisticated genetic, cell biology and bioinformatics tools available in the PI's lab, and the AI tools through a collaboration. • Project plan is systematic and based on strong preliminary data. Incorporates deep learning into prediction of dose response. Work on NPC less well developed. • The project is overall well designed, and the first two aims are likely to give meaningful results. This is less clear for Aim 3. The argument is that it is not practical to regenerate the tags in all the lines. However, tetraploidy may introduce a significant confounder in the interpretation of the results, and that is not well addressed. • Aim 3 has many potential flaws. Tetraploids may be genetically unstable. Compensatory effects of the partner genome may offset gene dosage. How much genetic diversity in this small panel of cell lines is unclear. • How well powered is this study to detect effects of variants in regulatory elements? How to validate individual hits which may be numerous?
Is the project feasible?

- Outstanding PI has extensive experience in cell modeling of neural crest development and disorders and chromatin biology and gene regulation.
- The team has appropriate leadership and outstanding expertise to carry out the project. The team has access to all needed resources. The budget and timeline are appropriate.
- The project is feasible given the existing resources and the track record of the lab. Reservations for Aim 3 remain.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- Study has a major focus on human genetic diversity. Project will incorporate genetic diversity into models.
- By examining gene dosage effects, this study directly addresses the influence of genetic, environmental and other external factors on disease manifestation.
- Yes, cell lines of diverse ancestry are proposed, but sex as a biological variable is not considered.

Application #	DISC0-17685
Title	Dissecting the cellular and molecular interactions between embryo and endometrium during human implantation.
Project Objective (as written by the applicant)	This research will define the dynamic mechanisms of human embryo implantation by integrating live imaging, spatial multi-omics, and gene editing in physiologically relevant stem cell-based models.
Impact (as written by the applicant)	This research will uncover factors driving embryo implantation and early pregnancy, address IVF implantation failure, and inform development of targeted therapies to improve fertility outcomes.
Major Proposed Activities (as written by the applicant)	• Developing a multi-modal atlas of human embryo implantation and stem cell embryo models using live imaging, clinical data and predictive models. • Deciphering embryo-endometrial interactions at implantation by spatial transcriptomics and proteomics. • Modeling pathological uterine tissue and uncovering genetic mechanisms of implantation and placenta formation using CRISPR-Cas12 gene editing.
Statement of Benefit to California (as written by the applicant)	The CDC 2020 report revealed 54% of IVF procedures in CA did not result in live birth, due to early pregnancy loss. Leveraging the diversity of our samples, this research offers significant benefits by addressing challenges in reproductive health. This study may lead to improved IVF outcomes, reducing infertility rates and pregnancy loss. These advancements will benefit underserved populations, improving reproductive outcomes and promoting equitable access to effective fertility treatments.
Funds Requested	$2,290,157
GWG Recommendation	**(85-100): Exceptional merit and warrants funding, if funds are available**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 90

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

Mean	89
Median	90
Standard Deviation	2
Highest	90
Lowest	85
Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	13
(1-84): Not recommended for funding	0

KEY QUESTIONS AND COMMENTS

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

Does the project hold the necessary significance and potential for impact?
• Strong potential to close a major gap in understanding human embryo implantation, an inaccessible developmental window. • High relevance to IVF failure and early pregnancy loss; strong translational upside. • Human implantation is crucial but difficult to experimentally study. The resubmission uses a further validated endometrial culture model and proposes a number of relevant experiments that will shed light on the initial steps of human embryo implantation. • Very exciting model that will give unprecedented insights (visual and molecular) into human implantation. • The project will provide key information on the process of human embryo implantation. This area has been unexplored due to a lack of NIH funding (congress mandate) and a lack of models to recapitulate the first two to three weeks of human development in vitro. It is relevant to human infertility and makes use of blastoids, i.e., in vitro produced structures that resemble the human blastocyst. • This is not a Team Track project; however, the PI, with expertise in human embryology, pluripotent stem cells, and in vitro embryo modeling, has reached out to two excellent collaborators, in which the PI contributes expertise on spatial proteomics (Aim 2) and the collaborator's spatial transcriptomics knowledge (Aim 2). • At times, the proposal seems overly ambitious (Aim 3).
Is the rationale sound?
• Sound and well-articulated; compelling background on embryo-maternal interface and disease relevance. • Yes, the model as well as the three (3) Aims to image, profile and perturb are logical and relevant. • The main objective is to understand the process of human embryo implantation. The three proposed aims are independent of each other and will contribute novel information on fertilized embryos recapitulating implantation in vitro, how well blastoids can model this process, and understanding the signaling processes between trophoblast, endometrial vasculature, and epithelial cells. They also propose modeling pathological conditions and genetic studies. • The preliminary data are solid. The PI has thoroughly addressed the previous reviewers' criticisms and has conducted significant preliminary experiments that give credit to the notion that the experiments are feasible.
Is the project well planned and designed?
• Aims now sharply defined; each includes benchmarks, validation criteria, and alternative strategies. • Use of blastoids as proxies for human embryos is innovative but introduces interpretive uncertainty. • Highly responsive to previous comments. Much improved proposal. • The project has three complementary aims built around the well established endometrial culture model. Aim 1 will provide high resolution imaging data/analysis. This will be complemented with the in depth spatial transcriptomics in Aim 2 and functional exploration in Aim 3. • The experiments are logically planned. Sub-aims are well thought out and will contribute to the knowledge independently as well. • Pitfalls are addressed, and alternative strategies are well thought-out. In some instances, like Aim 2.1, the alternative strategy appears to be safer than the proposed aim.
Is the project feasible?
• Substantial improvement from previous version, clear experimental numbers, stats plans, and outcome measures. • Yes, the team has key and complementary expertise. • The communication plan is well described and logical.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- Incorporates genetic background, maternal age, and diverse biobank sourcing.
- Explicit plan to correlate outcomes with demographic variables.
- Yes, to the extent that the scale of the model allow that.
- PI will work with different populations, including those with diverse genetic backgrounds and maternal ages.

Application #	DISC0-17946
Title	High-Throughput Discovery of Embryo Formation Factors Using Stem Cell-Based Human Embryo Models
Project Objective (as written by the applicant)	To integrate emulsion microfluidics-based encapsulation with AI-powered imaging to develop a high-throughput pipeline for stem cell-based embryo models.
Impact (as written by the applicant)	We will overcome key bottlenecks to drive breakthroughs in early development, infertility, and genetic and environmental influences on pregnancy that are currently unattainable.
Major Proposed Activities (as written by the applicant)	• Establish a microfluidics platform for efficient and accurate generation of human embryo models in large quantities for screening and modeling. • Develop AI-driven tools that automate embryo model analysis, phenotypic quantification and outcome prediction. • Refine and utilize human embryo models to replicate early development and disease, exploring genetic and environmental impacts on pregnancy outcomes.
Statement of Benefit to California (as written by the applicant)	Infertility is a widespread condition affecting millions of people in California and the US. Infertility rates also vary ethnically, with worse rates in commonly marginalized Black, Latina and Native American populations. In California, the cost of assisted reproductive technology can range from $14,000 to $30,000 or more per cycle, depending on the specific treatments needed. Our proposal aims to ameliorate the longstanding limitations that underpin the current status of the field.
Funds Requested	$2,872,697
GWG Recommendation	**(85-100): Exceptional merit and warrants funding, if funds are available**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 88

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

Mean	87
Median	88
Standard Deviation	2
Highest	90
Lowest	85
Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	13
(1-84): Not recommended for funding	0

KEY QUESTIONS AND COMMENTS

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

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

Does the project hold the necessary significance and potential for impact?
• An in vitro system in which human embryos can be examined is needed since direct observation of implantation events is impossible because they occur in utero. • The proposal addresses the scarcity/ethical implications of human embryos for research. • The proposal aims to build a scalable microfluidic platform for generating human blastoids (stem cell derived blastocyst models). Use ML to streamline quantitative assessments and apply this towards small molecules, supplements and rare mutations. • Aim 3 will serve as strong evidence for various future applications • This proposal aims to provide an extremely valuable stem cell-based resource for studying human developmental biology, reproductive medicine, infertility, and drug screening. It addresses all three areas that CIRM strives to improve understanding of. • The team is well-suited for this project. All three co-PIs have published extensively in the areas of early embryonic development and cell specification, microfluidics and high-throughput 3D culture, and advanced imaging and AI applications in embryology.
Is the rationale sound?
• Aim 2 was somewhat confusing. The goal is to generate an AI screening process for human blastoids, which indeed would be useful. The issue is that there are no good human blastoids (the goal of Aim 1 is to generate improved human blastoids). If human blastoids are not at a stage to be useful, how will an AI system be trained for something that doesn't exist (yet)? • Aim 3.3 could be a whole proposal by itself (but is interesting). • The field is already well established and the proposal would take this to the next level in terms of scalability and application. • The rationale is straightforward. In Aim 1, they will develop encapsulation and microfluidic devices to culture blastoids, aiming to improve yield, uniformity, and fidelity. In Aim 2, they will characterize the blastoids produced using the latest 3D imaging techniques, and in Aim 3, they will functionally test molecules to enhance blastoid production, as well as conduct toxicology and genetic studies. • Initially, it seems that the aims depend on the successful completion of the previous one. However, the PIs have proposed alternative approaches for Aims 2 and 3 that make them independent of each other. • The preliminary data is solid and plentiful, both published and unpublished.
Is the project well planned and designed?
• Preliminary data are in place for the proposed experiments. • Pitfalls and alternative approaches are identified and presented for each subaim. • All three Aims are outlined in sufficient detail and supported with enough data to demonstrate feasibility and meaningful execution. Aim 1 will establish the reproducibility and scale, Aim 2 will implement an unbiased and quantitative ML based appraoch to score and then apply both in Aim 3. • The project will generate a large amount of data regardless of whether the goal is achieved.
Is the project feasible?
• The combined skillset of the teams and leadership in place should allow the experiments to be accomplished. • The proposal uses the Team Track and would likely not be possible without the expertise of the different laboratories. • Yes, based on the description and preliminary data no concerns in this regard. The PI's lab has been a world-leader in this space. • Yes; the institutional resources available to the project are excellent.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• The proposal uses genetically distinct male and female hESC lines. • The data generated may help bring down the costs of performing fertility research, which may indirectly bring regenerative medicine discoveries to underserved populations. • This issue is extend to the extent possible with stem cell derived models (i.e., via the range of lines used). • Yes, the applicant will use male and female hECS lines, as well as lines representing ancestry-associated variation (HLA types), to capture population-level variation.

Application #	DISC0-17976
Title	Enhancing clinical predictability with novel models of iPSC-derived nociceptor for chronic pain.
Project Objective (as written by the applicant)	1. Develop a better chronic pain in vitro model using iPSC-derived nociceptors. 2. Understand the mechanism of action behind chronic pain nociception. 3. Attributes to establish a robust model.
Impact (as written by the applicant)	Translating pain drugs is limited by in vitro model relevance. We propose a novel human iPSC-derived nociceptor model that can provide enhanced clinical predictability for chronic pain.
Major Proposed Activities (as written by the applicant)	• Determine the best cell line to create the in vitro chronic pain model. • Determine the mechanism of action behind chronic pain nociception. • Validation of the model using additional analgesics. • Validation of the model by external collaborators. • Establish QC attributes of the model for robustness.
Statement of Benefit to California (as written by the applicant)	In California, studies indicate that the prevalence of pain-associated conditions ranges from 10% to 40%. Specifically, in 2023, 24.3% of adults had chronic pain, and 8.5% had high-impact chronic pain. The vast majority of the research proposed will be conducted in California. Most of the academic collaborators and some of the CROs are also based in California. We have the potential to patent, publish or find a Californian partner for commercialization of the in vitro model.
Funds Requested	$1,498,623
GWG Recommendation	**(85-100): Exceptional merit and warrants funding, if funds are available**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 88

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

Mean	87
Median	88
Standard Deviation	3
Highest	90
Lowest	80
Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	12
(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.

Does the project hold the necessary significance and potential for impact?
• Addresses critical unmet need in chronic pain therapeutics with novel iPSC-based model. • Leverages patient-derived voltage sodium channel (Nav) GoF nociceptors showing spontaneous synchronous bursts, a phenotype mimicking chronic pain. • Potential to transform preclinical testing and enable personalized in vitro validation of analgesics. • Overall, this is a well-considered proposal using a novel iPSC-based model for studying chronic pain. It has the potential to transform the identification and preclinical testing of new analgesics. • This project addresses a critical and persistent translational gap in chronic pain therapeutics: the lack of a reliable, scalable, and functionally relevant human in vitro model for sustained pain. Current preclinical models, including rodent assays and human iPSC-derived neurons, inadequately model chronic pain states or patient variability. • The proposed iPSC-derived nociceptor platform demonstrates spontaneous, sustained electrophysiological activity—mirroring some human chronic pain phenotypes—and offers an objective functional readout via MEA. If validated, it could substantially enhance preclinical predictability and reduce reliance on animal models. • If successful such models could help de-risk pain therapeutics development. However, the model is based on rare Nav mutations, which may limit generalizability to broader pain populations unless successfully extended. • For example, primary erythremelagia occurs in 1 in 100 to 250K individuals. Having said that Nav selective blockers are areas of active clinical testing and the applicant has an agent that represses the SCN9A promoter. • The collaborations are strategically structured and brings important advantages: • *[Company name redacted]* provides independent validation and scalability testing on high-density MEA platforms. • *[Company name redacted]* contributes cutting-edge proteomics to support omics-based phenotyping. • *[Company name redacted]* ensures quality-controlled iPSC line derivation and differentiation. • NCATS collaboration ensures alignment with regulatory translation and public-sector perspectives. These partnerships collectively enhance reproducibility, scalability, and translational relevance—critical for platform adoption.
Is the rationale sound?
• Model distinguishes chronic from acute pain via functional electrophysiological output. • There is very strong preliminary data that validates the use of iPSC-derived nociceptors and their electrophysiological readout as a relevant model for chronic pain. • While the project is based on sound scientific rationale and supported by strong preliminary data, there are also a number of blind spots. Pertaining to Aim 1, the small number of iPSC lines is not sufficient to investigate sex-specific differences in nociception, especially since the male and female line has different mutations in the SCN9A gene. Even if there are differences in spontaneous burst activities, these could be attributed to intrinsic variability between individuals. • The rationale is based on known pathophysiology: gain-of-function (GoF) mutations in SCN9A (Nav) are clinically validated causes of chronic pain conditions such as erythromelalgia. The spontaneous bursting phenotype observed in patient-derived nociceptors mimics clinical symptoms and offers a biologically coherent model. Importantly, the model's pharmacologic response to SCN9A repression strengthens its validity. • Is this truly modeling "chronic pain," or just spontaneous electrophysiology? The link between the MEA spontaneous bursts and true chronic pain signaling is correlative, not causative.The interpretation that synchronized bursts represent chronic pain is plausible but not fully validated. • Uses a patient-derived model with a Nav GoF mutation, directly tied to known human disease. • Functional outputs (MEA) are quantitative and scalable. • Strong team with experience and collaborations.
Is the project well planned and designed?

- Solid mechanistic hypothesis linking Nav GoF mutations to spontaneous neuronal bursting.
- The project is well designed and planned. Pitfalls are mostly well-considered.
- The study plan is well-structured and addresses both biological validation (Aim 1) and mechanistic exploration (Aim 2), while Aim 3 focuses on reproducibility and preclinical utility. Use of non-human cells (murine satellite glia) is justified for initial co-culture support; the applicant plans to move toward human-only systems later. The use of multiple iPSC lines with different mutations and patient backgrounds enhances the robustness of findings.
- The goal of Aim 1 is to demonstrate that the bursting activity observed in the initial model is reproducible across multiple SCN9A mutant iPSC lines and is clinically correlated with pain severity. This will be tested in 3 donor lines via MEA recordings and compared to healthy controls.
- The response to temperature will also be tested, along with the ability to silence with ZFP. This is all conducted in mixed cell culture, including iPSC neurons and murine glia.
- Aim 2 is designed to use omics to understand signaling mechanisms. This is the aim most likely to uncover novel molecular signatures.
- Aim 3, transfer the model to *[company name redacted]* for validation studies and drug testing, including TRPV1 agonists, gabapentin, and anti-inflammatories. Generate SOPs.
- Risks—such as inter-line variability, lack of phenotype in some donors, or limited translation to non-Nav-driven pain—are acknowledged. Mitigation strategies include:
    - Inclusion of multiple genotypes,
    - Multi-modal readouts (MEA + omics),
    - Use of internal controls and independent replication,
    - However, alternatives to co-culture and contingency plans for proteomic complexity could be more developed.

Is the project feasible?

- The team has a strong funding track record and relevant expertise including epigenetic modulation and pain models.
- The team includes collaborators experienced with MEA, nociceptor culture, and data analysis.
- Given the track record of the PI and the team, as well as the strong preliminary data presented in this proposal, this is highly feasible.
- The team has in-house capabilities for iPSC handling, MEA platforms, and molecular biology. Through partners, they access advanced proteomics, external MEA platforms, and QC-certified iPSC manufacturing. The resource base is fully adequate.
- The proposed timeline (3 years) and budget (~$2.6M) are appropriate given the scope and technical complexity. Cost-sharing with collaborators and existing infrastructure further supports feasibility. A detailed milestone structure is provided.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- The proposal incorporates sex differences and mutation heterogeneity; temperature sensitivity modeled.
- There is some attempt to maximize the impact of successful outcomes, such as in Aim 1 and Aim 3. While it is important to correlate clinical severity to the MEA readout, the sample size (n = 2) is simply too small.
- Aim 3 is critical in ensuring the robustness of the protocol and model for widespread applications. This is an important aspect of the proposal and appreciate that the PI has included this validation aim.
- The proposal explicitly includes iPSC lines from patients of diverse sex and ancestry and notes the differential expression of ion channels and pain thresholds across populations. Aim 1 is designed to assess sex-based and genotype-specific variation in phenotype expression.
- Thoughtful attention to sex/genotype variability and real-world translational constraints.
- By defining critical quality attributes (CQAs) and creating a scalable SOP-driven platform, the model can be expanded to test pain mechanisms beyond Nav and possibly apply to other pain-related mutations or idiopathic chronic pain. The platform might also be used for patient stratification in future trials.
- The team shows dedication to community engagement through collaboration with pain advocacy groups and gathering patient input. They intend to publish SOPs, MEA protocols, and QC pipelines to encourage wider use.

Application #	DISC0-18130
Title	Unlocking the regenerative potential of hepatocyte plasticity for diseases of the biliary system
Project Objective (as written by the applicant)	The research will generate the know-how for replacing lost or injured bile ducts in the liver by reprogramming an abundant and related cell type that normally has a very different function.
Impact (as written by the applicant)	Knowing how to generate bile ducts by reprogramming of other cell types would make a gene therapy for diseases possible that can currently only be cured by liver transplantation.
Major Proposed Activities (as written by the applicant)	• Analysis of gene expression changes in areas where bile ducts are injured in human liver samples • Analysis of regulation of gene expression changes based on chromatin accessibility, including linkage to area-specific gene expression analysis • Analysis of stability of changes in gene expression based on DNA methylation • Discovery of combinations of CRISPR perturbations that facilitate complete and therapeutic reprogramming • Discovery of enhancers that serve as barriers to complete and therapeutic reprogramming • Testing of CRISPR epigenetic editors that write DNA methylation for ability to stabilize long-term reprogramming
Statement of Benefit to California (as written by the applicant)	Facilitating a gene therapy for diseases of the biliary system will improve patient outcomes. Many of these diseases can only be cured by liver transplantation. Because of a donor liver shortage, not all patients can be transplanted, particularly in California which has one of the longest transplant waiting lists in the US. Delaying or bypassing liver transplantation by gene therapy will reduce mortality of patients on the waiting list, allow more patients to be listed and free up donor livers.
Funds Requested	$4,871,000
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: 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	87
Median	86
Standard Deviation	3
Highest	90
Lowest	80
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	13
(1-84): Not recommended for funding	2

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.

Does the project hold the necessary significance and potential for impact?

- The project has significant potential for impact in applying genetic research relevant to the pathobiology of primary sclerosing cholangitis (PSC).
- By studying the gene regulatory networks (epigenetics) of transdifferentiation of hepatocytes to cholangiocytes, the project should yield information that could enable gene therapy approaches to treating this rare but devastating disease. Effective treatment of PSC is a major unmet need currently requiring liver transplant (which is expensive and risky) to avoid a fatal outcome.
- The collaboration between the PI who has great experience in genetic control of transdifferentiation in the liver, and Co-PI who is an expert in high throughput CRISPR screens to elucidate gene regulatory networks offers synergism in achieving the project's goals. Additional personnel cited, including experienced data manager, further strengthen the project team.
- This is a highly detailed, well written and sophisticated proposal designed to identify the molecular mechanisms of human hepatocyte plasticity as it relates to cholangiocyte biology. It is based on a discovery approach that holds potential to resolve a knowledge gap in understanding the pathogenesis of PSC and is, therefore, relevant to human biology, disease and regenerative medicine.
- Key to the success of the project is identifying specific genes based on cutting edge spatial and single-nuclei profiling to identify factors that are required for the formation of mature, functioning non-inflammatory cholangiocytes and functional bile ducts from genetically engineered hepatocytes.
- The proposed collaborations within the Team Track offer a definite synergy and advantage that increase the potential impact and success of the project. While not unique, the senior PI and Co-I are highly suited to the project and provide complementary and integrated expertise targeted to each of the specific aims of the proposal. Their leadership approach, governance and organizational structure are adequate and appropriate to the project.
- Diseases affecting the cholangiocytes of the biliary tree are life threatening and recent studies have shown that transdifferentiation of hepatocytes into cholangiocytes could help to repair the epithelium in extreme situations.
- The goal of this grant is to uncover the molecular mechanisms involved in this process and to control them to promote liver regeneration.
- The collaboration will bring unique combination of expertise (liver/regeneration and CRISPR screen) which will strongly increase the feasibility and impact of the project.

Is the rationale sound?

- There is a sound scientific rationale that forms the basis for the proposed project. Transdifferentiation has been documented in a number of organs including the liver. The ability to stimulate hepatocytes to differentiate into cholangiocytes, and the ability of the latter to form biliary ducts draining bile, offers the prospect of ameliorating the liver damage caused by destruction of the biliary drainage system in PSC.
- The proposed project addresses an important clinical problem and a critical barrier to progress in the field. The studies are based on a relatively sound, albeit somewhat risky scientific rationale that if successfully developed, could possibly result in improved clinical care for patients with PSC and other biliary tract disorders. The approach is based on generating significant data that may or may not address the underlying pathogenesis of PSC and origin of the inflammatory cholangiocyte.
- The rationale for the project is supported by a relatively substantial body of data that supports the potential feasibility of the studies. The studies themselves are highly sophisticated using cutting edge technology to identify factors that may be involved in the ability of hepatocytes to morph into functional, normal cholangiocytes that are not inflammatory in nature. While the rationale is supported by data, the project itself is relatively high risk in achieving its overarching goal.
- The project is well written and each part is rationally articulated. The goal is to study transdifferentiation between hepatocytes and cholangiocytes. This goal is definitely in line with the focus of the field. Controlling this process could help to promote regeneration while blocking further injury.
- The biliary tree is a complex but organized network of tubes. Promoting the production of cholangiocytes might not result in a functional tree.

- The rational between inflammatory and mature cholangicoytes is difficult to follow. There is little information about the differences between these two populations which looks very similar on their single cell analyses.

Is the project well planned and designed?

- The project is appropriately designed with two principal aims: 1) identify genes and enhancers that block hepatocyte plasticity to transdifferentiate to cholangiocytes; 2) Identify chromatin perturbations that activate hepatocyte plasticity by using high throughput CRISPR screens to enhance or inhibit various gene expressions.
- An appeal of the project is that it is highly translational, studying human liver explants available to the PI. The use of mice is appropriate because of the relevance of the mouse model and its utility as a recipient for transplant of human liver tissue in in vivo experiments.
- The project is appropriately planned and designed to generate meaningful results. The studies use a combination of human liver tissue from PSC patients, controls and a well-established and studied mouse model that is well-described and highly familiar to the laboratory of the PI. The combination of resources is adequately justified and probably necessary to the success of the project.
- The overall strategy, methodologies, and analyses are well-reasoned and appropriate to accomplish the specific aims of the project. Of note, the scientific environment at the host institution will contribute significantly to the project's potential for success.
- The project is highly collaborative; and therefore, requires a well-constructed plan for the PI and team communications and management of all aspects of the project. The proposal provides such a plan. The project organization and management plan is well-written, and addresses issues of communication, resource sharing, budgetary issues and conflict resolution.
- Potential pitfalls are identified for each of the specific aims and alternative approaches are presented. That said, several of the potential pitfalls, while recognized, are relatively brief in description and accompanied by alternative approaches that are only moderately developed. Some additional detail would have been helpful.
- The project is well designed and will bring exciting results. Further, functional validations of the gene identified in the AGS model would have increase the impact of this program. Nonetheless, there is no doubt that this team will produce important results that will inform future clinical developments.
- The proposal beautifully mixes human and mouse models but the focus is clearly on human.
- The proposal includes back up plans but they are very optimistic. There are many technological aspects which are high risk. For example, the CRISPR screen are extremely complex and could result in a diversity of false positive. The FNRG mice is a difficult model with variable efficiency. The applicants are definitely very skilled and experienced but the technical aspect is beyond what seems currently possible in the field.

Is the project feasible?

- The PI is an acknowledged leader in the relevant field, and his co-PI brings vital capabilities to the studies involved in Aim 2 of the project.
- The resources and staff are appropriate to carry out the project. These include the NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases) supported liver center which the PI directs, a center for regenerative medicine and stem cell research, and overall resources at the host institution such as a research resources program.
- The leadership of the team is outstanding for both the PI and the Co-Investigator. The investigators have complementary and integrated expertise, as well as their respective teams. Of note, the PI is a world-renowned investigator; and together with the Co-I, their leadership approach, governance and organizational structure are all appropriate for the project.
- The PI will oversee all aspects of the project related to multiomic analysis in Specific Aim 1 and generation of humanized liver mice and adeno-associated viral vectors for Aim 2. The Co-I will oversee all aspects of the project related to CRISPR perturbations, particularly high-throughput screens using Perturb-seq in Aim 2, including experimentation, computational analysis, and data sharing and management. He has great expertise in this area.
- The host institution is a world famous research institute. Simply stated, the scientific environment in which the work will be done will undoubtedly contribute to the probability of success. The proposed studies benefit from unique features of the scientific environment, together with access to the PSC subject population. There is no evidence of additional institutional support; and a single letter of support is provided for tissue samples from PSC patients.
- The timeline appears appropriate for the proposed research. The budget appears excessive for the proposed studies and not entirely justified. This is particularly true for the supplies in years 2 and 3.

- The applicants are well established and are working in leading institutions. They have all the resource necessary for this project.
- The budget is relatively high. However, this is a complex project relying heavily on mouse models.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- While the primary focus of the project is on the specific disease PSC, knowledge gained on genetic regulation of epithelial cell transdifferentiation, and on the autoimmune basis for PSC could eventually prove useful in treatment of other organ failure diseases and other autoimmune diseases.
- It appears that the research team has assembled liver samples from a cohort of 20 PSC patients that faithfully reflect the epidemiology of the disease. It is recognized in the research proposal that the litany of perturbations do not target the underlying autoimmunity for PSC. This is a critical interface with the successful plasticity of hepatocytes into non-inflammatory cholangiocytes that are capable of forming normal biliary tracts.
- This is a cutting edge, high risk, albeit innovative and novel approach to an immunological disease that affects the biliary tract system. The potential success of the project with its focus on hepatocyte plasticity could potentially extend its application via regenerative medicine to additional affected populations, patients and communities. That said, implementing the project results into successful therapy for PSC would be a significant advance in the treatment of liver diseases.
- In the section on population impact, the PI indicates that he attended an annual PSC conference in late 2024 to get feedback on the proposed research and get involved in the PSC community. As indicated, the response was very positive from both the experts and the patients, which is not at all surprising. The PI will continue to regularly attend the conference. The PI has also connected with local patients with PSC via a patient advocacy group.
- Proposed plans for additional outreach, partnership and education activities to inform the progress of the research project are described.
- This is basic research project and thus, the main aim is to generate important knowledge. However, this knowledge will be essential to develop regenerative therapies against cholangiopathies.
- The applicant has described efforts related to outreach.
- PSC is considered an autoimmune disease that has a strong association with IBD (Inflammatory bowel disease). A number of genetic loci have been associated with higher risk to PSC. The proposed studies do not explicitly address and account for the influence of genetic, environmental and/or other external factors that may impact their research findings. That said, the PI and Co-I mention that genetic risk could be correlated with the transcriptomic and epigenetic analyses of hepatocyte plasticity.
- The genetic aspect is mentioned. There is a strong sex dimension in PSC with a stronger incidence in women. However, there is little information about sex consideration in the proposal. For example, the applicant could use male and female hepatocytes for their in vitro experiments. Furthermore, only female FNRG are used for transplantation experiments. Similarly, the question concerning genetic diversity is not addressed in the proposal. This is a missed opportunity.

Application #	DISC0-17315
Title	IFN-γ suppresses AT2 cell regeneration to promote lung fibrosis
Project Objective (as written by the applicant)	We will determine how viral infections alter lung progenitor cells thereby impairing their ability to regenerate the lungs and in turn promoting lung fibrosis.
Impact (as written by the applicant)	We will define mechanisms promoting fibrosis after viral infection. Our work will also provide information on the pathobiology of other types of lung fibrosis, such as idiopathic pulmonary fibrosis.
Major Proposed Activities (as written by the applicant)	• Define how immunological signals such as IFN-γ impair lung progenitor cells from appropriately repairing the lungs. • Create novel mouse models to study IFN-γ signaling in lung progenitor cells. • Develop novel methods using alveolospheres and precision cut lung slices in studying viral-mediated impairment of alveolar regeneration after injury. • Develop a system to track the long-term effects of viral infections in reprogramming lung progenitor cells. • Continued expansion of a human biorepository that will support the current and future studies in lung fibrosis. • Generate scRNA-seq and spatial transcriptomic data for these studies and provided to the community to study viral signals in mediating lung fibrosis.
Statement of Benefit to California (as written by the applicant)	Californians are routinely exposed to respiratory viruses such as influenza and COVID and are at risk for the acute infection and potential chronic sequelae afterwards. Severe damage to the lungs can cause lung fibrosis, and our studies will provide insight into the mechanisms causing these destructive complications. Moreover, our studies will provide information into how viral-induced signals drive other forms of lung fibrosis, which is estimated in 2019 to affect over 60,000 Californians.
Funds Requested	$4,525,190
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	3
Highest	90
Lowest	80
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	11
(1-84): Not recommended for funding	4

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.

Does the project hold the necessary significance and potential for impact?
• This is an interesting and well written application exploring the hypothesis that chronic IFN-γ activation after viral infections impair alveolar regeneration and activate maladaptive programs to promote lung fibrosis. • Emphasis on post-viral fatigue with specific reference to lung damage is a major problem. This needs to be identified earlier and then treated. • The main goal of this application is to study the mechanisms by which acute infection can result in permanent lung damage or persistent pulmonary fibrosis (PASC-PF). For that, the applicant wants to focus on the role of IFNg since they have shown in previous studies that this pro-inflammatory cytokine can alter the capacity alveolar epithelial cell (AT2) cells to differentiate in AT1 cells. • PASC-PF and its chronic version or idiopathic pulmonary fibrosis (IPF) are major and growing health care challenge. Thus, this application aims to address an important disease mechanism with a broad societal impact. • The link to stem cell is not incredibly obvious especially since the mechanisms by which AT2 cells are involved in regeneration need to be better characterized. • Most of the work will be performed in the lead applicant laboratory. There is little information about collaborators or their role.
Is the rationale sound?
• A growing body of evidence has demonstrated a link between impaired alveolar repair and the viral infection. This proposal will explore how viral-mediated signals such as IFN-γ influence AT2 cell differentiation into AT1 cells and accumulation of aberrant basaloid cells which promote lung fibrosis. • Critical here is the sequential experiments in mice - which are very hard to recapitulate in humans - in the absence of sequential human challenge experiments. Ethically you would never get permission to deliberately cause lung damage. • The importance of inflammation in fibrosis is well established. However, link with regeneration has always been difficult to establish. The role of IFNg is definitely interesting and its role in the control of AT2 cells proliferation and differentiation is interesting,
Is the project well planned and designed?
• The project has 3 logical aims underpinned by advanced methodology, utilizing cutting edge in vivo, ex vivo and in vitro models. • The balance between mice and human and ex vivo work is a strength. • Some part of the project are incredibly complicated and could have been better justified. For example Aim 1 will use complex mouse genetic model combined with virus infection to study the importance of IFNg using lung spheroids grown in vitro and lung tissue slides. The same experiment could be performed by using wild type spheroid grown in the presence/absence of IFNg and then by monitoring senescence and differentiation. • A large part of the program will rely on mouse models (in vivo and in vitro). The hypothesis is mature enough to transferred to human models (spheroids and tissue slides). The proposal could have started directly by validations in human tissues. Some aspects of the pathology are also challenging to model in mice especially the aging aspect. • A large part of the program seems to repeat in more details existing studies. • Otherwise, the plan is rational and the hypothesis is interesting. The role of IFNg on the capacity of AT2 to differentiate into AT1 is exciting and well justified. • Each sub aims include back-up plans which seems well planned and rational.
Is the project feasible?
• The PI and collaborators have all necessary expertise to carry out these studies and strong track record of working together.

- This is a well resourced project and adequately costed with adequate resource. The team is excellent.
- The team seems to have access to all the resource necessary to achieve their program of research.
- The timeline of 36 months will be difficult to achieve especially as the program will rely in part on 2-year-old mice. This is a complex project combining animal models, patients data, computational analyses etc. Concerning the budget, it seems relatively high especially on personnel costs.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- Lung fibrosis is an age-related disease. Murine studies into mechanisms of AT2 regeneration will use aged mice in many of the in vivo studies. Studies using human samples will account for sex, age, and racio-ethnicity when doing a comparative analysis of viral-mediated lung fibrosis and IPF.
- This is a problem that affects all ages, but gets worse the older we get. Therefore, ultimately (and not as a result of this grant) if a therapeutic intervention was possible then this would lead to healthier lungs in a significant proportion of people.
- The application will access biobank of human tissues representative of the local population. However, there is little information how human genetic diversity will be considered in their studies. It might be too early for such consideration.
- There is little information on these aspects. Their institution has several programs that they will use.

Application #	DISC0-17998
Title	Global profiling of miRNA-based gene activation to enable a new category of genetic medicine
Project Objective (as written by the applicant)	The proposed research will address key knowledge gaps in small activating RNAs (saRNAs) and could overcome bottlenecks limiting the broader adoption of this technology for regenerative medicine
Impact (as written by the applicant)	If successful, the proposed technology could activate the expression of many different genes throughout the genome, with applications to both rare disease and highly prevalent conditions.
Major Proposed Activities (as written by the applicant)	• Profile small activating RNA (saRNA) activity across ten diverse genetic targets • Computationally analyze the Activity 1 results. Develop an algorithm that can be used by both academics and drug developers to design activating RNAs • Systematically study the mechanism and selectivity of small activating RNAs to advance their therapeutic potential
Statement of Benefit to California (as written by the applicant)	The proposed research has the potential to unlock a new category of genetic medicine that could treat diseases as diverse as severe childhood epilepsy, cardiomyopathy, polycystic ovary syndrome, and hypercholesterolemia. These indications in aggregate affect millions of Californians and represent major unmet medical needs.
Funds Requested	$2,997,574
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	2
Highest	90
Lowest	80
Count	12
(85-100): Exceptional merit and warrants funding, if funds are available	10
(1-84): Not recommended for funding	2

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.

Does the project hold the necessary significance and potential for impact?
• Many clinically relevant genes are regulated by small activating RNAs (saRNAs) and are theoretically amenable to genetic therapy. This would be a new category of genetic medicine. • Knowledge gaps include: 1) What is the true scope of genetic targets amenable to activation by small activating RNAs (saRNAs); 2) How can one rationally design potent and selective saRNAs; and 3) What is the exact mechanism by which saRNAs drive transcription through paRNA targeting? • Academic and biotech team leverages the strengths of both environments. • Excited to read about this bold program of work. • The proposal addresses a critical limitation in genetic medicine: the lack of scalable technologies for gene activation. Most approved oligonucleotide therapies are limited to gene silencing, and only a small percentage (10–35%) of genes are currently targetable with splicing-based activation. This project proposes a novel and generalizable strategy using small activating RNAs (saRNAs) that target promoter-associated RNAs (paRNAs) to enable programmable, reversible gene upregulation. • If successful this could catalyze a new class of regenerative gene therapies. It is grounded in knowledge that endogenous miRNAs can activate genes through paRNA targeting. Preliminary data showed 2-4x activation of a target gene. • This is a compelling proposal that outlines a coherent, innovative, and well-supported plan to develop a new therapeutic modality in regenerative medicine. The integration of computational modeling, thorough experimental profiling, and mechanistic investigation demonstrates a mature and ambitious vision. Improvements in community engagement and project management structure could enhance the proposal further. • The partnership between biotech applicant and co-Investigator at an academic medical center is a major strength. The company offers drug development knowledge and clinically focused screening methods, while the academic lab provides advanced technologies in transcriptomics and RNA-protein interaction profiling.. Their collaboration is clear in the experimental design and mechanistic validation plan, supporting both therapeutic development and basic science.
Is the rationale sound?
• Endogenous miRNAs have been shown to mediate gene activation through AGO-mediated binding to a paRNA. • Several groups have engineered artificial miRNAs, termed small activating RNAs (saRNAs) capable of activating a target gene 10-20x fold through paRNA targeting. • This is a high-risk, high-reward project. • The proposal includes good preliminary data. • The project is built on a strong scientific foundation involving nuclear RNAi, AGO2-mediated (Argonaute 2) gene regulation, and miRNA biology. Previous studies have shown that endogenous or synthetic miRNAs can activate transcription through interactions with paRNAs. Using both binding and cutting variants of saRNAs enhances the mechanistic approach. AGO2 eCLIP (enhanced crosslinking and immunoprecipitation) is especially useful for identifying the targets of miRNAs.
Is the project well planned and designed?
• The three specific aims follow a logical sequence:1.Screen a well-designed saRNA library across 10 clinically relevant targets. 2. Build predictive models based on RNA features, structure, and RBP binding. 3. Conduct mechanistic studies to confirm AGO2 recruitment and Pol II activation. • The inclusion of diverse gene targets, suitable cell lines, and both sequence- and structure-guided design strategies supports broad applicability. Controls (e.g., binding vs. cutting RNAs) are carefully incorporated but positive controls are lacking. • The proposal anticipates several potential pitfalls, including: Variability in saRNA activity•Limited antibody availability; challenges in selecting optimal target sites along paRNAs. Alternative approaches are provided, such as using mass spectrometry, expanding search windows, or secondary structure modeling. However, the diversity of mechanisms across gene targets is acknowledged but not examined in depth. • Given that a stated weakness is a lack of proteins, and only ten genes are to be targeted, it is unclear why the endogenous gene is not targeted with a fluorescent reporter to assess knockdown by FACS without WB. • Targeting so many diseases might be overwhelming; it may be suitable to target a smaller range of conditions.

- There is variability in predicted rates of gene activation, so the applicant includes additional technical and biological replicates – but why not test across cell types to explore the cell type-specific nature of effects?
- Limited alternative strategies are considered for Aims 2 and 3. But what if insufficient data are generated in Aim 1?
- Weakness: Interdependence of all aims – why not include one validated saRNA and target?
- Aims 2 and 3 are highly dependent on Aim 1 success.
- Go/no go criteria for target prioritization are needed.

Is the project feasible?

- Both sites provide excellent infrastructure offering access to advanced molecular equipment and high-throughput sequencing, eCLIP, ChIP-seq, and machine learning. Access to large computational clusters and long-term storage is thoroughly documented.
- The proposed 3-year time-frame is suitable for discovery-stage research, although the scope is ambitious. Without a detailed budget, assessing the cost's appropriateness is limited. Prioritization may be necessary to ensure all aims are achieved.
- Excellent facilities. It is unclear the extent to which [redacted: applicant] can conduct paRNA screening at this scale.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- Selected targets associated with highly prevalent indications (e.g., hypercholesterolemia), severe pediatric indications (e.g., SynGAP1 deficiency and LAMA2 muscular dystophy), indications affecting women's health and reproduction (e.g., polycystic ovary syndrome), and indications disproportionately affecting people of color (e.g., sickle cell disease).
- Yes, diseases and risk variants affect diverse communities.
- Ongoing relationships with leading patient organizations; working to reduce bias in hiring and lab community.
- The team selected target genes relevant to diverse populations, including pediatric (SynGAP1), women's health (PCOS), and underrepresented groups (sickle cell disease). They also plan to use cell lines from individuals of different sexes and genetic backgrounds.
- The goal of creating an open-source saRNA design algorithm significantly broadens the usefulness of this work beyond just the initial 10 genes. The method is built to be scalable and applicable to a wide range of diseases, including those that currently lack genetic therapies.
- The team maintains connections with patient advocacy groups and emphasizes patient-informed views. However, there are few specific plans to engage patient communities throughout the project or to ensure the return of results to affected groups.

Application #	DISC0-17566
Title	In neurons and beyond: how protein interactions shape the cellular response to Huntington's Disease
Project Objective (as written by the applicant)	New knowledge relating to protein-protein interactions that drive vulnerability of different cell types to neurodegeneration disease mutations using Huntington's disease as a paradigm.
Impact (as written by the applicant)	The studies will inform current gaps in knowledge about drivers of cell-type specific functions of Huntington and cell vulnerability in Huntington's disease and other neurodegenerative diseases.
Major Proposed Activities (as written by the applicant)	• Differentiate iPSCs into medium spiny neurons, cortical neurons, astrocytes, and microglia. • Carry out proximity labeling following by mass spectrometry and RNA-sequencing on TurboID iPSC-MSNs, cortical neurons, astrocytes and microglia. • Carry out quality control, data processing and analysis of transcriptomic and proteomic data • Perform total RNAseq and ribosome profiling of differentiated TurboID lines followed by data analysis and comparison to protein/RNA interactors. • Evaluate gain and loss of HTT function mechanisms using KOLF2.1 engineered isogenic series. • Build networks to investigate HTT's role in posttranscriptional regulation
Statement of Benefit to California (as written by the applicant)	The disability, loss of personal freedom and earning potential, and costly institutional care of Huntington's disease (HD) and other neurodegenerative diseases is profound. Understanding mechanisms that can further inform treatments for HD and other neurodegenerative diseases will benefit the State through new technologies and intellectual property, resulting in possible job creation and revenues in new companies, and potential reductions in individual suffering, medical and care-giving costs.
Funds Requested	$2,056,195
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	85
Median	86
Standard Deviation	2
Highest	90
Lowest	80
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	11

(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.

Does the project hold the necessary significance and potential for impact?

- Huntington's disease (HD) is a devastating genetically inherited neurodegenerative disease for which there are no cures or effective treatments. The current project aims to identify network-based drivers of selective degeneration in HD which can identify novel therapeutic targets.
- This project addresses a fundamental knowledge gap in understanding cell-type specific vulnerability in Huntington's disease. Using isogenic iPSC lines with TurboID proximity labeling offers an innovative way to map HTT protein interactions across various brain cell types. This could yield important mechanistic insights into why medium spiny neurons are especially vulnerable while other cells are relatively unaffected.
- The systematic comparison across four disease-relevant cell types using identical genetic backgrounds is methodologically sound and could establish important principles for other neurodegenerative diseases.
- While the approach is innovative, its immediate therapeutic relevance may be limited. The study emphasizes basic mechanistic understanding rather than quick translational applications. The use of a single genetic background (female, unspecified ethnicity) may restrict the broader applicability of the results.
- This is a well-designed, innovative study addressing a significant biological question with strong preliminary data and suitable expertise. The TurboID approach represents a significant methodological advance for studying protein interactions in disease-relevant cell types. However, the ambitious scope, dependence on key personnel, and limited immediate translational relevance prevent a higher score. The project would benefit from more targeted aims and improved contingency planning.
- The proposed collaboration provides essential expertise in mass spectrometry and ribosome profiling that significantly enhances the project's technical capabilities.
- The established track record of collaboration and publications shows successful prior partnership. The complementary expertise of key personnel fosters genuine synergy.
- Proposal connects a leader in HD with a leader in proteomics.

Is the rationale sound?

- The project will identify cell type specific protein and cognate mRNA networks that may drive neurodegeneration or cause cellular dysfunction with good scientific rationale.
- The scientific rationale is solidly based on established HD biology. The hypothesis that cell-type specific HTT interactions cause selective vulnerability makes sense and is backed by known disease patterns. The preliminary data showing different protein binding in MSNs and cortical neurons provides a strong foundation. The focus on RNA-binding proteins fits with emerging knowledge of RNA problems in neurodegeneration.
- The project is supported by a large body of available data from the applicant's lab as well as others.
- Understanding how HTT protein interactions drive its function in different cell types, how they change in HD, and why medium spiny neurons (MSNs) are so uniquely vulnerable will provide insights into mechanisms that drive disease pathology and inform future therapeutic strategies that take into account different cell responses to the mutation.
- Previous HTT interaction studies identified RNA-Binding Proteins (RBPs) as prominent HTT binding partners, but cell type specific comparisons have not been undertaken. Could this not be predicted in silico by restricted to genes expressed in each cell type?
- The assumption that protein interactions alone explain cell-type vulnerability may be oversimplified. Other factors like metabolic demands, protein clearance capacity, or developmental origins could also play an important role.

Is the project well planned and designed?

- The experimental design is thorough and well-organized across three clear objectives. Using isogenic controls removes confounding genetic background factors. Multiple validation methods will be used to

reinforce the findings. Combining proteomics, transcriptomics, and ribosome profiling offers a layered, comprehensive analysis. Non-human studies are minimal and properly justified for validation.

- Systematic aims with sufficient technical details to understand experimental design and analysis.
- The project is well designed and planned. It has a logical, step wise design and builds on already generated cell lines and established methods. The project covers all steps from discovery to validation. It is based on stem cell differentiation into disease relevant neurons (MSNs and cortical) as well as astrocytes, and microglia. The extent of how similar these in vitro generated cells are to their in vivo counterparts are not well documented.
- Thoughtful scientific design with careful statistics, expected outcomes, and alternative strategies.
- Strong aims that complement each other.
- In Aim 3, the focus on comparisons of RNAseq and Ribo-seq only is unclear. What if interactions with the protein are not identified in Aim 1?
- They propose to test if knockdown of selected candidate interactors rescues HD-associated transcriptomic and translational abnormalities in mHTT-expressing neurons. Is there any evidence that the protein knockdown ameliorates HD phenotypes?
- The ambitious scope might be difficult to complete within the timeline or budget. Some validation experiments (Aim 3b) test only three interactors, which may not fully capture the complexity. The aggregation studies (Aim 1c) introduce additional complexity that could weaken focus from the main objectives.
- Good identification of potential technical issues (protein turnover effects, differentiation efficiency variations). Alternative validation approaches are clearly outlined. Contingency plans for siRNA knockdown failure demonstrate thoughtful planning.
- Limited discussion of what occurs if key hypotheses are wrong (e.g., if HTT interactions don't account for cell-type vulnerability). Lack of backup plans for significant technical failures or if collaboration encounters problems.

Is the project feasible?

- It is an excellent team well poised to carry out the experiments and all resources and techniques is in place,
- Excellent access to iPSC facilities, microscopy, and sequencing cores. A collaborative institution offers state-of-the-art mass spectrometry facilities. Robust computational resources are available as well as established cell line repositories and differentiation protocols.
- Reliance on the external facility for key analyses may cause scheduling or access problems. No clear backup plan if mass spectrometry facilities become unavailable.
- The 3-year timeline seems realistic for the proposed scope. Budget allocation appears suitable for the necessary personnel, reagents, and analyses. The collaboration funding structure is efficient.
- The broad scope (4 cell types, multiple analyses) might be optimistic for the timeline. No mention of potential cost overruns for mass spec analyses. Some preliminary work is still ongoing, which could delay the project start.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- The PI was a co-founder of the HD clinic at their institution and is involved in HD organizations nationally and is a co-Director of Research for an institute at their organization where one of the tenets of the institute is the equitable deployment of health care.
- Acknowledges the limitation of a single genetic background and proposes future validation using diverse patient-derived lines. Outlines a plan to extend findings to a broader HD population. Recognizes that factors beyond genetics may also influence disease manifestation.
- The current study design doesn't actively address population diversity. It offers limited consideration of how environmental factors might influence protein interactions. Relying on a single cell line limits immediate generalizability.
- Clear explanation of how findings could apply to other neurodegenerative diseases. Mentions availability of diverse patient iPSC lines for future research. Approach methodology could be widely useful across different diseases and populations.
- No current plans to test findings across diverse populations. Limited link between basic interaction studies and clinical variability. No defined strategies for validation in underrepresented groups.
- Existing strong partnerships with HD patient organizations, such as HDSA and HD CARE, provide a solid foundation. The PI's leadership roles within the HD community offer an excellent outreach platform. Plans include data sharing and developing community resources.

- There are limited specific plans for engaging diverse communities in this project. Outreach activities appear more connected to existing clinical work than to this basic research project.

Application #	DISC0-17276
Title	Modeling Rett syndrome neurological disorder with human pluripotent stem cells to develop in cellulo screening platforms.
Project Objective (as written by the applicant)	Study the neurological disorder Rett syndrome using neurons derived from genetically engineered human pluripotent stem cells and develop platforms to screen drugs for this disease in live cells.
Impact (as written by the applicant)	Rett syndrome is the second most common cause of girls' intellectual disability, still lacking effective treatment. We hope to accelerate the discovery of more effective drugs for this orphan disease.
Major Proposed Activities (as written by the applicant)	• Model Rett syndrome neurological disease by genome-editing human pluripotent cells to carry a fluorescent MeCP2 protein and introduce Rett mutations. • Convert the modified pluripotent cells to neurons and determine how MeCP2 mutations affect protein levels and DNA binding by live cell imaging. • Miniaturize the conversion of human pluripotent cells to neurons to test hundreds of samples at once. • Develop an automated microscopy platform to measure MeCP2 binding to DNA in real time in live neurons at high speed and throughput. • Develop an automated microscopy platform to measure MeCP2 protein amounts and neurons' characteristics at high throughput. • Perform an exploratory screening of drugs approved by the Food and Drug Administration to test how they affect MeCP2 function and protein amounts.
Statement of Benefit to California (as written by the applicant)	Our proposal enhances and combines California's preeminence in stem cell and single-molecule biology. Based on previous work, the investigators co-founded a $600+ million California company using live cell single-molecule imaging for drug discovery. Our proposal will apply this imaging technology to more complex, disease-relevant systems based on human pluripotent cells, enabling the next generation of therapeutics that will benefit Californians and the world.
Funds Requested	$2,393,281
GWG Recommendation	**(85-100): Exceptional merit and warrants funding, if funds are available**
Process Vote	All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG." Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."

SCORING DATA

Final Score: 85

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

Mean	86
Median	85
Standard Deviation	1
Highest	90
Lowest	85
Count	14

(85-100): Exceptional merit and warrants funding, if funds are available	14
(1-84): Not recommended for funding	0

KEY QUESTIONS AND COMMENTS

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

Does the project hold the necessary significance and potential for impact?

- There are limited therapeutic options available for Rett syndrome. This proposal addresses the most common molecular disease mechanism.
- Project will use mutant cell lines in a novel screening approach for drugs that might restore function to MeCP2 protein.
- This resubmission addresses two major concerns in the original proposal. 1. About neuron differentiation: They have now provided preliminary data to demonstrate they robustly derive and image excitatory neurons hiPSCs. 2. About studying a single mutation MECP2 in one cell line: They now plan to test the hit drug with additional RTT mutations and will confirm hit specificity in neurons differentiated from patient-derived hiPSCs.
- The team has extensive imaging and high-throughput drug discovery expertise and have expertise of Rett syndrome and MECP2 mutations.

Is the rationale sound?

- Imaging approach to measuring chromatin interactions is well founded. Post-translational modification affects protein stability and chromatin function therefore could be targeted by drugs.
- MeCP2 levels are tightly regulated in neurons, and MeCP2 overexpression leads to another neurological disorder. Pharmacological approaches to directly restore MeCP2 levels or function are currently lacking. Males carrying the MECP2 mutation provide an invaluable resource to understand RTT pathology and test therapies, as X-inactivation does not occur in males and all cells thus express the mutant protein.
- This proposal aims to model this MECP2 mutation in a male patient hiPSCs-derived neurons and developing imaging screening platforms to quantify MeCP2 DNA binding ability and protein levels by advanced microscopy.
- Preliminary data showed that the mutation reduces MeCP2 protein levels and DNA binding in the patient-derived iPSCs derived neurons, and the mouse brain with mutation knocked-in mouse model. The preliminary data suggested the single-molecule tracking is an appropriate approach to distinguish physiological from pathological protein dynamics in live cells.
- Preliminary data also suggest they can achieve successful neuronal differentiation.
- Overall, the data support the proposed project. One concern remains on whether a homogeneous neuronal population and similar maturation can be achieved in 96- or 384-well plate formats, as this may affect the drug screening platform. The team has identified this potential pitfall and will use imaging platforms with immortalized human cell lines as a backup plan.
- The rationale for the repurposed library and relationship between chromatin association and neuronal function could be clarified further.

Is the project well planned and designed?

- The project is appropriately planned and designed.
- The imaging technology is well developed and iPSC models well-conceived.
- The high throughput screening methodology looks feasible and has been thoroughly considered.
- Data on positive controls for screen (which the applicant has identified) would be important in the future.
- It is unclear whether secondary screening is satisfactory - multiple cell line validation on the basis of neuronal morphology seems reasonable but is not explained in detail.
- Aim 3: What if there are no hits? How will hits be pursued? This could be clarified but is a minor concern.

Is the project feasible?

- The PI has extensive experience in chromatin imaging in live cells and collaboration with disease experts.
- The proposed cell lines, imaging and high throughput technology are all available.

• The team has extensive imaging and high-throughput drug discovery expertise and now provide data for neuron differentiation from iPSCs.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• Yes, a small molecule therapy would likely be more accessible than gene therapy approaches. • The study focuses on male iPSCs carrying a novel mutation. This averts the cellular mosaicism in female cells when studying disease mechanisms and evaluating therapeutic strategies. In this setting, all neurons derived from male hiPSCs will be affected, as they have only one X chromosome, which simplifies data interpretation. • The potential drug can be extended to validation in female cells. This is now discussed in the proposal. • The team plan to employ the strategies for outreach or educational activities, including Drug Discovery Bootcamp and community outreach events.

Application #	DISC0-17488
Title	A novel platform to rescue neurodevelopmental disorders caused by haploinsufficiency
Project Objective (as written by the applicant)	We develop a powerful platform to correct gene expression defects caused by haploinsufficiency and will show its ability to identify novel targets using iPSC-derived neurons and cortical organoids.
Impact (as written by the applicant)	De-repressing haploinsufficient genes from endogenous regulation has lacked high-throughput and resolution tools. Our technical innovations overcome this bottleneck enabling a new discovery platform.
Major Proposed Activities (as written by the applicant)	• We will generate a list of miRNA- and RNA binding protein (RBP)-target interactions within haploinsufficient genes in iPSC-derived neurons. • We will screen regulatory elements for their de-repression potential and identify those that rescue protein levels in haploinsufficient disorders. • We will test phenotypic rescue in disease models to show that our strategy can identify novel therapies for neurodevelopmental disorders.
Statement of Benefit to California (as written by the applicant)	In California, one out of fifty children are diagnosed with a neurodevelopmental disorder (NDD). Haploinsufficiency, when one allele is not enough for normal gene function, is a prevalent etiology in NDDs, making it crucial to develop therapies that increase protein levels of NDD genes. The systematic identification of repressive regulatory elements within haploinsufficiency NDD genes proposed here, and the ASOs targeting them, could support clinical trial efforts.
Funds Requested	$4,086,486
GWG Recommendation	**(85-100): Exceptional merit and warrants funding, if funds are available**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

SCORING DATA

Final Score: 85

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

Mean	86
Median	85
Standard Deviation	2
Highest	90
Lowest	84
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	13
(1-84): Not recommended for funding	1

KEY QUESTIONS AND COMMENTS

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

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

Does the project hold the necessary significance and potential for impact?
• In California, one out of fifty children is diagnosed with a neurodevelopmental disorder (NDD), with conditions like severe epilepsy and autism. The burden to the child and caretaker is enormous, both emotionally as well as financially. An expected outcomes is identification of the most promising negative regulatory elements that, when blocked, restores protein level and rescue haploinsufficiency. • The proposed approach can be applied to many NDDs. Applicant will also generate a publicly available library of iPSC lines from NDD patients. • Focus on patients that have severe, treatment-resistant epilepsy, which is a major co-morbidity in NDDs increases significance and is of high urgency. • Conceptually exciting. • This is an outstanding team proposal from an outstanding team. The potential for impact is strong, but one concern is that the proposal does not address the fact that regulatory elements are, to this reviewer's knowledge, not generally gene-specific. Thus identification and blockade of negative regulatory elements on haploinsufficiency NDD genes would likely have extensive effects on expression of many other genes, potentially limiting the potential usage of this approach. • The motivation here is that many NDDs are due to loss of one gene copy. Antisense oligonucleotides are a new and exciting therapeutic modality that can upregulate expression of genes but they are limited because we often don't know where the best targets in the transcript are. • Good use of stem cell-derived neurons to upregulate gene expression in the relevant cell types. • The project is synergistic based on techniques from the PI and Co-Investigator's (Co-Is) labs and patient stem cell resources from another collaborator's lab.
Is the rationale sound?
• The observation that NDD haploinsufficient genes are regulated by miRNAs and RBPs, and that blocking miRNA-mediated repression boosts their protein levels, support the feasibility of the approach. • Preliminary data are interesting and support the approach. • The direct experimental mapping of MTIs at scale and with high sensitivity and specificity is far superior to modeling approaches that generate many false hits. • Yes. The team proposes to profile NDD haploinsufficient genes to find miRNA target sites which they call MTIs and RNA binding protein target sites which they call RBPTIs. They use stem-cell derived neurons and focus on eight (8) haploinsufficient genes.
Is the project well planned and designed?
• The three Aims are logical: (1) Map the regulatory landscape of haploinsufficient genes in iPSC-derived neurons derived form healthy individuals across time; (2) Screen MTIs and RBTIs for their de-repression potential. (3) Assess phenotypic rescue in disease models. • Innovative tools. • Discussion of specificity could have been more defined and clearer - what is the risk of off target-or long term effects? • Could nonsense mediated decay play an interfering role? This is not discussed. • Yes. Aim 1 maps microRNA binding sites in iPSC-derived neurons using Ago2 eCLIP. The Co-Investigator is one of the world experts in this technique. Then RBP binding sites are mapped for 35 RBPs involved in RNA translation or stability using antibody-barcoded eCLIP, a new technique. The Aim also proposes to use a different technique called SAP-seq to map MTIs and RBPTIs in organoids. • Aim 2 proposes a screen with an RNA-targeting CRISPR (Cas13) on the 8 prioritized haploinsufficient genes at the MTIs and RBPTIs. It uses another recent technique from the Co-I to quantify translation. Blocking RNA regulatory sites will be correlated with translation. This is a very innovative idea. • Aim 2 also includes work with the clinical collaborator in patient-derived neurons for validation and development of ASOs. • Aim 3 tests Cas13 and ASOs in human neurons and organoids for SCN1a and SCN2a and in humanized mouse models for SYNGAP1 and STXBP1. It's great to see the team completing the loop all the way to preclinical work.
Is the project feasible?

- The critical tool called "Ribo-STAMP" is shown to measure translation in the brain at single cell resolution and was highly sensitive in detecting differences in translation rate for genes that have similar RNA levels across different cell types - this tool makes the approach feasible.
- Effort capitalizes on a prior CIRM-funded project where they generated an iPSC line library from NDD patients, that includes their genetic mutations.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- Significant effort is made to ensure that the identified regulatory elements are conserved across different ethnicity, sexes, ages, and disease states.
- The approach could be extended to many disease that are driven by haploinsufficiency and is adaptable to other cell types.

Application #	DISC0-18038
Title	Base Editing, Single-Cell Multiomics, and Cardiac Organoids to Decode Genetic Variants
Project Objective (as written by the applicant)	Develop a high-throughput platform combining iPSC-derived cardiac organoids and CRISPR base editing to functionally assess missense variants in hypertrophic cardiomyopathy.
Impact (as written by the applicant)	(i) Limited tools for functional interpretation of missense variants, (ii) lack of scalable human cardiac models, and (iii) poor understanding of variant effects across genetic backgrounds.
Major Proposed Activities (as written by the applicant)	• Generate dual-reporter iPSC lines (NPPA-TagRFP/ACTA2-Clover2) for real-time phenotyping in cardiac organoids. • Optimize and scale 3D cardiac organoid culture in 96-well format for genetic screening applications. • Construct and deliver pooled lentiviral sgRNA library for CRISPR base editing of HCM-associated variants. • Sort edited organoids by reporter activity and perform next-generation sequencing to quantify variant impact. • Validate top candidate variants in individual organoid lines and assess phenotypic consequences. • Conduct single-cell multiomics to map variant-associated gene networks across diverse iPSC-derived organoids.
Statement of Benefit to California (as written by the applicant)	Hypertrophic cardiomyopathy affects an estimated 78,000 Californians, yet many remain undiagnosed due to limited tools for interpreting genetic variants. This project will develop stem cell–based cardiac organoids and CRISPR editing to improve diagnosis and risk assessment. By modeling disease in genetically diverse populations, it promotes equitable precision medicine while supporting California's biotech workforce and advancing public health across the state.
Funds Requested	$4,606,248
GWG Recommendation	**(85-100): Exceptional merit and warrants funding, if funds are available**
Process Vote	All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG." Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."

Scoring Data

Final Score: 85

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

Mean	85
Median	85
Standard Deviation	2
Highest	89
Lowest	83
Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	10
(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.

Does the project hold the necessary significance and potential for impact?
• Hypertrophic cardiomyopathy represents an important disease entity. A variety of genes contribute to the disease risk and each of these disease genes present with a broad spectrum of associated disease variants with various impacts on the disease severity, progression and prognosis. The proposed experiment aims to functionally explore the mutational effect. This is highly relevant and will provide important insights. • There is a major barrier to interpreting the clinical significance of genetic variants for disease due to the limited ability to study genetic variants. Current methodologies rely on animal models or 2D cell cultures which often fail to recapitulate human-specific complexities. • The molecular mechanisms linking genetic variants to hypertrophic cardiomyopathy (HCM) pathogenesis remain poorly understood. • This project integrates novel iPSC-derived cardiac organoid models with CRISPR base editing techniques as well as "cell villages" and single-cell multi-omics to evaluate the functional impact of missense variants across genetically diverse populations. If successful, it will resolve the knowledge of the function of the missense variants in HCM, and provide tools sets and cell models for studying genetic variants in disease which is currently challenging. • It is a Team Track project involving a large collaboration. The PI will bring expertise in iPSC and cardiac biology, and co-investigators will bring expertise in gene editing and computational biology. There are other multiple collaborators listed in the proposal that contribute strengths in CRISPR screening, 3D organoid engineering, and multi-omics data analysis. • The investigators are highly qualified and are synergistic given unique experience and expertise.
Is the rationale sound?
• The proposal aims to use stem cell derived cells to investigate the impact of selected disease variants. This is innovative and well supported by preliminary data. • The team provided large amount of data to support the proposed project. They provided multiple figures from previously published papers demonstrating a strong track record in the development and application of iPSC-derived cardiac organoids. These include generating micropatterned organoids to model human cardiac vascularization, establishing organoid-based platforms for clinical drug testing, and developing a cocktail-induced organoid model of cardiomyopathy. • For screening, they have optimized a delivery system for gene editing in organoids. They have established a lentiviral infection protocol for the 3D cardiac organoid system to achieve infection rates as high as 80%. This finding strengthens the feasibility of the base editing screens within cardiac organoid systems. • For "cell villages" together with single-cell sequencing, they provided preliminary data from using a "cell village" of iPSC-CMs from 10 different donors, they applied single-cell RNA sequencing and used a tool to trace cells back to identify each. • Finally, the team has already used "organoid villages" to study Down syndrome-congenital heart disease (DS-CHD) and developed a machine-learning based-tool to predict functional genome. This platform allows the team to study HCM genetic variation in the diverse cardiac organoids village proposed in this project.
Is the project well planned and designed?
• The proposal has numerous strengths. The overall design is supported by outstanding preliminary data. The proposed experiments are well justified. • The proposed experiments are very comprehensive and all phenotypes which will be collected are well justified. The data analysis is described in sufficient detail and is state of the art. • Aim 1 builds the foundation of the proposed experiments and is highly innovative. Aim 2 proposes base editing introducing selected disease variants. Finally Aim 3 proposes a highly innovative 'village' approach. This is an innovative study design which will allows for the analysis of a larger number of variants in a diverse set of cell lines.

- Aim 1 is appropriately planned and designed.
- The challenge in Aim 2 will be analysis of "true hits" in the ABE screen. The base editing efficiency can vary significantly depending on loci. This variability complicates the analysis of base editing screens, even though the team plans to implement a recently developed pipeline for base editor screen analysis.
- In Aim 3, a minor question: does each cell village contain iPSC lines from a single ethnic population or does it include a mix of five populations?
- The proposal aims to describe the underlying disease mechanisms and functional and structural impact. One minor concern is a potential lack of correlation with the actual disease presentation in patients. While the overall goal is to determine the impact of disease variants, the study is not designed to correlate with disease severity and disease progression.
- The inclusion of a few cell lines or the correlation of variants with patient phenotypes and the functional studies results would further strengthen the proposal.
- Editing efficiency in some areas appears slightly optimistic. This is a minor concern.
- Pitfalls are discussed and alternative approaches are well described.
- Research plans should better account for paracrine effects.

Is the project feasible?

- The PI and co-Is have appropriate leadership and expertise to carry out the proposed activities. The budget is appropriate justified. Due to the large project with multiple collaborations, the applicant should detail a clear timeline for tracking the project progress in the proposal.
- The resources and staff are highly supportive.
- The budget is appropriate.
- The proposed work with cell villages appears unfeasible within the requested budget.
- Aim 1 and Aim 3 are very risky.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- It’s a highly innovative project and the combination of a human iPSC-derived cardiac organoid platform, base editing screening, and the organoid village approach will accelerate the study of disease-associated genetic variants. The optimized cell model and data analysis pipeline optimized for base editing screening will facilitate the study of other genetic variants in the future.
- The proposal addresses the impact of genetic variability.
- The results are applicable to affected patient populations.
- Outreach and dissemination of findings are adequately addressed.

Application #	DISC0-17513
Title	COPA Syndrome as a paradigm to define mechanisms of epithelial progenitor cell dysfunction in autoimmune lung disease
Project Objective (as written by the applicant)	This work examines mechanisms driving autoimmune ILD (AI-ILD) in COPA syndrome, a monogenic autoimmune disease, focusing on epithelial progenitor cell dysfunction and contribution of immune cells.
Impact (as written by the applicant)	Autoimmune ILD is a progressive, often fatal condition, for which there is no cure. Our findings will support the urgent need for regenerative and precision medicine approaches in AI-ILD.
Major Proposed Activities (as written by the applicant)	• Use mouse models of COPA to assess intrinsic defects in epithelial progenitor cells and contribution to interstitial lung disease (ILD). • Ask whether defects in epithelial progenitor cells can be corrected to reverse ILD in COPA mouse models. • Use gene editing of iPSC-derived lung epithelial cells from COPA patients to restore function to epithelial progenitor cells • Determine how epithelial-mesenchymal cell interactions are altered in COPA using iPSC-derived AT2 and mesenchymal cells. • Examine the role of neutrophils and NETosis in COPA syndrome using mouse models of COPA and autoimmune ILD • Assess how COPA neutrophils might exacerbate epithelial cell dysfunction using iPSC-derived AT2 cells and neutrophils or NETs from COPA patients.
Statement of Benefit to California (as written by the applicant)	Autoimmune-associated interstitial lung disease (AI-ILD) affects approximately 40% of patients with autoimmune disorders, with over 64,000 individuals in California alone impacted—predominantly women. By using COPA syndrome as a model (where patients universally develop AI-ILD) we will define the cells and pathways that contribute to AI-ILD, directly benefitting the wide spectrum of AI-ILD patients in California by better informing treatment strategies.
Funds Requested	$4,034,724
GWG Recommendation	**(85-100): Exceptional merit and warrants funding, if funds are available**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

Scoring Data

Final Score: 85

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

Mean	83
Median	85
Standard Deviation	2
Highest	86
Lowest	79
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.

Does the project hold the necessary significance and potential for impact?
• This is a strong, well written proposal with a clear potential to enhance our understanding of the contribution (and underpinning mechanisms) of alveolar epithelial progenitors to the development of the interstitial lung diseases. • To dissect how epithelial progenitor cell defects and immune/neutrophil dysregulation interact to drive autoimmune interstitial lung disease in a monogenic disease from COPA mutation resulting in constitutive IFN activation and ER stress. The hypothesis is that mutant COPA causes an AT2 cell-intrinsic progenitor cell defect that causes lung disease and associated immune activation and fibrosis. • This work leverages the COPA syndrome to understand how aberrant neutrophil responses drive autoimmune-associated interstitial lung disease, which affects more than 64,000 Californians. Lung transplants are the only curative therapy for these patients. • This is a team track application that leverages synergy of investigator experise in lung stem cell biology, immunogenetics, and iPSC biology.
Is the rationale sound?
• The application focuses on the study of COPA syndrome and specifically mutations in the COPA gene to get insights into the biological pathways underpinning development of lung fibrosis in autoimmune interstitial lung diseases. The application is supported by strong preliminary data. • There is scientific soundness in the rationale - the preliminary data shows that mutant COPA triggers unfolded protein response and interferon stress in AT2 cells and causes interstitial thickening in conditional mice, which supports the hypothesis that epithelial defects initiate the fibrosis. • Overall okay, but more consideration of immune cell-intrinsic effects of COPA mutations is warranted.
Is the project well planned and designed?
• The project is well planned with three clear research aims and clear research methodologies utilizing advanced in vivo models with whole body and tissue specific COPA genetically modified mice, primary mouse and human AT2 (Alveolar Type 2 cells) cells in organoid cultures, human iPSCs lines from COPA patients. • The number of mice used in experiments may be too low, given the heterogeneity expected in scRNAseq experiments. • There are pitfalls to address drift of the iPSC cultures, variability in readouts, and backups for pharmacologics. • This reviewer sees a missed opportunity here - the project focuses on deciphering molecular disease pathways in epithelial and neutrophil functions, and largely lacks studies of other immune cell populations. ScRNA seq studies of the whole body KO proposed in the Aims 1 and 3 of the application would provide excellent opportunity to gain insights into immune cell mediated mechanisms and cell-cell interactions at baseline and after injury. • It is not quite clear how the work in Aims 1 and 2 will inform Aim 3. • The proposal lacks assessment of mutant COPA in immune cells to define cell-intrinsic immune dysregulation (as opposed to immune effects secondary to ER stress in AT2 cells). The PR8 infection and bleomycin models of lung perturbation are a strength. However, the connection to STING remains hypothetical and is somewhat tacked on without sufficient context. Moreover, the Aim 3.3 goal of extending to more common autoimmune ILD is laudable but only loosely connected with the overall proposal. • Aim 3 is weak. • Aim 3 is not well developed.
Is the project feasible?
• The main applicant and collaborators have very strong track record and all necessary expertise to carry out such study.

• Host institution has excellent clinical infrastructure; core facilities are strong and the laboratory resources are appropriate. • Aim 3.2 has a broad range of profiling approaches that could use more justification.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• The study does incorporate ethnically diverse samples from biobanks and will assess sex as a biological variable. Environmental triggers are modeled, which may generalize the findings. • There is potential to extend the reach of this therapeutic to lupus and systemic sclerosis. • There are standing partnerships with two foundations. • Patients of both genders and various ethnicities will be included into the study, however no information on how this will be taken into account in the experimental plans is provided.

Application #	DISC0-17507
Title	Unraveling nuclear Tau functions using age-equivalent human induced neurons from healthy aging donors and tauopathy patients
Project Objective (as written by the applicant)	This project investigates how normal tau protein protects aging neurons. We'll use aging human neurons to understand how tau helps repairing DNA and why this protection fails disease.
Impact (as written by the applicant)	This research would address a key bottleneck in understanding tauopathies by revealing nuclear Tau's role in DNA repair using physiologically authentic aging neurons that current models cannot provide
Major Proposed Activities (as written by the applicant)	• Generation and validation of aging and tauopathy induced neurons (iNs) • Aging, FTD and AD iN transcriptome analysis • Conditional, spatial distribution of nuclear Tau in aging and tauopathy iNs • RepairSeq for quantifying and mapping DNA damage repair sites in response to hyperexcitation • Tau-ChIPseq for mapping Tau-DNA interaction sites in response to hyperexcitation, and bioinformatic analysis of RepairSeq and ChIPseq • Assessment of treatment strategies to promote nuclear Tau translocation to improve neuronal resilience and identity
Statement of Benefit to California (as written by the applicant)	This research will benefit California by addressing critical knowledge gaps in dementias, which affect thousands of Californians. Using an innovative human model, we'll uncover how Tau protein dysfunction contributes to brain vulnerability in aging. Our findings will advance understanding of age-related brain diseases and potentially identify novel therapeutic targets, ultimately reducing healthcare costs and improving quality of life for California's aging population and their families.
Funds Requested	$2,137,778
GWG Recommendation	**(85-100): Exceptional merit and warrants funding, if funds are available**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

Scoring Data

Final Score: 85

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

Mean	83
Median	85
Standard Deviation	3
Highest	86
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.

Does the project hold the necessary significance and potential for impact?

- The project addresses a crucial gap in understanding the age- and disease-related changes in the nuclear translocation of Tau, and thus its interaction partners and role in the DNA damage response changes. This is of relevance for normal biological aging as well as neurodegenerative diseases with tauopathies.
- The project is well positioned to establish novel datasets and resources to study the effect of aging on human neurons in healthy vs disease (AD and FTD) individuals, with a focus on nuclear Tau functions and interactors, along with testing possible treatment avenues.
- High risk, high reward – what if there is no role for Tau in the nucleus?
- Strong team with inclusion of collaborative investigators in proteomics and AD biology but unclear the extent to which this team is already working together.

Is the rationale sound?

- The rationale that the induced neuron (iN) model is uniquely promising to study aging and tauopathies is compelling.
- The hypothesis is based in the role of nuclear Tau in preserving neuronal health and resilience by facilitating its translocation to the nucleus and regulating the DNA damage response. And that disruption of these processes—especially under conditions of acute neuronal stress—may play a key role in age-related neurodegeneration. The choice of cell model (patient derived iNs) is excellent as it maintains age of donor allowing the investigators to distinguish between healthy aging and disease.
- The rationale for the role of Tau in the nucleus is reasonably backed by literature and data, but clear evidence is lacking.
- While Tau protein has been extensively studied in the context of neurodegenerative disorders, age- and disease-related changes in its nuclear translocation, interaction partners, and role in the DNA damage response are not well understood. Proposal to study Tau in age context overcomes limitations of fetal-like state of traditional hiPSC models.
- While team cites papers that show detection of Tau in nucleus, little evidence of function of Tau in nucleus; no data from team supports role of Tau in nucleus.

Is the project well planned and designed?

- The project is well planned and designed, and collectively the investigative team has the necessary expertise to ensure successful execution. The sample size of 30 individuals - spanning younger and older individuals, healthy controls and FTD and AD patients - is a unique and powerful resource for this project.
- The use of iNs is well described in proposal and has gained clear attraction over iPSCs broadly, based on previous studies where the applicant has been one of the driving teams.
- Use of PROTACs for selective protein degradation is novel and interesting.
- Aim 1 is a well powered study. In Aim 2 it is unclear which donors/ages these experiments focus on. Test effect of future PROTACs and ASOs on Tau, but these therapies are not yet developed.
- It's unclear whether over-expression for CHIP will alter phenotypes and/or represent physiologically relevant endogenous Tau levels.

Is the project feasible?

- Yes, however, the project might benefit from validation of key results in human post-mortem tissue, given the availability of these resources. Additionally, the possibility that the treatment strategies do not reverse the observed phenotypes, or do not do so in all patient cells, is not considered.
- The main applicant is an expert in the cell model and its use in modeling age-related pathology/AD. The co-applicants have clear roles and contribute specific expertise needed and thus the team has the competence and technical expertise needed.
- Collaborative team has resources and expertise to conduct proposed studies.

• Unclear whether cell culture and sequencing costs are reasonable given scope of work proposed.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• The plans to address the influence of genetic factors with a genetically diverse cohort and external factors using application of glutamate to induce DNA damage are appropriate. • Induced neurons (iNs) derived from patients representing different ages, sexes, and ethnic backgrounds will be used. There are some limited plans for maximizing impact across all patient populations. • There is diversity in the biosamples used for this project: biobank of patient samples that includes Latino (20%), Asian (13%), and African American (4%) communities. • Likely yes, given the large impact of Tau mutations across ancestries.

Application #	DISC0-17954
Title	APOE4 compromises BBB integrity through pericyte-microglia cross talk
Project Objective (as written by the applicant)	Our proposal aims to utilize human iPSC models to unravel the intrinsic cross-talk between pericytes and microglia and how APOE4 disrupts this cross-talk, helping our understanding of CAA and ARIA-E.
Impact (as written by the applicant)	APOE4 carriers have higher risk of ARIA-E during Aβ mAb treatment, and CAA. Our approach will enable us to investigate how APOE4 influences this susceptibility and provide a model to test new drugs.
Major Proposed Activities (as written by the applicant)	• Investigate the cell autonomous impact of APOE haplotype in pericytes • Characterize how APOE4 impacts state shifts of microglia to BAMs near vasculature • Dissect the impact of APOE4 microglia and pericytes on Aβ accumulation and CAA
Statement of Benefit to California (as written by the applicant)	APOE44 homozygotes represent 15-25% of all late onset Alzheimer's diseases (AD) case, however including APOE4 heterozygotes, that number increases to 50%. Researchers estimated 720,000 people in California live with AD dementia, more than in any other U.S. state, ranking 4th in terms of prevalence. Current FDA approved drugs put APOE4 carriers at risk for adverse effects. Our proposed work would uncover how APOE4 impacts the brain vasculature and provide a model to test new drugs.
Funds Requested	$1,499,999
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: 85

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

Mean	83
Median	85
Standard Deviation	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.

Does the project hold the necessary significance and potential for impact?
• The proposed study uses iPSC model to assess the role of APOE4 variant in blood brain barrier (BBB) integrity in Alzheimer's disease (AD). This is an important but inadequately understood aspect of AD pathology. The study's focus is on pericytes and microglia, which may have a key role in BBB disruption. It addresses cerebral amyloid angiopathy, which is an important component of the disease. • APOE4 is the strongest genetic risk factor for AD and cerebral amyloid angiopathy (CAA), with studies linking APOE4 to accelerated amyloid pathology in both brain tissue and cerebrovascular structures. The specific role of different APOE genotypes (APOE33, APOE44) in the systemic breakdown of the BBB remains uncertain. • This application addresses a fundamental and under-explored mechanism in AD: how APOE4 alters vascular function and immune interactions to drive cerebral amyloid angiopathy (CAA) and BBB dysfunction. The focus on human iPSC-derived pericytes, endothelial cells, and microglia offers a stem cell-based platform to resolve critical questions about APOE4 pathobiology that animal models have failed to answer. • If successful, this work would advance understanding of vascular contributions to AD, inform the development of safer therapeutic strategies, and offer new regenerative medicine targets. It fills a major gap in AD research by focusing on the vascular-immune interface, often neglected in favor of neuronal models. • The proposal integrates synergistic expertise: The PI's group provides world-leading experience in human iPSC modeling and single-cell analytics, while collaborators bring strengths in BBB biology, lipidomics, and microglial immunology. This collaborative structure substantially strengthens the application.
Is the rationale sound?
• Compromise of BBB in AD is important but not well understood; microglia may play a role, along with pericytes. The applicant's proposed models capture the key cellular players. • The rationale is compelling. APOE4 is the most prevalent genetic risk factor for late-onset AD, and increasing evidence points to its role in vascular dysfunction and microglial dysregulation. The hypothesis—that APOE4 impairs pericyte and microglial function, leading to ECM thickening, amyloid trapping, and a pro-inflammatory environment—is supported by previous mouse studies and preliminary human iPSC data.
Is the project well planned and designed?

• Project plan concerns:
  ◦ Is the composition of 3D vascular co-cultures reproducible?
  ◦ Is the pericyte component reproducible?
  ◦ Does vasculature show a tissue-specific gene expression signature?
  ◦ The team's experience with BBB on a chip assays (Aim 3) is limited.
  ◦ Will BBB on a chip produce bonafide basement membrane, or enable assessment of the multiple endpoints in Aim 3?
• Aims 2 and 3 are interdependent and ask highly related questions; it’s not clear to what extent both are necessary. Moreover, it is unclear the extent to which co-culture, trans-well, and organ-on-a-chip models are established at the scale necessary to undertake the proposed experiments.
• A reasonable number of isogenic pairs is proposed (1-3 per experiment), but otherwise, technical details of the project plan are frequently vague, both in terms of how co-cultures will be established and how data will be analyzed.
• The hypothesis is unclear. Mechanistic dissection is not proposed for any phenotypes to be uncovered. In addition, although preliminary data repeatedly highlights the potential of fibronectin to ameliorate/reverse APOE4 effects, this is not tested.
• The planned methods to optimize co-culture medias are not detailed.
• Planned methods to purify microglia to account for low cell numbers are highlighted. However, the ability to conduct and resolve APOE3 vs APOE4 lipidomic and metabolic effects is unclear, particularly within complex co-cultures.
• The project employs a comprehensive design using human isogenic iPSCs (APOE3 and APOE4) differentiated into relevant vascular and immune cell types. It integrates co-culture systems, vascularized organoids, and organ-on-chip models. Multi-omics approaches (proteomics, lipidomics, scRNA-seq) are

well-matched to the project's complexity. The organ-on-chip assays for Aβ deposition and mAb-associated ARIA risk modeling are particularly innovative. • The proposal acknowledges the complexity of modeling BBB and microglial interactions and the limitations of current in vitro tools. Alternative cell lines, Aβ sources, and LXR rescue strategies are included. However, mitigation plans for limited throughput or inconsistency in organ-on-chip performance could be strengthened.
Is the project feasible?
• Sophisticated analysis of cell interactions is critical to pathogenesis. • Given the scope of work proposed, this proposal might have been better suited to a larger collaborative team. • There are some concerns about low budget for cell culture costs ($30,000 per year) and scRNAseq ($6,400 per year). In addition, proposal describes extensive cell culture to be conducted by a single full-time postdoc. • The host institution provides access to cutting-edge facilities, including imaging, omics cores, iPSC derivation and differentiation infrastructure, and microfluidic modeling platforms. The application also leverages robust pre-existing iPSC cohorts and proprietary organ-on-chip systems. • The proposed budget appears reasonable given the technical demands of the project. A 3-year timeline for each aim is plausible but may require contingency planning for delays in organoid maturation or microfluidic assay optimization.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• The proposal notes genetic variation in sensitivity to APOE4 variant and disproportionate burden of disease in underrepresented groups. • The project will expand the diversity of stem cell lines under evaluation. • The project includes 22 donors, but the proposal does not provide a specific breakdown by ancestry. • APOE has strong risk effects across populations. • The applicant describes limited outreach to training of diverse scientists through established programs such as CIRM-Bridges. • The application directly addresses APOE4 risk stratification across ethnicities and sex. It includes isogenic iPSC lines derived from underrepresented backgrounds, allowing exploration of gene-environment and sex-genotype interactions. • Because APOE4 is prevalent across racial and ethnic groups (especially in individuals of African, Hispanic, and East Asian ancestry), findings could be broadly generalizable. The identification of modifiable vascular risk mechanisms may also be relevant to CAA and TBI populations. • The PI and institution have established partnerships with the AD patient community, and there are clear commitments to data and resource sharing (e.g., scRNA-seq datasets, iPSC lines). However, more detail on specific outreach strategies could improve this section.

Application #	DISC0-17263
Title	Neuroprotective discovery for Parkinson's disease (PD) using human iPSC models
Project Objective (as written by the applicant)	Using hiPSC-derived mDA neurons, we aim to uncover new mechanisms for PD, and to discover synergistic neuroprotective compounds that can be translated into new disease-modifying therapeutics for PD.
Impact (as written by the applicant)	If successful, this project will bridge the knowledge gap in understanding neurodegenerative disease mechanisms and address the bottleneck in discovering disease-modifying therapeutics for PD.
Major Proposed Activities (as written by the applicant)	• Develop a high content screening platform to follow survival of hiPSC derived mDA neurons using TH-reporter hiPSC cell line suitable for live imaging • Investigate the neuroprotective potential of RAAS inhibitors and other candidate drugs in human mDA neurons and neuron-glia co-culture models • Develop a 3D midbrain neuron-glia assembloid models to evaluate the neuroprotective potential of candidate drugs in an 'in vivo like' environment • Engineer multiple hiPSC lines from different background with TH-reporter using CRISPR to discover therapeutics that will benefit all individuals • Develop a CRISPRi/a screening platform to identify new mDA neuron specific survival genes and pathways that can enhance neuroprotection. • Integrate PD patient derived, CRISPR edited and various background models to address the needs of a global population suffering from PD.
Statement of Benefit to California (as written by the applicant)	Parkinson's disease (PD) is the most common movement disorder and the second most common neurodegenerative disease, affecting 0.3% of the population—1% over 60 and 5% over 80 (de Lau & Breteler, 2006). In the U.S., the economic burden exceeds $51.9 billion annually (Yang et al., 2020). In California alone, over 116,000 people live with PD (CDPH, 2022). Despite its impact, no disease-modifying therapies exist beyond symptomatic treatments.
Funds Requested	$2,244,452
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: 84

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

Mean	82
Median	84
Standard Deviation	2
Highest	86
Lowest	80
Count	14

(85-100): Exceptional merit and warrants funding, if funds are available	1
(1-84): Not recommended for funding	13

Key Questions and Comments

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

Does the project hold the necessary significance and potential for impact?

- The project is aimed to repurpose existing drugs and to identify new drugs for neuroprotection in Parkinson's disease (PD). There are currently no effective disease-modifying therapeutics for PD available and the project therefore meets an unmet need. Stem cells are a necessary tool for these studies.
- The project has potentially important impact in understanding PD pathophysiology.
- The project addresses a critical unmet need in PD disease-modifying therapeutics.
- Yes, this project uses hiPSCs derived models with CRISPRa/i screens to identify neuroprotective pathways relevant to PD.
- The proposal includes a nice set of preliminary data. Additional expertise is brought in with collaborators with expertise in PD pathology.

Is the rationale sound?

- Strong preliminary data linking a signaling pathway to SNCA regulation and neuroprotection.
- The proposal is based in previous work in zebrafish performed by the co-PI that have uncovered a series of compounds that counter the loss of DA neurons by inhibiting proteins associated with the renin-angiotensin-aldosterone (RAAS) system. Independent studies using single-cell genomic profiling of human mDA neurons in PD patients have also shown that a subset of mDA neurons express the RAAS pathway receptor and thus supports findings in zebrafish.
- This proposal is significantly improved upon resubmission. The applicant has also added patient-derived and CRISPR edited hiPSCs, additional PD-relevant features to be assessed, and new preliminary data showing neuroprotective effects of inhibitors have improved the rationale.
- The preliminary data are strengthened with CRISPRi knockdown replicating the neuroprotective effect of the inhibitors, which gets at mechanism. It's also encouraging that neuroprotective effects were seen even when inhibitors were applied after neurodegeneration induction.

Is the project well planned and designed?

- A CRISPRi/a screen limited to druggable genome enhances focus.
- The project is hypothesis driven, well planned and designed. The model for neurodegeneration does not necessarily mimic degeneration in PD.
- Some concern was raised over the neurodegeneration model that is being used for this project.
- The scope has been focused based on new preliminary data. The rotenone model has been removed. Hits from the screen will be validated individually and tested with pharmacological validation. Animal model work has been removed.

Is the project feasible?

- The project has strong institutional support.
- Feasibility is reasonable for the proposed 2-aim structure and data load.
- The team is strong but the lead PI is relatively inexperienced with limited publications/activities as senior PI. The proposal is based on previous data and results from the lab where the applicant is now based, but not performed by applicant. The main applicant is not an expert in PD or DA neuron differentiation (core to the proposal). The project relies too much on collaborations for core expertise such as DA neuron differentiation and CRISPR based screens. There are limited preliminary data on screens.
- There is concern about the PI not having expertise in the area of the grant. The PI has done a great job of bringing others together that do have relevant expertise, but it is unclear whether the PI is bringing PI-level expertise. Otherwise, the improvement in the grant and the PI's success in bringing these folks together are impressive.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• The project directly addresses PD heterogeneity via hiPSC lines from diverse donors and PD mutations. • Yes. PD is mostly found in White populations. They will start in White British male line and move to ethnically diverse and female lines from CIRM hiPSC repository. • Proposed plans for outreach are primarily via the lab where the PI is based.

Application #	DISC0-18123
Title	Maternal/embryonic/fetal communication, from implantation through the first trimester: Development of physiologic placentation models from stem cells.
Project Objective (as written by the applicant)	We will better understand how communication between maternal endometrium and the embryo and trophoblast cells lead to successful human embryo implantation and placentation for a healthy pregnancy.
Impact (as written by the applicant)	We anticipate that successful completion of these studies will lead to models of human implantation and placentation to improve overall reproductive efficiency and successful healthy pregnancies.
Major Proposed Activities (as written by the applicant)	• We will identify and characterize secreted proteins and extracellular vesicles from embryos that alter the endometrium for implantation. • We will develop a physiologic human implantation model that can be used to study factors contributing to successful and unsuccessful implantation. • We will characterize how maternal decidua (uterine lining) and immune cells communicate to trophoblast stem cells for healthy placental development. • We will develop physiologic trophoblast models that reflect the dynamic changes in the first trimester of pregnancy.
Statement of Benefit to California (as written by the applicant)	California's birth rate is at its lowest level in more than 100 years. This is due to younger populations postponing family building and utilizing fertility preservation and older populations due to infertility. In California, fertility coverage will be become more readily available. As a result a better understanding of successful implantation to improve efficiency and placentation for a healthy pregnancy is imperative to improve our population growth.
Funds Requested	$2,304,886
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: 83

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

Mean	83
Median	83
Standard Deviation	3
Highest	90
Lowest	80
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	4
(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.

Does the project hold the necessary significance and potential for impact?
• At the core of successful pregnancies is the very challenging interaction of the maternal endometrium and the embryo during implantation and placentation. A miscommunication during this stage is likely to result in failed pregnancies and/or infertility, which represent an increasing public health problem. • The main focus of this application is to understand how (i) stem cells of the developing embryo communicate to the maternal endometrium to facilitate successful implantation how (ii) how does the communication from the maternal surface impact trophoblast stem cell development that then leads to successful placentation and a healthy pregnancy. • California has experienced a steep decline in birth rates. Factors include delay of pregnancy and a decline in fertility that is poorly understood. The model system established in this application will allow to address potential underlying causes. • This project is goal-oriented but will also provide foundational information for future hypothesis-driven experiments. The validation of in vitro models of trophoblast differentiation is necessary. • The proposal investigates a crucial aspect of human reproductive biology with a focus on the communication of extra-embryonic (trophoblast) cells and the endometrium. The two aims will collect large data sets covering secreted factors (EVs, proteins, RNA) and the tissue response.
Is the rationale sound?
• The proposal builds on exciting data from the applicant who identified embryo-secreted proteins and identified extracellular vesicles (EV) and secreted factors that are likely playing a role in preparing the endometrium for implantation and for the establishment of a healthy placenta. • In preliminary experiments the applicant identified secreted proteins from embryonic cells and EV composition. • Factors were tested for their ability to define changes in cell populations and cell specific differentially expressed genes of the endometrium. To achieve this the applicant collected human endometrium during the window of implantation which were exposed to either embryo conditioned or embryo free media and found significant changes in cell proportions of endometrium. • Following the initial signaling from the embryo, embryo derived trophoblast stem cells differentiate into defined progenitor cells which are critical for a healthy placenta and can thus be used as a proxy for placentation. The applicant has collected human trophoblast stem cells following collection of chorionic villi which they differentiated into placenta-relevant progenitor cells. These studies show differences in the progenitor population depending on the sex and age of the placenta. • The rationale is sound; the model is a reasonable reductionist approach. • The majority of studies focus on the maternal (endometrium) or the embryo compartments individually, and many use mouse models and lack translatability studies. • The assumption is made that all cross-talk occurs via EVs - other sources of cross talk are not adequately discussed. • It is a goal-oriented project. • More considerations for alternative mechanisms would have been desirable.
Is the project well planned and designed?
• The first aim they will ask the question whether proteins and EV secreted by different embryonic cells alter endometrial and immune cell populations for implantation. The second aim will test whether early placental development and placentation depends on maternal signaling from the maternal decidua and immune cells that are unique at different time points. The aims are logical and approaches are appropriate and well-described. • Aim 1 is described in great experimental detail and hence seems well thought through on the sample handling and processing side. • Access to resources and recruitment of pregnant women to harvest the tissues is described.

- During Aim 1, endometrial biopsies will be collected from individuals undergoing mock embryo transfers. Without knowing the specific stage of the endometrium that the PI wants to study, it is unclear how representative this tissue will be of the endometrium of a naturally cycling woman.
- The proposal lacks some details of the experimental design, which should be improved. Specifically, the stage of development of cultured embryos from which the conditioned media is used is not described.
- Culture conditions could have been better defined. Why do the applicants not use a defined medium?
- Aim 2 is comparatively short (only one page). A lot of data are collected and while the basic processing is well described it is less clear what and how meaningful biology will be extracted. Lots of data packages are mentioned, but running them is only the beginning of the analysis.
- During Aim 2, the PI will use placenta/decidua from pregnancies that are being terminated. Will this impact the interpretation of the results? Is there an exclusion criterion for these samples?
- Pitfalls are not very informative as they do not discuss unanticipated outcomes.

Is the project feasible?

- The PI serves as director of the biorepository which has enrolled over 3500 patients with over 118,0000 samples including blood samples from individuals undergoing fertility treatment, maternal and paternal blood samples, cord blood (birth), endometrium, embryo conditioned media, chorionic villus samples, maternal decidua, first, second and third trimester placenta are stored in dedicated -80°C freezers. This is a unique resource that will aid in the feasibility of the project.
- Yes, the resources at the applicant institution are excellent, and the access to human material is a strong positive.
- Preliminary data support feasibility.
- No pitfalls or alternative approaches are listed.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- The proposed studies incorporate all backgrounds, different maternal age groups, gestational ages of trophoblast cells, fetal sex, and the sex of the trophoblast.
- Background and maternal age will be considered.

Application #	DISC0-17822
Title	Role of stem-like T cells in autoimmune diseases
Project Objective (as written by the applicant)	We have evidence that T cells with stem cell features (Tsc) are responsible for autoimmune diseases. We will identify molecules that allow Tsc to thrive, providing targets to improve patient outcomes
Impact (as written by the applicant)	Treatments for autoimmune diseases generally do not provide cures. They often focus on the pathogenic cells, which might be short-lived, rather than the cellular source of the disease causing cells.
Major Proposed Activities (as written by the applicant)	• Analyze tissues from patients with ulcerative colitis, Crohn's disease, rheumatoid arthritis, and psoriasis for stem-like T cells. • Verify the expression of key proteins specific for disease-causing stem-like T cells (Tsc) in these autoimmune diseases. • Determine if the response to treatment is correlated with a change or decrease in Tsc. • Analyze the cells neighboring Tsc in tissues to identify the signals that allow Tsc to self-renew and give rise to inflammatory cells. • With a model system and gene deletion, validate target molecules that disease-causing Tsc require, which could provide novel treatments.
Statement of Benefit to California (as written by the applicant)	There are more than 80 autoimmune diseases in which the immune system attacks the body. These debilitating diseases can affect every organ, and their incidence is increasing. In California, nearly 5% of the population suffers from an autoimmune disorder, with a great health care cost. Treatments for autoimmune diseases often fail and do not provide cures. Our research provides a new approach to understanding cells that cause autoimmune disease, which could lead to new treatments.
Funds Requested	$4,603,793
GWG Recommendation	**(1-84): Not recommended for funding**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

Scoring Data

Final Score: 83

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

Mean	82
Median	83
Standard Deviation	4
Highest	85
Lowest	75
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	7*
(1-84): Not recommended for funding	8

* See Minority Report below

Key Questions and Comments

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

Does the project hold the necessary significance and potential for impact?
• The objective of this proposal is to determine the prevalence of stem-like T cells fueling ulcerative colitis (UC), Crohn's disease (CD), rheumatoid arthritis (RA), and psoriasis, which is significant. However, UC and CD are not classical autoimmune diseases but rather auto-inflammatory disorders with complex interplay of causal factors from epithelial, innate, and adaptive immune cells. This potentially confounds the T cell objectives of this proposal since IBD is not clearly initiated by T cells like classic autoimmune diseases. • The key goal of the project is to identify new therapeutic targets for autoimmune diseases, mainly RA, UC, CD and psoriasis, driven by T cells, by means of identifying factors that sustain T cells with stem/progenitor cell properties that are presumed to give rise to effector T cells that sustain autoimmunity. Current therapies do not generally provide long-lasting benefits and are targeted against factors that target short-lived effector T cells. • Co-investigators provide complementary expertise in computational analyses and clinical aspects of autoimmune diseases. • The team has track record for collaborating in IBD field.
Is the rationale sound?
• The concept of a stem-like T cell pool fueling disease in UC, CD, RA, and psoriasis is based on observations in chronic infection and cancer, which also results in chronic stimulation and an exhausted (rather than classical memory) lineage of CD8 T cells. The idea of a stem/resource pool in autoimmunity is less well defined but proposed to enable persisting responses of T cells recognizing auto-antigen. • The only preliminary data provided in support of a functional impact are in Fig 4 (CD4 T cell transfer colitis), are quite modest, and do not consider other potential effects of deleting Tcf7 from T cells. The CRISPR experiments in Aim 2 will go beyond profiling to functionally interrogate these cells, but assessing 100-200 targets is diffuse and use of the T cell transfer colitis model may limit interpretability based on Fig 4 data. • The premise that T cells with stem cell properties that give rise to effector T cells may exist and play important role in autoimmune diseases is reasonable, albeit supported by rather limited data. The group has performed previous studies supporting this rationale. • Yes. For UC, the rationale to investigate prevalence and biology of human TSC from patients is based on (i) this group's findings suggesting that loss of these cells protects in mouse models of intestinal inflammation and (ii) their higher prevalence in inflamed tissue from patients. • The rationale to expand beyond UC to CD, RA, and psoriasis is less supported. Data are included showing cells are present in CD and RA patients, however, these data lack comparison to samples from healthy controls. • Aim 2 is higher risk, as there are limited data on the appropriate direction for functional studies.
Is the project well planned and designed?
• Aim 1 is well designed. There is concern regarding readiness to perform the functional screen in Aim 2. There are no preliminary studies to establish feasibility and this Aim is rather open-ended. • There is discussion of pitfalls and alternatives, however some pitfalls of the screening approach, such as relevance to human T cells and potential technical problems, may be somewhat understated. • Yes, logical rationale and technical approaches are proposed. • The project employs cutting edge analytics to help identify regulatory targets in TSCs and how these cells communicate with others in the tissue. • Healthy patients do not seem to be included for comparison of TSC in different cohorts, this will likely be important. • It's unclear how the applicant will assess whether TSCs are driving disease with these approaches, particularly in relation to the new diseases they are expanding into. While all may be categorized broadly as autoimmune, pathogenesis and mechanisms likely vary significantly.

• The functional analyses for the targets identified in RA and psoriases are unclear. • Partially; however, there are conceptual concerns and lack of a specific hypotheses for the CRISPR work as described above. • Additional discussion of models beyond CD4 T cell transfer colitis is needed. • Issues with Aim 2.
Is the project feasible?
• The patient tissues and technologies are available. • This is an ambitious proposal (300 patient samples, and extensive -omics in both aims). The scope proposed may be difficult, however, this team does have the appropriate expertise and track record, mostly in relation to UC. • The budget is appropriate, the projected timeline for Aims is not clear.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• There are plans to extend studies to Latino populations. • There are outreach and education activities proposed.

Minority Report

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

Scoring was within a tight range (75 to 85), with all reviewers in agreement that the assembled team and resources for the project were strengths of the proposal, and all in agreement that the project plan has limitations. Supportive reviewers additionally commented on the project novelty, translational potential, team's track record in the field, two key preliminary findings supporting the rationale, and cutting-edge analytics.

Application #	DISC0-17737
Title	Multi-Ome Profiling in Neurodevelopmental Patient iNeurons to Identify Therapy-Responsive Biomarkers
Project Objective (as written by the applicant)	Using patient derived neurons, we will perform a comprehensive biomarker assessment using cutting-edge technologies. Results will be applied to advance knowledge and advance novel therapies.
Impact (as written by the applicant)	Neurodevelopmental diseases in include autism, epilepsy and intellectual disability, impacting >4% of Californians. Our effect will lead to new mechanistic understanding of these conditions and new treatments.
Major Proposed Activities (as written by the applicant)	• Validate patient derived and control IPSCs for genotype and pluripotency • Generate iNeurons from each of 3 patient and control lines for each of 6 condition. • Generate multi-ome datasets from each patient and control cell lines • Assemble gene regulatory networks, reveal mechanisms of disease, and identify biomarkers of each gene mutation • Confirm altered biomarkers in separate set of iNeurons, and confirm normalization of biomarkers in iNeurons after ASO treatment. • Confirm biomarker signatures are reproduced directly in patient tissues and fluids.
Statement of Benefit to California (as written by the applicant)	Approximately 4% of children in California suffer from neurodevelopmental diseases, and almost none have targeted treatments available. We are partnered with California-based n-Lorem Foundation that is developing personalized medicine for these California-based patients, that will be benchmarked here. Patients were prioritized using PI considerations, so a greater swath of the population can benefit. Moreover these results and these drugs could be commercialized to support the economy.
Funds Requested	$2,349,271
GWG Recommendation	**(1-84): Not recommended for funding**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

Scoring Data

Final Score: 83

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

Mean	82
Median	83
Standard Deviation	2
Highest	86
Lowest	80
Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	1
(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.

Does the project hold the necessary significance and potential for impact?
• This project holds high promise to solve a key knowledge gap in the impact of gain of function patient mutations in multiple NDD genes by studying their effect in multimodal datasets generated from iNeurons. They will additionally test whether ASO will correct these cellular phenotypes and validate their biomarker findings using fluid samples collected from patients. • With many NDD genes potentially suitable for ASO treatment, and clinical trials underway, biomarkers to track efficacy are sorely needed. Towards this, multi-omic analysis of patient neurons with and without ASO, will identify systems level changes that accompany correction. That being said, whether differences in cultured neurons can uncover in vivo biomarkers is unclear. An improved design might also incorporate animal models of NDD. • Use of stem cell models to identify biomarkers for neurodevelopmental disorders. Biomarkers important for rare NDD to understand patient response to therapies such as ASO. Not clear how applicable this in-depth approach will be across the board in rare diseases-substantial amount of work for these six diseases. • PI to collaborate with co-I for computational multi-omic analysis. • Designed to prioritize ASOs already in clinical trials (n=1) by n-Lorem. Unclear if patient iNeurons are to match patients in n-lorem ASO trial, which would be a remarkable design.
Is the rationale sound?
• The project is based on a compelling scientific rationale, mainly that risk NDD genes have been identified, but underlying mechanisms are still unclear. To address this, they will use multiple patient iPSC lines harboring mutations in each of 6 genes, generate multimodal data to profile iNeurons derived from the lines and matched controls, integrate the data, and finally try to rescue the phenotypes using ASOs, and validate the identified biomarkers in patient samples. • PI showed that a gene-associated NDD is a lipid disorder treatable with a dietary lipid in a recent publication. • Many GOF mutations do not alter transcriptome markedly-how to screen for efficacy of ASO is unclear. Clinical assessment of response to ASO intervention is often not straightforward.
Is the project well planned and designed?
• The project is reasonably planned and likely to generate useful data. However, given the variability and heterogeneity in patient clinical phenotypes, even ones carrying the same mutation, they may not be sufficiently powered to detect strong molecular signatures. • Aim 1. Patient iPSCs from genetically distinct single gene NDDs will undergo reprogramming to iNeurons and harvested for multi-omic analysis. Unclear if design is proposing technical replicates, biological replicates (different differentiations or hiPSC clones), or, most ideal, 3 different patient iPSCs for each condition. Likewise, it is unclear if controls are isogenic CRISPR corrected. • Aim 2. Integrate multi-omic profiles to define disease-associated regulatory networks. This aim is entirely dependent on success of aim 1. Unclear power to resolve effects. • Aim 3. Evaluate the effects of ASO therapy on disease-associated multi-omic signatures. Test in isogenic H9 neurons and patient biofluids-do patients match genotype of hiPSC neurons? • No discussion of impact of differences in cell type composition, which is highly likely for neurodevelopmental disorder knockouts. • It is possible that alterations in omics profile in iNeuron may not reflect changes in critical target tissue in the patient. Metabolic alterations in cultured iNeurons may not reflect changes in vivo. It is possible that genetic background might affect omics readouts in the aim 3 validation. Study identifies networks affected by mutation but how to choose a single biomarker on that basis-analysis across network may not be practical. • Successful preliminary work focused on metabolic disorders which are constitutive and more easily modeled with any cell type. Targets for this proposal may have cell type specific effects not captured in the iNeuron model.

- Pitfalls focused on variation between replicates without considering variation between patient-specific mutations and donor backgrounds. Brief discussion of sample outliers but not how they will be identified and accounted for.

Is the project feasible?

- Wet lab informatics and pediatric genetics expertise all well represented by strong PIs.
- The team has the demonstrated expertise, track record and leadership to carry out the different components of the proposal.
- Concerns about limited number of donors and replicates for what will essentially be n-of-1 analyses.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- Study is focused on disease genetics. Plans for educational outreach are described, as well as outreach to rare disease community.
- The team is prioritizing diversity in patient-derived iNeurons by identifying multi-omic biomarkers across diverse populations to enhance the precision of future targeted interventions, ensuring equitable access to the benefits of regenerative medicine.
- The team articulates that the project plan incorporates the influence of diverse genetic populations. However, specific information about this is missing from the proposal. For example, it is unclear how many male versus female lines will be studied, and how will sex as a biological variable be studied, what is the specific breakdown by genetic ancestry of the patient compared to the control lines.
- Insufficient genetic backgrounds tested to understand.
- Limited.

Application #	DISC0-17690
Title	A function-based screening platform for identifying implantation-competent embryos
Project Objective (as written by the applicant)	We are developing a foundation to extend female reproductive lifespan by building function-based IVF screens to identify the small proportion of embryos from older women that will lead to pregnancy.
Impact (as written by the applicant)	Current IVF protocols screen embryos based on genetic abnormalities and general morphology, not function. For women over 35, these screens yield only a 5-10% success rate for a full-term pregnancy.
Major Proposed Activities (as written by the applicant)	• Fill key knowledge gap on basis of impaired implantation/pregnancy for embryos from older women. • Extend findings from highly manipulable mouse studies to human embryos and IVF patients. • Improve our ability to link existing clinical Embryoscope data with pregnancy outcomes to form the basis of next-generation diagnostics. • Develop a new function-based pipeline for determining viable embryos from older women that could form the basis of next-generation diagnostics
Statement of Benefit to California (as written by the applicant)	By the time a woman reaches the age of 35, she only has a 5% chance of a successful pregnancy. We aim to reduce the duration of IVF treatment for all women, reduce the incidence of failed embryo transfers, and lower rates of clinical miscarriage, which in turn would lower IVF costs. In California, the cost for a single IVF cycle can exceed $40,000. Our goal is to lessen both the burden related to age-related biological constraints and the economics of IVF procedures for all women in California.
Funds Requested	$3,675,887
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	4
Highest	85
Lowest	70
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.

Does the project hold the necessary significance and potential for impact?
• This proposal aims to develop a method for identifying viable pre-implantation human embryos, thereby increasing pregnancy rates. If successful, it will reduce the number of cycles required for women undergoing IVF. It will also help select embryos from aged mothers. It is an ambitious project. It proposes to develop in vitro assays to predict successful implantation based on the embryo's mechanics. • The overall goal is to develop prognostic tools to select for implantation-competent embryos and/or predict implantation success based on specific criteria. • Aim 4 is exciting. It is an analysis of already available human embryo images where outcomes as well as age are known (retrospective study). • Women over 35 have only a 5-10% success rate of full-term pregnancy and must often undergo multiple costly IVF cycles. Stated that this is an inequity as the success rate is higher for younger women. • The project addresses a major unmet need in IVF-screening for implantation competence beyond genetic normality. • This is a pioneering use of embryo mechanics to inform reproductive success and IVF outcomes. • The collaboration between *[redacted PI name]* (expert on mechanotransduction, advanced microscopy, embryonic development) and *[redacted PI name]* (human embryology, embryo imaging) is advantageous. All other key personnel listed are significant contributors to the success of this proposal.
Is the rationale sound?
• The proposal has a clear hypothesis that mechanical properties of embryos influence implantation. • The project represents a strong conceptual advance over morphology and PGT-A-based screening. • An issue with Aims 1 and 2 is that the preliminary data use the entire embryo for implantation assays. • There are significant assumptions that make this proposal somewhat risky. Major criticisms relate to the reliance on the success of aim 1 for executing aim 2, and the partial dependence of aim 4 on aim 3. • Preliminary data linking any of the assays proposed to pregnancy outcomes are lacking. The PIs are working on gathering such data. In Page 18, second paragraph, the applicants' mention: "We are currently extending this work to verify our ability to identify the small proportion of embryos from aged mothers that are competent to implant ... and lead to successful pregnancies ..."
Is the project well planned and designed?
• The project takes a thoughtful multi-pronged approach (invasive + non-invasive). • The project includes both *in vitro* and *in vivo* validation in mice and humans. • The proposal has a clear link to current IVF protocols and potential for clinical translatability. • Yes, the results will be meaningful, independently of their success in finding a non-invasive assay to identify implantation-competent embryos. • A proposal with only aims 3 and 4 could change the way embryos are chosen for IVF. • This reads as two separate grants. aims 1 and 2 are one project and aims 2 and 3 are a second project.
Is the project feasible?
• This proposal uses the team model to its advantage. • This is an accomplished PI and co-PI with complementary skill sets. • The team is well qualified to perform the studies proposed and resources at *[redacted host institution]* and *[redacted host institution]* are excellent.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• The applicant plans to examine embryoscope data from *[redacted hospital name]'s* IVF Clinic, which contains embryo data from a range of ages and genetic backgrounds.

- The project addresses biological age-related disparities directly.
- The project uses diverse human embryo data from national biobank.
- The outcomes will benefit the population of women who are older or have lower economic status. Access to embryos from women of different ages and ethnic backgrounds is considered.

Application #	DISC0-17756
Title	De Novo Epigenetic Programming of Human Regulatory T Cells
Project Objective (as written by the applicant)	We aim to develop a generalizable CRISPR-based epigenetic platform to reprogram human naive T cells into stable, functional regulatory T cells for use in adoptive regulatory T cell therapies.
Impact (as written by the applicant)	Our work will expand treatment access for patients lacking functional regulatory T cells, reduce risk of destabilized regulatory T cells, and reduce reliance on complex manufacturing pipelines.
Major Proposed Activities (as written by the applicant)	• Fine-map Treg differentially methylated regions (DMRs) of key regulators to prioritize functional subregions for epigenetic reprogramming. • Perform pooled CRISPRon demethylation of fine-mapped Treg DMRs, validate targets in an arrayed format, and assess FOXP3 stability over time. • Test combinations of fine-mapped Treg DMRs in an arrayed format to evaluate synergistic effects and monitor FOXP3 stability over time. • Profile transcriptomic, chromatin, and epigenetic features of epiTregs and perform phenotypic validation to define and compare cell states. • Evaluate epiTreg suppressive function using in vitro suppression assays. • Validate epiTreg suppressive function in vivo in a graft-versus-host disease (GvHD) model.
Statement of Benefit to California (as written by the applicant)	Our proposed research will benefit the State of California by creating "epiTregs," engineered immune cells capable of suppressing autoimmunity, inflammation, and transplant rejection. With millions affected by autoimmune diseases alone, there is a critical need for new treatment approaches. While regulatory T cell immunotherapies represent promising treatment strategies, current approaches are limited in efficacy and accessibility - challenges our proposed research seeks to overcome.
Funds Requested	$2,609,641
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	5
Highest	90
Lowest	70
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.

Does the project hold the necessary significance and potential for impact?
• The significance of using epigenetic reprogramming to convert conventional human T cells into functional Tregs (regulatory T cells) is high. Some of this has already been achieved by other groups (e.g., Wilk, et al 2022). • This proposal aims to generate a scalable approach to convert conventional T cells into regulatory T cells by targeting the methylated TSDR (Treg-specific demethylated regions) of FOXP3 with CRISPR activators (CRISPRon). • This is a single PI application from an investigator with expertise in high-throughput genetic manipulation of T cells. • Novel method for modified cell product generation with implications beyond Tregs. • Potential for generating genomically stable and intact cells from autologous donors. • Alternative methods require large blood draws or intensive in vitro stimulation protocols.
Is the rationale sound?
• Strong preliminary data on the CRISPRon/off approach are provided. • Epigenetic drivers of the Treg lineage, particularly methylation of the FOXP3 locus, is well defined in the literature. Therefore, there is a solid mechanistic foundation for the proposed studies. • There are numerous conditions in which autologous Treg therapy can be applied. • The actual application of the cells the therapy isn't discussed much— it is unclear what would be the initial indication. • Weaknesses: It is not clear if Tregs the best comparison for these transcriptionally and if a broader survey should be done. • Yes; however, the aims are somewhat interdependent. Use of CRISPRon with TET1 (ten-eleven translocation methylcytosine dioxygenase 1) demethylase to demethylate specific loci in CD4 Tconv (conventional T cells) to direct them to become Tregs is rational; however, the antigen specificity of the Tregs is insufficiently addressed.
Is the project well planned and designed?
• The project is designed with three aims, including refining the regions differentially methylated regions that should be targeted, either singly or multiplexed, characterizing the cell states of the edited cells, and functional validation, including in vivo validation in a model of GvHD. This is a logical flow of aims for this type of proposal. • The aims logically follow the development of the technology from in vitro to in vivo validation. • The iterative inclusion of targets from Perturb-seq is a power tool for identifying relevant DMRs and regulatory regions. • Several pitfalls are identified that include the artificial nature of human xenogeneic GvHD assays and have proposed syngeneic models to complement their studies. Additionally, the edits proposed may not be enough to generate stable regulatory programs - up to 33 edits could be used. • Avoiding double-stranded DNA breaks from traditional CRISPR editing is an advantage, and the proposed fine mapping of Treg-associated DMRs has strong potential to yield new insights to aid in T cell engineering. The in vitro and in vivo (xeno) suppression assays are well described; however, using only 2-4 human donors for the NSG GvHD (graft-versus-host disease) models is low for capturing human-to-human variability. • Weaknesses: Some more detail for the methods in the *[redacted paper]* would have been helpful since this is where some of the novelty lies, in terms of manipulating primary cells. • The comparisons in Aim 3 are unclear, in different parts of the aim, different comparisons are highlighted.
Is the project feasible?
• The TCR specificity of the Tregs is not at all addressed.

• The resources at the host institution has been sufficient to complete prior, related studies, and are sufficient here. • The budget is within DISC0 limits.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• Yes, patients whose Tregs cannot be isolated/expanded can still benefit from this reprogramming effort. • The plan has included usage of donors with variable sex, age, and ethnicity to mirror California census demographics. • This platform approach could broadly be used to target several inflammation-driven diseases. • The main activities in this area include engagement with other institutions and participation in summer internship programs.

Application #	DISC0-17973
Title	Leveraging Area Specific Cortical Organoid Models to Understand Neurodevelopment Disorders
Project Objective (as written by the applicant)	We will uncover how genes and brain connections guide the formation of specific brain regions using stem cells, advancing models to study and eventually treat brain development disorders.
Impact (as written by the applicant)	If successful, this work will overcome the bottleneck of creating brain region-specific organoids, enabling better models for neurodevelopmental disorders like autism and schizophrenia.
Major Proposed Activities (as written by the applicant)	• Perform CRISPRa screens in cortical organoids to identify transcription factors that drive brain area-specific cell identities. • Use thalamocortical fusion organoids to study how external brain inputs shape regional identity during human brain development. • Validate key gene targets and signaling mechanisms through single-cell multiomic analyses and quantitative imaging approaches.
Statement of Benefit to California (as written by the applicant)	This research will advance California's leadership in stem cell innovation by developing next-generation models of human brain development. These models will accelerate discoveries into the causes of neurodevelopmental disorders like autism and schizophrenia, which affect many Californians. By enabling earlier diagnosis and therapeutic development, this work has the potential to improve health outcomes and reduce long-term care costs across the state.
Funds Requested	$2,342,488
GWG Recommendation	**(1-84): Not recommended for funding**
Process Vote	All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG." Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."

Scoring Data

Final Score: 80

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

Mean	80
Median	80
Standard Deviation	4
Highest	91
Lowest	75
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	2
(1-84): Not recommended for funding	12

Key Questions and Comments

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

Does the project hold the necessary significance and potential for impact?

- My prediction is that many labs would be able to use the advances proposed by this research, and it will advance the field in a meaningful way - not just incremental progress but a significant leap forward.
- Well-revised proposal from an outstanding team, but flaws in premise reduce enthusiasm for impact.
- There are no concerns about the ability of the team to complete the work proposed.
- This can also be generalized for other organoid systems that are similarly affected by geographically influence differentiation that is not possible to now recapitulate.
- Agree with a previous review that this work is unlikely to be funded through traditional mechanisms and requires an investment of scale beyond usual pilot funding. This makes it a good fit for this funding mechanism.
- Yes; a key gap is that we don't know the transcription factors (TFs) that drive arealization in stem cell-derived organoids. Identifying these TFs (and/or thalamic input) would be helpful in making more brain-like organoids.
- Yes - it is a significant gap in our technical and scientific knowledge that area-specific cortical organoids are not reproducible for directed differentiation. This limits the ability to test specific populations of cells and their connections to other parts of the brain.

Is the rationale sound?

- Yes - this is an innovative but feasible way to address this difficult knowledge gap.
- Most studies of TCA function have naturally been carried out in rodent models, as they are the most accessible for the manipulation of thalamic projection formation. However, characterizations of TCAs have shown that in primates, including humans, TCAs form synaptic connections to cells in the subplate (62,63). The subplate is an expanded, transient, pre-plate structure in primates and humans that is not fully characterized but is thought to give rise to layer VIb of the cortical plate.
- The implication seems to be that rodents do not have thalamic innervation of the cortical subplate, which is inaccurate. In addition, the subplate is well established to critically influence cortical arealization in rodents. While this does not suggest a lack of importance for studying arealization in humans, but does suggest a disturbing lack of appreciation for the overall highly remarkable conservation of arealization across mammalian species.
- Among the candidate transcription factors (TFs), it is unclear whether any exhibit prefrontal cortex (PFC)-selective expression. In a related note, Aim 1 is based on the hypothesis that there exist key pioneer and maintenance TFs that generate and perpetuate terminal areal cell fate. Such TFs have been extensively searched for in rodents but have not been identified. Given the remarkable overall conservation of cortical arealization across mammals, it is unclear why humans would differ significantly in this regard. While there are TFs required to establish and maintain fate, but at least in rodents those seem not to function in an area-exclusive manner, but to work within a multifactorial system based on intrinsic and extrinsic factors. That said, there is very high enthusiasm for attempting to study the influences of thalamic-like projections into neocortical-like organoids on gene expression related to arealization.

Is the project well planned and designed?

- Yes - this reviewer appreciates the areas where redundancy have been built in and orthogonal methods have been proposed (especially in this revised form).
- It is reasonable to use a more concise transcription factor (TF) library. This proposal does not read as being a discovery project for new TF that are involved in specific cortical development, but more of a categorization of those ones that have already been identified.
- The small library size (and lack of combinatorial TF considerations) raises doubts as to whether arealization will be achieved.
- The prior application had several critiques (CRISPRa method, small library size, ORF alternative) that were not addressed in this resubmission. Although these are somewhat mitigated by the successful screen performed for PFC, it would've been better to address these critiques with data.
- The chimeroid-based delivery system is an interesting addition. Although more detailed data comparisons would have been preferable, what is presented is sufficient to lower the initial concerns.

• Overall, the project is well-planned. However, key aspects of the phenotypic screen such as the PFC/V1 similarity score should be explained in detail and are missing.
Is the project feasible?
• No concerns.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• Yes - no concerns.

Application #	DISC0-17580
Title	Developing a scalable platform for ex vivo manufacturing of universal red blood cells
Project Objective (as written by the applicant)	Our objective is to uncover pathways enabling high-density erythropoiesis and to engineer stem cells for scalable, antigen-optimized RBC production with minimal reliance on costly external factors.
Impact (as written by the applicant)	This work addresses major bottlenecks in ex vivo RBC manufacturing, including high cost, low yield, and poor antigen compatibility, with the goal of enabling scalable, equitable transfusion solutions.
Major Proposed Activities (as written by the applicant)	• Engineer HSPCs with synthetic cytokine pathways to eliminate reliance on exogenous factors for RBC maturation. • Evaluate and optimize stem/progenitor cell sources for high-yield, scalable RBC production in bioreactors. • Adapt and validate stirred-tank bioprocesses for efficient, high-density erythroid culture at scale. • Perform genome-wide CRISPRa/i and base editing screens to identify regulators of erythropoiesis and enucleation. • Knock out RBC surface antigens to generate functional rare and universal donor cells for transfusion compatibility. • Benchmark engineered RBCs for yield, function, and antigen expression; share validated tools with research community.
Statement of Benefit to California (as written by the applicant)	This research will enable scalable, cost-effective manufacturing of red blood cells, reducing reliance on donor blood and addressing shortages that disproportionately affect California's diverse populations. By developing universal and antigen-matched RBC products, this work will improve transfusion safety and equity, support innovation in regenerative medicine, and create new tools and technologies with statewide clinical and economic impact.
Funds Requested	$4,787,600
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	86
Lowest	80
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	1

(1-84): Not recommended for funding	13

Key Questions and Comments

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

Does the project hold the necessary significance and potential for impact?

- Yes. There is a clear need for blood for transfusions, especially in underserved communities. Successful completion of this project could enable ex vivo production of RBCs. Aims 1 and 2 will provide useful and actionable information, while Aims 3 and 4 are more exploratory and less well supported by preliminary data.
- Aim 1 is feasible and likely to be achieved by this talented team. Aims 2 and 3 may be far from any potential translation. Aim 3 may be closer to being achievable.
- The strengths of the project are Aims 1 and 2. However, for Aim 2, insufficient data are presented to demonstrate that manufacturing can be accomplished at scale in a cost-effective way. Although Aims 3 and 4 are interesting, they appear overly ambitious and raise significant safety issues that have not been sufficiently addressed.
- The team includes strong clinical and research scientists with access to the necessary patient cells, as well as expertise in genome editing, GMP operations, and hematopathology.
- This is an ambitious, high-risk, high-reward project with multiple potential challenges. The investigator is an expert in gene editing, and the goal of producing RBCs ex vivo in sufficient numbers to be clinically impactful is potentially significant. However, much more work may be required for the approach to become feasible and cost effective.
- The team is very strong overall.

Is the rationale sound?

- Aims 1 and 2 are strongly supported with preliminary data and appropriate. Aim 3 and 4 seem like they could be independent grants and lack preliminary data. Aim 4 is the least supported and could likely be removed and still be a lofty proposal.
- Aim 4 has a lot of potential limitations: 1) multiplexing gene knockout as proposed will result in deletions, inversions, and large chromosomal aberrations even with one or two edits. Sequential editing of primary HSCs will also prove toxic and may alter cell viability, expression of gene, and ultimately usability of the RBCs they are trying to generate. This could be alleviated with base editing strategies.
- Yes, but these are feasibility issues. Unknown cost and risk of multiple edits that may be necessary. Ultimately some DNA or intact cells with nuclei with multiple edits may likely exist after manufacturing. Release testing may be difficult, costly and challenging.

Is the project well planned and designed?

- Aims 3 and 4 make the project too ambitious for this mechanism. Focus on Aims 1-2 would be more appropriate.
- Aim 1 and 2 are planned well and likely to succeed. Aim 1 could be a grant on its own. I'm very enthusiastic about 1 and 2 but 3 and 4 are too ambitious to include in this one grant.
- Densely written very ambitious project which has many aspirational aspects but numerous feasibility challenges.
- Some significant progress has been made in Aim 1 which has well defined, feasible with good preliminary data.
- Aim 2 is very challenging to do in a clinically relevant scaled up version. The suggestion that manufacturing advances have been achieved by the PI are not clearly defined in the application, data supporting this would have strengthened the application.
- Aim 3 is likewise challenging.
- If Aim 2 and 3 fail then Aim 4 becomes less relevant, although with significant translational impact.
- Aim 4 is potentially impactful but bringing each edit to the clinic represents significant challenges. Effect on rheology, thrombosis, sickling, survival, oxygen delivery etc. remain and each will need to be tested and measured.
- Pitfalls were not well discussed.

Is the project feasible?
• If the project was only Aims 1 and 2, it would have been feasible. • The project seems overly ambitious for the time period. Aim 4 is particularly ambitious. I would prefer to see some in vivo work following up on Aim 1 and 2. • The project would be more feasible without Aim 3 and 4. • No issues with budget. May take longer than expected.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• Plan incorporates antigen prioritization based on regional transfusion needs. • Community outreach is a bit weak. They say they will implement a strategy that includes community engagement, but the only real engagement seems to be through interactions with clinicians and researchers in the community.

Application #	DISC0-17322
Title	Profiling the molecular landscape of the injured newborn brain may inform how neural stem cells preserve & may recreate neural circuitry
Project Objective (as written by the applicant)	Better understand how neural stem cells rescue damaged brain in babies following perinatal asphyxia so that their actions can be improved, including repairing regions & nerves that can't be rescued.
Impact (as written by the applicant)	To enable neural stem cells to be more than just neuroprotective but also render them capable of replacing lost neural circuits, a goal heretofore not well-achieved in the brain using stem cells.
Major Proposed Activities (as written by the applicant)	• Genetic characterization of the hNSCs prior to & following engraftment into the penumbra, correlated with genetic changes in the penumbra. • Characterization of the proteins made by single cells in the injured brain with & without neural stem cell transplantation. • Characterize neuronal fibers within & passing through the penumbra & determine if they are being rescued & are functional. • Determine whether putting "developmental genes" into the grafted neural stem cells may allow them to replace neural circuits that couldn't be rescued. • Correlate penumbra's molecular & cellular profile & integrity of rescued & new neuronal fibers with degree of behavioral improvement.
Statement of Benefit to California (as written by the applicant)	Perinatal asphyxia, the lead cause of pediatric neurologic disability, occurs in 2/1000 births in CA. The stated cost-of-care of such a child ($48,000/yr) is actually 2-5x greater because of indirect costs to the family (lost wages, out-of-pocket expenses, ER visits, disruption of family psychodynamics, etc.) It accounts for 11% of deaths in kids <5 y/o & 22% in the 1st month-of-life. Standard-of-care is inadequate. Treating with neuroprotective neural stem cells could improve lives & finances.
Funds Requested	$4,586,156
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	78
Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	13

Key Questions and Comments

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

Does the project hold the necessary significance and potential for impact?
• The objectives of this proposal are to address the gaps in knowledge relating to the mechanisms of action of stem cell mediated benefit in the treatment of perinatal hypoxic-ischemic brain injury, to provide insight into the extent of damage of connectivity altered by hNSC transplant and to assess whether neuronal specification ex vivo can yield a more efficacious cell replacement strategy. • Yes, with important caveats. Perinatal hypoxic-ischemic encephalopathy (HIE) affects ~10,000 U.S. babies annually (1-3/1000) and ten times more in low- and middle-income countries, representing a major unmet medical need. Current standard-of-care therapeutic hypothermia is only partially effective, limited by narrow treatment windows (<6hrs), and ineffective in severe cases. • This preclinical study proposes to understand the molecular mechanisms by which human neural stem cells (hNSCs) might augment the brain's endogenous repair system in a rat model of HIE. • The team has held pre-IND meetings and been encouraged to proceed toward clinical trials, indicating regulatory feasibility. • Critical knowledge gap addressed: The project investigates whether neonatal brain injury triggers "fetal reversion"—reactivation of developmental repair programs—and whether hNSC transplantation can extend this natural repair window. This mechanism-of-action understanding is essential for optimizing cell therapy approaches. • Limitations: The "fetal reversion" hypothesis represents a significant mechanistic leap requiring validation. The observed molecular signatures may reflect injury stress responses rather than authentic developmental reversion. • Yes, strong synergistic potential. The core team of two investigators offers complementary expertise: The PI brings neuroprotection and clinical HIE expertise, while the Co-I contributes circuit reconstruction knowledge and transcriptional programming approaches. • The interdisciplinary combination of molecular characterization (Aim 1), circuit analysis (Aim 2), genetic enhancement (Aim 3), and behavioral validation (Aim 4) creates a comprehensive research framework that neither investigator could achieve independently. The Co-Is transcriptional code approach provides a novel strategy for enhancing hNSC therapeutic potential beyond neuroprotection to actual neural replacement.
Is the rationale sound?
• The need for improved therapeutics in perinatal hypoxic-ischemic brain injury is clear. The current standard of care involving therapeutic hypothermia is insufficient in the majority of cases to elicit a meaningful benefit and cannot treat severe cases. • There were some concerns with the presentation and rigor of the snRNAseq data and interpretation of brain volume data (Fig 5). The representative image for HI+Tr shows clear swelling of the lateral ventricle and cortical thinning which would seem to contradict the assertion that 100% of brain volume was preserved in these rats. Several figures lacked any appropriate quantification which was also a concern. • Partially sound but built on significant assumptions. The central "fetal reversion" hypothesis is based on rapid appearance of specific gene expressing cells and protein upregulation after injury. The applicants argue these represent fetal cerebrogenic astrocytes activated by hypoxic-ischemic injury. • Mechanistic innovation: This challenges classical niche hypotheses by suggesting endogenous "replacement" cells already exist in situ. The proposed mechanism suggests hNSCs work through instructive/paracrine actions—"coaching" the injured brain to maintain its natural repair state longer. • Testable predictions: (1) Timing matters—hNSCs should be most effective during the natural fetal reversion window; (2) Location specificity—hNSCs should preferentially engraft where fetal signatures are strongest; (3) Phenotype adoption—transplanted hNSCs should become fetal astrocytes. • Strengths of the proposal are based on the established Rice-Vannucci model of HIE. The novel MRI algorithm enables real-time distinction between penumbra and core, which is an important technical advancement. There is also biological plausibility because of the mammalian brain's inherent injury response mediated by endogenous NSCs. NSCs delivered ICV may home to injured tissue. Would these MRIs be used to select for transplantation?

- There is biological plausibility due to the mammalian brain's intrinsic injury response via endogenous NSCs and strong preliminary data of penumbra rescue and synergy with hypothermia. If ICV delivery works it is compatible with NICU care.
- The MRI algorithm allows real-time mapping of the penumbra vs. core to guide treatment and is plausible. Although it is not clear how many human HIE show a well-defined penumbra.

Is the project well planned and designed?

- It is not very clear what will be quantified in Aim 2. What is the extent of disruption of any of these metrics induced by HIE? The application lacks quantitative preliminary data providing such evidence. Thus it is unclear whether this aim can be completed and if it will yield meaningful results.
- It is unclear if this code to induce neuronal fate has been validated in vitro and what degree of heterogeneity of expression is present after this procedure, i.e. what proportion of neurons express all of the factors? What proportion express other non-induced genes indicative of fate conversion? Do these neurons display hallmark morphological or electrophysiological characteristics that is consistent with fate conversion? Aim 3 appears to be underdeveloped and will be very high risk.
- Aim 1 strengths: Spatial proteomics preserves tissue architecture (major advantage over traditional scRNAseq). Imaging guidance allows precise molecular characterization. Multi-timepoint analysis captures dynamic processes. Multi-timepoint analysis captures dynamic processes; includes fetal brain comparisons for validation. Will create valuable reference atlases.
- Aim 1 critical limitations: There is no systematic comparison to actual fetal brain tissue posing a fundamental validation gap. Methodological concerns include fixation artifacts and limited antibody panels (~40-60 proteins). Punch biopsies may miss regional heterogeneity an introduce sampling bias. Molecular signatures may reflect injury response rather than functional reversion posing interpretation challenges.
- Aim 2 feasibility concerns: DTT sensitivity limitations in immature myelination and injection precision challenges for consistent BDA placement. Focus on transcallosal fibers may miss other critical pathways limiting the scope.
- Aim 3 represents the highest-risk component with transcriptional codes derived from adult stroke models applied to neonatal "fetal reversion" environments.
- Aim 4 has appropriate statistical power with proper controls.
- The Aim 3 transcriptional codes approach appears disconnected from Aims 1-2 and could represent a separate project entirely.
- Insufficient consideration of alternatives. The proposal shows over-reliance on the single fetal reversion framework with limited exploration of alternative mechanisms if this hypothesis proves incorrect. Risk mitigation strategies are needed, particularly for the high-risk Aim 3.

Is the project feasible?

- The two leads are accomplished investigators who have appropriate experience and expertise to lead these activities.
- The environment at the home institution is excellent with appropriate core facilities available to the investigators.
- Yes, feasibility appears adequate. Institutional affiliations provide access to specialized equipment. Experienced team members with relevant technical expertise.
- The timeline is compressed for scope. While sequential organization helps, four major aims with complex multi-omics, extensive behavioral testing, and novel therapeutic approaches represent an ambitious 3-year timeline. Aim 3 particularly represents a major technological leap that may require separate optimization.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- HIE is the most common neurological disability in US children.

  The applicant reports a 10-fold more prevalence in low-and-middle income countries.
- Yes, partially addressed. The project includes plans for a diverse patient population and comprehensive outreach planning. However, more detailed consideration of how genetic and environmental factors might influence the fetal reversion response would strengthen the approach.
- Unclear path to broader impact. The allogeneic transplantation approach may have limited applicability in resource-limited settings where HIE burden is highest. The requirement for sophisticated imaging and ICV delivery may limit broader applicability, particularly in low or middle income countries.

- Adequately described for preclinical stage. Community Advisory Board, committee participation, training programs, and budget allocation for outreach activities are outlined. Appropriate level of community engagement for discovery-stage research.

Application #	DISC0-17391
Title	A Multimodal Atlas of Redox Signaling and Organelle Dysfunction in Stem Cell Models of Neurodegeneration and Aging
Project Objective (as written by the applicant)	To create a multimodal atlas and deep learning models linking redox signaling, oxidative damage, and organelle dysfunction in stem cell–derived neurons for disease modeling.
Impact (as written by the applicant)	This project will address key bottlenecks in modeling neurodegeneration by resolving how redox stress and organelle dysfunction drive disease in stem cell–derived neurons.
Major Proposed Activities (as written by the applicant)	• Engineer and deploy genetically encoded redox biosensors in stem cell–derived neurons. • Map oxidative damage to RNA, proteins, and lipids using mass spectrometry and sequencing. • Capture live-cell imaging of organelle dynamics using label-free microscopy and fluorescence. • Develop and apply deep learning models to quantify and predict redox-linked phenotypes. • Compare redox and organelle phenotypes across iPSC-derived and directly reprogrammed neurons. • Disseminate all datasets, tools, and models via open-access platforms with detailed documentation.
Statement of Benefit to California (as written by the applicant)	This research will strengthen California's leadership in regenerative medicine by developing tools to better model and understand neurodegenerative diseases. It will improve stem cell–based models, enable redox-targeted therapeutic discovery, and support progress toward treatments for conditions affecting the state's aging population. All tools and data will be shared openly to drive innovation across California's academic, clinical, and biotech sectors.
Funds Requested	$4,354,071
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	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.

Does the project hold the necessary significance and potential for impact?
• Proposal addresses fundamental gap in understanding redox biology and oxidative damage in iPSC-Ns (iPSC-derived neurons) vs. iN (directly reprogrammed neurons). • The applicant will specifically focus on human stem cell derived neurons and will determine whether and how redox regulations in neurons are altered in disease states and are affected by reprogramming methods. • Addresses limitations of current stem cell-based disease models, especially redox-resetting in iPSCs. • Redox dysregulation occurs during again and in nearly all neurodegenerative diseases. Tools available to survey and modulate redox states in an appropriate manner are largely missing. This lack of insight represents a significant knowledge gap. • Large body of data generated (not hypothesis driven) will have little impact if it is not disseminated in an accessible form. • While the study of disease models is a strength, the relevance of any differences remains unclear as no benchmarking to primary neurons is included. • It is not described how the data will eventually be used and how they might impact the field. Without the ability to "restore" a normal redox state, which in itself is not defined, there is no discussion of how to incorporate the data to any iPSC work. • Project may create tools and data that enhance the use of iPSC models in the study of disease. Applicant is innovative in developing tools to probe redox status and organelle integrity-key factors in many disorders. Useful tools will be generated in Aim 1 but unclear how they will be distributed to the research community. Not clear how interested individuals will be able to query the atlas-what will it look like at the user interface.
Is the rationale sound?
• Redox status and organelle integrity are affected by many disorders. Tools to probe these factors in living cells will likely find widespread applicability. • Clear, compelling rationale linking redox state to organelle dysfunction and disease phenotypes. • Direct testing of hypotheses with sub-cellular resolution in relevant models. • It is well documented that redox regulation plays are role during normal development and aging and dysregulation occurs in nearly all neurodegenerative diseases. • Direct re-programmed neurons have been suggested to retain their metabolic state but a formal comparison regarding redox state retention has yet to be made.
Is the project well planned and designed?
• Strong modular design, enabling cross-validation and flexibility. • Interesting use of iNs vs. iPSC-Ns for modeling aging and pediatric vs. adult disease. • State-of-the-art sophisticated methods to define the redox state and oxidative damage in neurons derived via different methods. Use of biosensors, sub-cellular controlled oxidative stress induction, label free high throughput live cell imaging and deep learning based analysis. • It is unclear how well integrated is Aim 1 into Aim 2 and 3. The project has a very large scope. No application to neuronal target cells discussed. Aim 2 cellular models are not adequately described. No causal link between observed molecular changes and disease phenotype. Most relationships described will be correlative. Aim 3 deep learning approach to track organelles through live imaging is impressive. Organelle tethering concept is poorly described. • Overall many appropriate approaches but - it is a major shortcoming that is remains unclear what a "normal redox state" actually means. One could argue that the study is analyzing potential artifacts that are due to culturing variations - no discussion is provided about the "fidelity" of the data in relation to primary cells.

- Fluorescent labeling cells itself might cause oxidative stress. It is not clear how the applicant will control for generating redox stress by labeling cells with fluorescent activated proteins. A discussion should have been included.
- Redox states might depend on culture condition and oxygen incubator levels. It is not discussed how such experimental changes influence measurements and outcomes making it difficult to understand how generalizable results will be.
- Analysis of functional consequences of redox manipulations are not clearly discussed.
- Cell lines, cell culture methodology, and controls not adequately described.

Is the project feasible?

- The team has limited expertise in disease modeling but otherwise the three PIs have appropriate domain expertise.
- Ambitious but well justified given the depth and platform-building nature of the work.
- Highly feasible and excellent team.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- The proposal addresses inclusion of diverse diseases, ages, and genetic backgrounds (APOE, PINK1, SOD1, MFN2).
- Yes, cells from both sexes will be used.
- Some statements in this section are generic.

Application #	DISC0-17579
Title	Gene-edited CD19 CAR-T cells with superior proliferation, persistence and serial-killing activity
Project Objective (as written by the applicant)	Our studies are designed to modify the DNA inside of T cells, so that they can function better in treating people who suffer from blood cancers such as leukemia or lymphoma.
Impact (as written by the applicant)	Patients who receive a cancer therapy called CAR-T often see good initial results, but then the tumor comes back. If successful, our study could allow patients to have longer remissions or cures.
Major Proposed Activities (as written by the applicant)	• Modify a gene in anti-cancer cells called CAR-Ts. The gene can be altered in many different ways; determine which changes make CAR-Ts work the best. • In test-tube experiments, test how well our modified CAR-Ts kill tumor cells from several different types of leukemia and lymphoma. • Study the reasons why modifying this gene makes CAR-T cells more effective against cancer. • Develop a strategy to consistently make this gene change in CAR-T cells. This is to ensure that our special CAR-Ts can work in all kinds of patients. • Make sure that our special modified CAR-Ts are safe for use in patients. • Test improved CAR-Ts in mice implanted with human cancer cells. Determine if the modified CAR-Ts are safe and work better than FDA-approved CAR-Ts.
Statement of Benefit to California (as written by the applicant)	According to statistics compiled by the Leukemia and Lymphoma Society, there were a predicted 10,860 new cases of blood cancers (leukemia, non-Hodgkin lymphoma, Hodgkin lymphoma and myeloma) in California in 2021, with 5,840 deaths from those malignancies. These numbers are second highest in the country behind Florida. Improvements to CAR-T therapy, which is so far only approved for blood cancers, could potentially impact thousands of cancer patients, existing and new, in California.
Funds Requested	$2,429,480
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	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.

Does the project hold the necessary significance and potential for impact?

- This project aims to extend the in vivo persistence of CAR-T cells, as lack of persistence is one of the main causes of post-infusion relapse.
- This is an interesting project with significant translational potential, as it focuses on mutations in a specific gene. Identifying the portion of the gene or optimal mutation to enhance CAR-T expansion and persistence could be clinically transformative if proven safe. Therefore, the project has the potential for significant impact.
- The proposed project is led by a single PI, a physician-scientist, with a research team that includes expertise in pre-clinical mouse modeling.
- Collaborations are limited.

Is the rationale sound?

- The rationale is supported mainly by in vitro data showing that mutated CAR-T cells can withstand a large number of in vitro tumor re-challenges while retaining cytotoxicity.
- The proposal includes in vivo data demonstrating that monogenic mutations in this gene result in a lymphoproliferative disease and an immunodeficiency syndrome. Preliminary data from the PI suggest that inserting this mutation into a CAR-T construct phenocopies what is observed in normal T cells with this mutation.

Is the project well planned and designed?

- The design includes aims to identify the best genotype from pooled CRISPR edits, to establish the safety profile of edited cells, to incorporate a safety switch, and to evaluate the final product in clinically relevant xenograft mouse models.
- Prime editing is proposed as an alternative strategy if HDR knock-ins prove inefficient.
- The approach is very simple and elegant. Tumor refeeding generates convergence toward a dominant gene variant, which will then be tested both in vitro and in vivo.
- A weakness of the plan is that efforts to evaluate toxicity (CRS, ICANS, GvHD transformation, etc.) are not included.
- The studies are highly descriptive and unlikely to yield substantial mechanistic insights. However, the pooled CRISPR screens may provide useful insights. These screens will be performed both in vitro and in vivo, which is a strength.

Is the project feasible?

- The resources at the applicant institution are satisfactory to complete the proposed studies.
- The budget is satisfactory and within DISC0 limits.
- All reagents are available to the investigators.
- There are no concerns with the proposed budget or timeline.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- Donor blood samples will be collected from individuals that mirror California census demographics, and a sufficient number of independent donors will be evaluated.
- CAR-T cells with enhanced persistence could be applied to multiple CAR designs and targets, providing potential for broad clinical utility.

  The applicant states that the applicant institution has a major goal of extending access to CAR-T cell products for populations that currently have no access.

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Application #	DISC0-17428
Project Objective (as written by the applicant)	The objective of this project is to develop an innovative, minimally invasive approach for monitoring cellular states, functionality, and survival within the retina.
Impact (as written by the applicant)	The proposed technology will overcome limitations of current tools, enabling minimally invasive real-time quantification of gene expression, cell survival, and activity of gene and cell therapy.
Major Proposed Activities (as written by the applicant)	• Systematically characterize RMA in retina • Monitor photoreceptor cell transplantation based therapy in vivo through RMAs • Monitor RPE cell transplantation based therapy in vivo through RMAs • Develop RMA monitoring system for target gene expression in gene therapy • Use RMAs to track neurotrophic factor expressions by transplanted cells
Statement of Benefit to California (as written by the applicant)	Vision is vital for daily life, yet retinal degeneration diseases like macular degeneration, diabetic retinopathy, and glaucoma affect over 3 million Californians. With limited treatment options and no cure, these conditions lead to irreversible vision loss. Our research aims at advancing gene and cell therapies by developing innovative tools to monitor targeted cells and gene expression, accelerating the development of novel treatments that will directly benefit affected patients in California.
Funds Requested	$2,030,050
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	79
Median	79
Standard Deviation	3
Highest	85
Lowest	75
Count	12
(85-100): Exceptional merit and warrants funding, if funds are available	1
(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.

Does the project hold the necessary significance and potential for impact?
• The team has limited expertise in disease modeling, but otherwise the three PIs have appropriate domain expertise. • Monitoring graft function in vivo via minimally invasive blood test using engineered secreted biomarkers has significant potential to improve our ability to refine cell therapy. • Yes, good collaboration between ophthalmologist and the inventor of technology. • Yes - no concerns. • The project seeks to generate neonatal Fc receptor (FcRN)-based Released Markers of Activity (RMA) to track cell therapy presence and function *in vivo*. The development of these capabilities would enable monitoring the safety and efficacy of cell therapies within patients in the future. • Yes - the bioengineering and gene therapy expertise brings synergy to enable in vivo monitoring, potentially in patients. • The project addresses major clinical need. • The authors identify a novel approach to be able to track gene expression and transplanted cell survival of in vivo retina. There is indeed need for this. • There is a good fit between two labs with complementary expertise.
Is the rationale sound?
• The key technology is recently published in a high impact journal. • Is AAV an episome or will it integrate? Duration of perdurance of signal indicates possibility of random integration with attendant safety risks. • Yes - no concerns. • Yes - the preliminary data shows proof of principle in murine models. Prior published studies in the brain establish the rationale. • The proposal includes lots of preliminary data. • The RMA technology is novel and well thought out.
Is the project well planned and designed?
• Aim 1 targets endogenous cells in eye. It's not clear why this is important if the concept is to follow the fate of grafted cells. Controls lacking the FcRN should be included. For the long term - are the constructs integrated into genome, what is the risk, and are they silenced? • The proposal lacks consideration of likely immune response to long-term expression of foreign protein. This is not even considered but could compromise graft survival. Safety issues concerning integration of AAV or lentivirus are not considered - why not target the construct into a safe harbor locus? • Immune response to foreign protein is not adequately considered. Genetic modification should be targeted rather than random if the construct can integrate. • The project plan is thorough. • Studies elucidating the immunogenicity of the RMA need to be included. • Yes - no concerns. • Species-specific differences in the FcRN transport processes relative to the human system should be well considered. To establish the mechanisms of action, a transgenic mouse that over-expresses the transferrin receptor could be used.
Is the project feasible?
• One investigator is an ophthalmologist, and the other developed RMA technology. • Facilities at both sites are appropriately equipped. • The budget (personnel, consumables, and animal costs) is adequate. • Yes - no concerns. • The institution has all the necessary staff and resources. • Good preliminary data and team.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• Responses are generic.

- The statement is minimal.
- Yes - no concerns.

Application #	DISC0-17269
Title	Microglia replacement with non-myeloablative hematopoietic stem cell transplantation for Alzheimer's disease (AD)
Project Objective (as written by the applicant)	Our objective is to resolve biological and pre-clinical bottlenecks to brain engraftment with microglia-like cells to hasten application of stem cell therapy for Alzheimer's (AD) and other CNS diseases.
Impact (as written by the applicant)	Our project impacts the biological and pre-clinical bottlenecks in promising experimental studies that currently prevent clinical trials of HSCT for Alzheimer's disease (AD).
Major Proposed Activities (as written by the applicant)	• Aim 1 will test new, safer approaches to translatability of HSCT as a potential route for regenerating brain microglia. • Aim 2 will test the effectiveness of HSCT to regenerate microglia-like cells in an Alzheimer's disease (AD) model. • Aim 3 will perform pre-clinical feasibility studies using human stem cells as a first step towards clinical trials.
Statement of Benefit to California (as written by the applicant)	In 2020, the people of CA approved Prop. 14, authorizing $5.5 billion to CIRM, of which $1.5 billion is dedicated to research of CNS- and brain-specific disease. CA has more people living with Alzheimer's disease (AD) than any state, with 3 counties in the national top 10. Bone marrow transplantation (BMT) is widely available in CA, covered by Medi-Cal, and rapidly becoming safer. Our project will use CA taxpayer funds towards developing BMT as a viable AD therapy to benefit Californians.
Funds Requested	$2,303,684
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	76
Median	79
Standard Deviation	7
Highest	86
Lowest	60
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	1
(1-84): Not recommended for funding	13

Key Questions and Comments

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

Does the project hold the necessary significance and potential for impact?

- The proposal aims to develop a hematopoietic stem cell transplantation (HSCT) therapy for Alzheimer's disease (AD). The project will determine the translatability (Aim 1), effectiveness (Aim 2), and preclinical feasibility (Aim 3) of HSCT as a treatment for AD. The application is relevant to human biology and disease and it pertains to stem cells and regenerative medicine.
- The PI brings neuroscience expertise and will lead proof-of-concept animal studies in their laboratory. A collaborator specializes in hematopoietic stem cell transplantation in humans, providing a complementary clinical perspective for this project.
- This resubmission is focused on determining the translatability, effectiveness, and preclinical feasibility of HSCT transplantation in a model of AD to replace or replenish dysfunctional microglia. If successful, the impact would be extremely significant.
- Microglia are known to play a major role in AD, and their progressive dysfunction during aging is thought to contribute to pathology. Regenerating microglia is therefore a therapeutic approach that could halt or reverse AD pathology.
- The approach could also be applicable to other tauopathies.
- The project has strong potential to advance understanding of how CNS glial cells are replaced, at least in part, by HSCs.
- Establishing the role of TLI/ATG in mouse models and assessing the benefit of adding CSF1R1 (PLX3397) to facilitate microglial replacement are important aspects.
- Mouse models in Aim 2 will provide information on potential reversibility versus prevention of disease establishment.
- Aim 3 will provide important information on human translation potential, including exploration of autologous versus allogeneic transplantation.
- Team collaborations provide synergy. The PI is an expert in neurocognitive issues and mouse models, while the main collaborator is an expert in non-myeloablative and reduced-intensity conditioning as well as allogeneic transplants.
- The proposal does not provide much information on the durability of microglial replacement.
- The lack of a strong basic immunologist on the team represents a weakness.

Is the rationale sound?

- Yes. Previous studies provide the rationale for HSCT to partially replace dysfunctional microglia with donor HSC-derived macrophages. Microglial depletion using CSF1R (colony-stimulating factor 1 receptor) inhibitors could enhance both safety and efficacy by creating available niches and facilitating engraftment. Given the protective roles of some microglial subsets, the proposal suggests preserving resident microglia while promoting integration of HSC-derived cells capable of compensating for dysfunctional microglia in AD.
- Microglia are known to play a major role in AD, and their progressive dysfunction during aging contributes to pathology. Timely microglia regeneration could therefore be a therapeutic approach to halt or reverse disease.
- Previous findings with a different collaborator (who provided a letter of support) have shown that replacement of yolk sac–derived microglia with HSC-derived microglia is predicted to be beneficial and safe, supporting the translational relevance of this approach.
- In the preliminary data for Aim 1 (translatability), the applicant tested optimization of HSCT conditions for brain engraftment. These findings support administering PLX3397 post-HSCT to enhance donor cell engraftment in the brain.
- In the preliminary data for Aim 2, the applicant showed that the [redacted animal model] exhibits impaired spatial memory, consistent with AD pathology. However, no preliminary data were provided regarding the effectiveness of HSC transplantation.
- Overall, the rationale is sound. However, microglia in the CNS are derived from yolk sac progenitors, not from the same cells as HSC-derived myeloid cells. This represents a major functional gap. It is also unclear whether syngeneic or allogeneic transplanted microglia will persist and function, and whether

"functioning" will result in measurable clinical benefit. The mouse models in Aims 2 and 3 should help address these uncertainties.

- Microglia depletion in animal models has also been shown to accelerate cerebral amyloid angiopathy, brain calcification, and hemorrhages. Thus, timing is crucial. Because the applicant proposes to delete microglia before transplantation, there will be a period when microglia are absent, which could have negative effects. This concern is not well addressed.

Is the project well planned and designed?

- The project is appropriately planned. It will test the translatability, effectiveness, and preclinical feasibility of HSCT as a treatment for AD. For translatability, the team will optimize PLX3397 timing under clinically relevant conditions in mice and identify molecular characteristics key to successful brain engraftment. For effectiveness, they will transplant bone marrow from APOE2-KI, APOE4-KI, or wild-type GFP+ mice into [redacted animal model].
- For preclinical feasibility, the team will test the ability of human HSCs (purchased) to engraft in the brains of NSG mice and use low-dose kainic acid (KA) to trigger a microglial gene response.
- Previous reviews raised major concerns about translatability, including (1) whether microglia need to be ablated, (2) whether endogenous microglia would interfere with engrafted HSCT-derived cells, and (3) whether the applicant fully appreciates the potential beneficial roles of endogenous microglia. These concerns are not adequately addressed.
- Behavioral defects in [redacted animal model] appear at 8 months, and Aim 2 uses 9 months as the latest time point, when mild defects are observed. It is unclear whether the approach models halting progression or reversing established defects. Scaling to human brains also remains unaddressed—specifically, how many cells would need to be replaced?
- A study using TLI/ATG conditioning showed that the one-year rate of extensive chronic GVHD was 22%. While this might be acceptable for patients with lethal cancers, it seems high for slowly progressing AD patients. It remains unclear when such a therapy would be initiated and whether older patients could tolerate it.
- The applicant acknowledges the protective role of microglia and proposes to preserve a pool of resident cells while promoting integration of HSC-derived cells. It is unclear how this delicate balance will be achieved.
- The main functional readout is glutamatergic neuron hyperexcitability. While tau contributes to hyperexcitability, Aβ can also have epileptogenic effects, even before plaque formation. In the APP/PS1 model, neurons exhibiting epileptic discharges were found to colocalize with Aβ plaques. The applicant's focus on PS19 mice may therefore limit insights into treatment efficacy in plaque-bearing brains.
- Overall, the project is well planned.
- All aims, especially Aims 2 and 3, are likely to be informative and feasible.
- The readouts from transplanted mice are not well described and remain vague.
- Better quantitative measures in mouse studies would strengthen the application.
- The APOE mouse experiments are elegant and well designed.

Is the project feasible?

- Published data suggest that the approach is feasible.
- There are no issues with the budget.
- The project will be managed collaboratively by the PI and co-investigator. The team has the appropriate expertise and has worked together to generate preliminary data. However, preliminary data for Aim 2 are limited. The budget and timeline are appropriately justified.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- The project will use genetically different donor and recipient mice, providing proof-of-concept for HLA-mismatched microglial replacement. Reducing the need for closely matched donors could enhance access to CNS-related stem cell therapies for all patients, particularly underserved and underrepresented communities.
- Sex is considered in the design.
- The proposal acknowledges mouse sex, but there is no mention of the human CD34+ cell source.
- The application does not address prevention versus progression of disease. It focuses on young animals, which do not represent the target human population.

Application #	DISC0-17603
Title	Cord-to-Cure: Uncovering Genomic Mechanisms of Placental Resilience and Vulnerability for Improved Maternal-Fetal Outcomes
Project Objective (as written by the applicant)	This project will uncover genomic mechanisms of stem cell resilience that will establish the molecular basis for the development of early interventions for placental injury and preterm birth.
Impact (as written by the applicant)	This project will advance the development of human mesenchymal stem cells as stem cell-based therapies for BPD by determining the impact of EMT on in utero genomic instability of MSC and TSC.
Major Proposed Activities (as written by the applicant)	• Determine the impact of MSC and TSC genomic instability on clinical neonatal and maternal outcomes in placental villous tissue and UC-MSCs. • Evaluate EMT-MET balance in MSC and TSC with respect to clinical outcomes leveraging whole transcriptome sequencing of MSC and TSC. • Develop a predictive model of BPD in preterm births based on genomic instability and impaired EMT-MET in MSC and iPSC-TSC. • Interrogate DNA repair machinery in mesenchymal and trophoblast stem cells in the healthy and diseased state. • Induce genotoxic stress in MSC and TSC by hypoxia, measure genomic instability and EMT in healthy and disease. • Develop a high-throughput patient-derived MSC and iPSC-TSC stem cell drug screening model to correct EMT-MET imbalance in development.
Statement of Benefit to California (as written by the applicant)	This work lays the foundation for optimizing treatments for preterm birth and neonatal disorders on a personalized basis leveraging patient-derived MSC and iPSC trophoblast stem cell models. The long term goal of this project is to improve maternal-fetal health and reduce the great societal and economic costs related to pregnancy complications for citizens of the State of California.
Funds Requested	$4,278,894
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	2
Highest	80
Lowest	75
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	14

Key Questions and Comments

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

Does the project hold the necessary significance and potential for impact?
• Preterm birth is associated with severe neonatal mortality and morbidity and over 13 million babies are born preterm (<37 weeks gestation) annually (~10% of all births). 15% off preterm neonates are born prior 32 weeks of gestation that often results in bronchopulmonary dysplasia (BPD) which is a chronic lung disease for which there is no cure. • BPD is characterized by dysregulated epithelia-mesenchymal transitions (EMT). EMTs and cell cycle progression drive EMT- linked DNA damage responses. The mechanisms by which EMT-linked DNA repair pathways operate in tissue-resident stem cells are unexplored. • Successful completion of this project will establish the molecular basis for the development of early interventions and/or improved clinical management for placental injury. • Yes. Placenta is a relatively under-appreciated contributor to developmental disorders, and this application uses human tissue and human stem cell derived systems to study an intriguing, and potentially targetable, hypothesis on DNA repair defects mediating some placental defects, including in the G x E context. • The project is a deep dive into molecular details that aim to improve outcomes and prevent pre-term births. Given the high numbers, this is clearly a relevant topic. The project itself is a mix of data gathering and very detailed characterization that are not convincingly enough presented.
Is the rationale sound?
• It is well established that TGFβ signaling is involved in the dysregulated epithelial-mesenchymal transitions (EMTs) in placental injury, preterm birth and neonatal disorders such as BPD Hypoxia and placental genomic instability are recognized as predictive biomarkers for preterm birth and preeclampsia and are known to be damaging. Variants in TGF-β production and stem cell differentiation pathways predict increased risk of preterm birth and abnormal placental pathology. • Oxidative stress is well known to cause damage to DNA and increases genomic instability. • Preliminary data suggest that the defective EMT and DNA repairs machinery drive genomic instability stem cells or conversely, genomic instability disrupts cellular processes. • Aim 1 doesn't sufficiently clarify how the 30x genome sequencing from bulk and likely very heterogeneous genomes will be analyzed/interpreted. It is not clear for Aim 2 how well it is understood that everything is genetic defects as the reprogramming will erase all other effects. The multiple rounds of transformations may also lead to clonal biases that need to be considered and it may have plenty of unknown confounders. • The subsequent analysis are very detailed, but it is unclear whether meaningful differences can be extracted from the reprogrammed and differentiated mesenchymal stem cells (MSCs). The use of only a few founder MSCs raises concerns about representation, which remains unclear.
Is the project well planned and designed?
• In Aim 1 the applicant will analyze placental villous tissue and the differentiation potential of primary UC-MSC and TSC from normal and injured placentas to generate a predictive model of BPD in preterm births based on genomic instability and impaired EMT-MET. UC-MSCs play a role in maturation in immature fetal lungs but their impact on fetal lung development is only assumed retrospectively and not directly tested. It will be unclear which specific factors contribute to development of BPD. • The applicant proposes to generate a predictive model but it is not clear how the validity of the model will be tested. • In Aim 2 the applicant will generate trophoblast stem cell (TSC) lines from human UC-MSC-derived induced pluripotent stem cells (iPSC) from the various pregnancies and test their differentiation, proliferation and survival potential, perform bulk RNA-seq assays, conduct genomic instability assays, DNA repair assays and single nucleus RNA + ATACseq (10x Multiome) to correlate defects with these outcomes and conduct a drug screen.

- These lines will then be used for manipulation experiments to test the impact of exogenous induced stress. Stressors are not well defined and benchmarked.
- The drug screening approach is poorly defined. The meaning of "correcting EMT–MET imbalance" is unclear - all pregnancies tested were premature. Would a normalized EMT-MRT transition not be full term?
- Overall it is not clear whether the proposal is seeking to understand/prevent prematurity or to affect the transition at a later stage, which could be highly cell type specific.
- Both Aim 1 and 2 have very good descriptions on what will be collected and done. The main concern as noted is the its not entirely clear or convincing that meaningful insights can be extracted from the proposed data collections/experiments.
- The application was hard to read, with some experiments seeming to duplicate goals across aims, but this was not clear. There also seemed to be conflation in some interpretation between cause and effect of DNA repair defects and placental outcomes.

Is the project feasible?

- Too short for both aims. For Aim 1 many other caveats in particular heterogeneity are not discussed. For Aim 2 only closing sentence states if no differences are found, then single candidate genes will be knockout out. Some of that should be done before to showcase the readouts are even able to pick up the effects. Or confirm that any relevant mutations are in the clones.
- A key challenge in this type of study is always in the detail, in particular the analysis. Of course, one can perform standard processing, but that isn't always a straight path to meaningful and actionable results.
- Technical aspects of the work seem feasible.
- Bioinformatic details are missing Integration of all data. Management of data and modeling seem to fall on the laboratory manager conducting cell culture experiments. A second year Ph.D. student in the institutional bioinformatics and systems biology graduate program will help. This seems inadequate for the vast an amount of data that are expected to be integrated and tested.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- No, it is unclear and the discussion is too short.
- Subjects chosen for placenta-derived MSC collection are chosen purely based on clinical and histopathological presentation, with no regard for age or racial/ethnic background. Banked cell lines are from a diverse patient population. Fetal (placental) sex is considered as a biological variable, and both male and female placenta-derived iPSCs will be included.
- Premature birth is a major problem and all future pregnancies could benefit from successful completion.

Application #	DISC0-17367
Title	Generation of Functional Proximal Tubules in Organoids through Gradual Developmental Mimicry for Kidney Injury Modeling
Project Objective (as written by the applicant)	We will develop protocols to generate clinically important kidney cell types that can be used for drug screening, disease modeling, and efforts to build an artificial kidney.
Impact (as written by the applicant)	Clinical trials can perform efficient nephrotoxicity assays at low costs, cells can be produced to build an artificial kidney, kidney disease can be studied.
Major Proposed Activities (as written by the applicant)	• Genetically engineer stem cells to expressed genes important for kidney development and test their ability to make functional kidney cell types. • Develop protocols using normal signaling cues to push human stem cells into kidney cell types. • Test the importance of a new target in acute kidney injury.
Statement of Benefit to California (as written by the applicant)	In California, 15% of the population have chronic kidney disease. This arises through several different paths but almost always, the proximal tubule cells in the nephron stop functioning as they should. We are developing ways to generate these cells from human stem cells. This will be an invaluable resource as they will allow us to develop safer drugs in clinical trials, allow us to understand the origins of kidney disease, and even pave the way to new kidney replacement therapies.
Funds Requested	$2,153,295
GWG Recommendation	**(1-84): Not recommended for funding**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

Scoring Data

Final Score: 75

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

Mean	74
Median	75
Standard Deviation	3
Highest	78
Lowest	70
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

Key Questions and Comments

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

Does the project hold the necessary significance and potential for impact?

- Kidney disease/failure is widespread and a major healthcare burden. Development of cell-based therapeutic (replacement) strategies would be feasible and have a high impact. The proposal aims to improve the differentiation (and molecular dissection) towards proximal tubule cells (PTCs) from pluripotent cells.
- The proposal focuses on maturing and enriching proximal tubules of kidney organoids, towards improving nephrotoxicity testing during drug discovery and facilitating regenerative medicine strategies. From preclinical to late-stage trials, lead candidates during drug discovery often fail for nephrotoxicity, with current human in vitro systems poorly predictive. By maturing stem cell-derived proximal tubules they may offer a more faithful platform for nephrotoxicity testing.
- Beyond drug discovery, mass production of these mature proximal tubules may benefit regenerative medicine strategies, as the proximal tubule is the workhorse of post-glomerular filtration and the fabrication of acellular glomerular filtration becoming a reality. The proposal builds off of the applicants' two bioRXiv submissions that proximalize and distalize nephrons of organoids by supporting Notch signaling and inhibiting bone morphogenetic proteins (BMP) signaling, respectively, as well as building off the collaborators' work regarding the persistence of SOX9 in injured proximal tubules being a marker of maladaptive repair.
- The overall objective of this application is to understand the limitations of current protocol allowing the differentiation of hPSCs into nephron proximal convoluted tubule cells (PTCs) in vitro. Indeed, the cells generated by this protocol remains fetal in nature. This issue is a common problem with hPSCs.
- Production of fully functional PTCs could be useful for a number of applications especially disease modeling and developmental studies.
- This is a single track PI application but it involves one major collaboration which seems synergistic and complementary. This collaboration will bring clinical expertise and also technical support regarding epigenome analyses.

Is the rationale sound?

- The preliminary data and literature clearly support a role of hepatocyte nuclear factors (HNF) and BMP and explore their contribution is reasonable. Concerns relate to the simplification of the hepatocyte nuclear factor 4 (HNF4) induction and lack of analysis details.
- Aim 1 proposes that enhanced HNF4A will facilitate proximal tubule maturation. While the bioengineering methods are to be appreciated, specifically regarding the generation of an inducible fluorescently-tagged HNF4A line superimposed on their existing HNF4A-YFP iPSC line to permit FACS sorting, their methods are not cell type specific. Moreover, there are three significant concerns.
    - #1: HNF4A is still expressed out to at least day 63 in organoids without driving the expression of the applicant's proposed mature proximal tubule markers.
    - #2: The belief that HNF4A, drives a differential transcriptomic program at different developmental stages is poorly supported. If proximal tubule maturation markers are driven by HNF4A, then why are they not expressed when HNF4A is high (day 14 – 28)? If HNF4A is part of the story, it is not acting alone and the proposed experiments are of less utility.
    - #3: A post-hoc analysis of "genes of strong interest using RNAscope and antibodies" may lead to experimental/reporting bias, as these genes should be predefined.
- The importance of HNF4 in PTCs is well established. However, the role of this factor in functional maturation is less clear. Indeed, HNF4 might be necessary to maintain PTCs identity but not really to drive their functional maturation. Nonetheless, the hypothesis needs to be tested and the proposal is convincing and supported by solid preliminary data.
- The cells generated by the current protocol seems to expressed HNF4a. The rationale to over-express HNF4 is not clear and could result in side effect since this factors is broadly expressed.
- The Aim 1 is based on the integration of inducible system which will be integrated in a genome safe harbor. This transgene will be silenced during differentiation and thus Aim 1 likely to fail. The use of insulator is essential. Similarly, the use of the KRAB-dCas9 CRISPRi system in Aim 3 will be challenging.
- The mutliomics approach is also very optimistic. Capturing 6,000 cells will be challenging.
- The Aim 3 is difficult to link with Aim 1 and 2. It seems artificially added to add a translational aspect. The link between chronic injury in adult organs and developmental process is always difficult to establish even if resemblance exists.

Is the project well planned and designed?

- The project is mainly based on hPSCs but will also take advantage of existing data on human tissue. This is a powerful combination and probably the only to identify mechanisms controlling human development.
- Back up plans are included in the proposal and they seem appropriate.

- Aim 3 proposes to investigate the interplay between BMP4 and (SRY-box Transcription Factor 9) SOX9 during maladaptive repair. This is based on proximal tubules dedifferentiating after injury, reducing maturity marker HNF4A and reactivating developmental markers SOX9 and BMP4, and the collaborator's demonstration that maladaptive repair is mediated by the persistence of SOX9 and BMP4 in failed repair PTCs. They postulate that knock-down of SOX9 and BMP4, made possible by a novel inducible dCas9-KRAB repressor line, may prevent maladaptive repair and promote recovery. There are three concerns for this Aim.
    - #1: There is a clear contradiction as Aim 2 hypothesizes that BMP signaling drives HNF4A expression, here the applicant suggests to inhibit BMP4 and monitor for HNF4A re-expression.
    - #2: Knockdown of SOX9 and BMP4 immediately after cisplatin injury would prevent healthy adaptive repair, as well as maladaptive repair, a better strategy would have been to knock-down SOX9 and BMP4 after the development of CDH6 positivity.
    - #3: Although the authors hypothesize that interplay between BMP and SOX9 perturbs the return to a normal proximal tubule state, they are simultaneously knocked down and an interplay uninvestigated.
- The overall steps taken for data collection are outlined sufficiently. The analysis is a bit short as it required multi-layered data integration. More details could be provided on how the regulatory dissection will then be turned in iterative rounds to produce therapeutically meaningful PTCs.
- Functional readouts are missing.

Is the project feasible?

- Overall many of the project goals are feasible though more details on the eventual translation of very basic molecular insights would be desirable.
- Additionally, SOX9 and BMP4 gRNA would best be delivered specifically to proximal tubules in organoids that have pan-cellular dCas9-KRAB induced.
- The team has access to all the necessary resource.
- The budget and time line seems appropriate for such project.
- Lentiviral delivery of SOX9 and BMP4 gRNA to proximal tubules of formed 3-dimensional organoids needs to be demonstrated, as delivery to (induced nephron progenitor cell) iNPCs in 2D culture is insufficient.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- The PI serves as a co-director of their institution's stem cell diversity oversight committee and has previously used iPSCs across ethnicities and genders.
- The applicant mentions the importance of genetic diversity in kidney diseases. However, this aspect is not clearly integrated in the experimental design. It is not clear how many female/male hPSC line will be used.
- This is a long term basic research application which could result in the development of new platform to model kidney disease.
- The applicant has outlined outreach and educational activities.
- Population impact is not a major focus, and not within the scope of the few targeted lines.

Application #	DISC0-17386
Title	Machine-Guided and Quantitative Solutions to the Unique Challenges in Gene Therapies for Neurogenetic Disorders
Project Objective (as written by the applicant)	We tackle two obstacles for gene therapy to treat brain disorders: how to ensure that our bodies do not reject such therapies as other, and that there is neither too much nor too little of payload.
Impact (as written by the applicant)	We will produce designs to be directly developed into gene therapies for four neurogenetic disorders. The platform will then be readily adaptable for creating therapies for hundreds of more diseases.
Major Proposed Activities (as written by the applicant)	• We will use machine learning to guide the creation of therapeutic proteins that both achieve the functional goals and masquerade as our own parts. • We will combine computation and experiments to create a collection of interaction biomolecules that maintain the Goldilocks regime for the payloads. • We will validate and optimize our innovations above in stem cell-derived models that recapitulate certain aspects of the corresponding diseases.
Statement of Benefit to California (as written by the applicant)	If successful, the therapies will benefit Californian patients and their caregivers. The later development will also likely be pursued through biotech startups, which would benefit Californian economy.
Funds Requested	$2,186,981
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	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.

Does the project hold the necessary significance and potential for impact?
• The proposed project will address an important gap in the application of gene therapy approaches to neurogenetic disorders using human cells, by developing constructs based on human-derived zinc finger transcription factors (ZF-TFs) that both have a low immunogenic potential and dosage-control of target gene expression levels. • This is a single PI proposal, leveraging their expertise in synthetic biology and biomolecular engineering, who is joined by a collaborator who brings their iPSC and neuronal differentiation expertise. In this way, the collaboration is synergistic. • The immunogenicity problem needs to be framed in a disease context. • The studies attempt to create de-immunized and functionally optimized transcription activators using machine-guided dual-objective engineering. The studies could have an impact on gene therapies using transcriptional activators. • The collaboration helps establish a clinically relevant system to test the novel technology. • Most gene therapies lack quantitative control and many neurogenetic disorders are also triple-sensitive. How do we get gene dosage just right? This proposal seeks to address both the dosage issue and developing gene therapies that are not immunogenic, pointing out that CRISPR proteins are bacterial in origin. It has the potential to address a key knowledge gap.
Is the rationale sound?
• The theoretical work and computational analysis is well considered. • The rationale describing the need to for constructs with low immunogenicity for delivery into human cells for therapeutic applications is compelling, but the rationale for the strategy is less sound. • The combination of technologies is not well articulated. • The degree to which reduced immunogenicity needs to be reduced is not clear for clinical impact. • The idea that precise dosage control is important for haploinsufficiency is a relevant and important problem. However, it is not clear that incoherent feed-forward loops (IFFL) ZF-TFs delivered by AAV can achieve precise dosage control over simpler approaches.
Is the project well planned and designed?
• The reduced immunogenicity of ZF-TFs is done via a straightforward plan and should give meaningful results. The ESM-2 use is intriguing but there are only high-level statements about making the ZF-TFs better and no supporting data for ESM-2-generated mutations. The proposed IFFL systems are quite different than the TEV protease shown in the preliminary data. • The pitfalls are overall well considered, with the exception of the heterogeneity that might be present in the patient populations, and the scale of work to address this. • More of a focus on clinical impact is needed. • The studies with immunogenicity need to be expanded. • The fluorescence polarization competition assay has not been demonstrated with any ZF-TFs. • Caveats are focused on the mathematical model but do not describe alternatives for the experimental approaches, which is needed given the high-risk of the proposal. • Overall, the iterative approach (in-silico design then optimizing using transient infection in HEK293 cells) is promising. How well this will translate in human neurons is unclear. The applicants will generate het mutations in iPSCs and restore gene expression using their constructs. While it is acknowledged that the edited mutations may behave differently than the patient ones, beyond a vague plan to acquire and modify “patient lines” (which would entail substantial additional work) this is not addressed.
Is the project feasible?
• The PI is an expert in synthetic biology and biomolecular engineering, and relies on the co-I expertise with iPSC and differentiation work, but some pitfalls are not well considered. • The team has the resources and staff necessary to conduct the work, with the exception of patient derived iPSCs. • The budget and timelines are appropriate. • Yes - no concerns.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• The analysis of HLA alleles adequately addresses variation in the US population.

- Yes. If successful, this approach will extend, and better, the applicability of regenerative medicine discoveries to additional affected populations.
- Reasonably well. Sex as a biological variable and genetic ancestry are not considered in this approach.
- The described plans are acceptable.
- Yes - no concerns.
- Yes, to some extent.

Application #	DISC0-17609
Title	Exploring Potential Drugs to Enhance Neural Recovery in Combination with Neural Stem Cells after Spinal Cord Injury (SCI)
Project Objective (as written by the applicant)	Identify the molecular mechanisms underlying plasticity mediated by rehabilitation after spinal cord injury (SCI) and develop novel pharmacological therapies that recapitulate these mechanisms.
Impact (as written by the applicant)	It will be a rapid path towards human intervention that can lead to greater anatomical and functional recovery in patients with spinal cord injury.
Major Proposed Activities (as written by the applicant)	• Pharmacological compounds screened in vitro • RNA-seq of cortical neurons in vitro • Testing drug optimal dosage and method of delivery • Spinal cord lesions and Transplanted H9scNSCs • Behavioral and Functional recovery analysis • Histological analysis
Statement of Benefit to California (as written by the applicant)	This research has the potential to significantly impact the lives of Californians living with SCI and contribute to a healthier and more prosperous future for the state. It will improve the quality of life for SCI patients by enhancing motor function, reduce healthcare costs by decreasing the need for long-term care, and consequently enhance economic benefits by increasing independence and participation in the workforce.
Funds Requested	$2,403,379
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	2
Highest	75
Lowest	70
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	14

Key Questions and Comments

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

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

Does the project hold the necessary significance and potential for impact?
• Acute rehabilitation following SCI can improve functional outcomes, but the precise cellular and molecular mechanisms that mediate these effects are not well known. • The proposal aims to understand the molecular mechanisms underlying the positive effects of rehabilitation after spinal cord injury and to use this knowledge to develop drugs that could replicate these effects and be combined with stem cell transplants. • The project addresses the lack of early-phase therapies for SCI patients who are unable to participate in immediate rehabilitation. • Large animal data with and without rehabilitation demonstrate clear clinical potential. • Previous work has identified rehabilitation-induced transcriptional changes and candidate compounds. • There is a strong collaborative framework between one institution performing the experimental work and another handling bioinformatics and sequencing. The PI is embedded within a world-class laboratory, ensuring access to critical resources. There is a clear division of expertise aligning with each partner's strengths. However, there is heavy reliance on external bioinformatics support and on personnel who have not yet been hired.
Is the rationale sound?
• The scientific premise for studying the molecular mechanisms underlying the benefits of rehabilitation is reasonable. The limitations of applying rehabilitation to patients acutely after injury are acknowledged, and the identification of a surrogate drug to replicate these effects has great potential. • It is well established that there is a critical time window for therapeutic interventions in spinal cord injuries. There is strong rationale for seeking the molecular mechanisms of this effect and for combining such approaches with cell transplantation. • In the introduction to the resubmission, the applicant states that “detailed information regarding the candidate drugs will be provided.” However, this information appears to be missing. • The research is based on the following concepts: (1) rehabilitation creates a “pro-recovery” transcriptional state; (2) RNA-seq performed on corticospinal neurons from animals with and without rehabilitation after SCI identified specific gene expression patterns at defined time points; (3) upregulated and downregulated genes were identified; and (4) these changes activated specific pathways. • Computational comparison of this “rehabilitation transcriptional signature” against drug profiles identified a couple dozen drugs that produce similar gene expression patterns when applied to cells. • This represents an oversimplified view of rehabilitation, which actually works through complex interactions between multiple brain regions, spinal circuits, and muscle feedback, where synaptic plasticity depends on the precise timing of volition and circuit activity. • It is not clear that this is discovery research. The transcriptional signatures are already known, the pathways have been identified, and candidate compounds have been computationally selected. This proposal represents validation research rather than discovery research.
Is the project well planned and designed?
• There is a logical progression from in vitro screening, to dose optimization, to combinatorial in vivo testing. • Improved selection criteria now specify that drugs must meet two-fold in vitro outgrowth thresholds and exhibit electrophysiological network activity before proceeding. • The proposal includes appropriate control groups and explicit use of both male and female animals. • The assay in Aim 1 appears to measure outgrowth in the absence of inhibitors known to be present in the injured cord. A negative result in vitro might therefore reflect the incompleteness of the model rather than a lack of efficacy, and no consideration of this limitation is outlined. • It appears that only one candidate will be tested in Aim 2 after initial pilot experiments. However, the design and endpoints of the PK-based pilot experiments are underdeveloped. • The project is generally well planned and designed. However, there is some disconnect between Aims 1 and 2, and it is not clearly established how relevant in vitro data will be for in vivo plasticity, reconstruction, and repair. • There is limited explanation of how the best compound will be definitively selected if multiple candidates show similar effects. • The application provides insufficient detail on how drug load will be monitored and optimized in vivo.

- A prior critique regarding the lack of rehabilitation-only comparison groups remains somewhat unresolved.
- The proposal includes an expanded contingency list, including viral gene therapy, compound prioritization strategies, and drug combination approaches. Fallback plans are described if drugs do not show behavioral efficacy. The applicant recognizes that multiple approaches may ultimately be needed.

Is the project feasible?

- The PI is a project scientist working in the group of *[name redacted]*. In the past five years, the PI has published three first-author papers but no senior-author publications.
- This work appears to conceptually derive from, and will be conducted by, members of the *[name redacted]* laboratory, raising questions regarding the PI's autonomy. A letter of support from the Department Chair or Dean indicating that a tenure-track position is forthcoming would help clarify institutional support and the applicant's independence.
- The experiments are feasible given the expertise of the applicant and co-applicant.
- A named institution provides comprehensive resources, including behavioral suites, imaging cores, and surgical facilities.
- H9-scNSC lines and adult cortical neuron protocols are established.
- The institution's genomics core provides world-class sequencing and analysis capabilities.
- Many key resources remain with collaborators rather than under the direct control of the PI.
- Twelve-week functional assessments align with established SCI preclinical timelines.
- Preliminary study plans for dosing studies are outlined prior to major experiments.
- Increasing the sample size from n=10 to n=12 addresses prior power concerns.
- Power analysis remains thin, with minimal justification for effect size.
- The timeline lacks detailed breakdown of milestones and resource allocation.
- The timeline and workload critically depend on the hiring and training of new research assistants.
- Completing comprehensive screening, optimization, and validation within three years may be challenging.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- Yes. The project acknowledges the increased prevalence of SCI in males.
- This issue is well described in the proposal.
- There is an excellent analysis of SCI epidemiology by sex (79% male), race, and socioeconomic factors. The proposal recognizes that pharmacological intervention could broaden access in settings lacking rehabilitation infrastructure.
- The focus on cervical injuries and upper extremity recovery aligns with patient priorities.
- The project addresses tetraplegia, the most prevalent injury type, with particular attention to upper extremity function and applicability to patients with chronic injuries.
- There is a robust framework for mentoring and open science principles.
- The proposal includes comprehensive plans for open access publication and data dissemination.
- The potential societal benefits are clearly articulated.
- There are no specific partnerships with SCI community organizations or patient advisory groups.
- Direct engagement with affected communities in study design is limited.

Application #	DISC0-17688
Title	Regulation of Cardiac Cell Reprogramming by Macrophages
Project Objective (as written by the applicant)	This project is to elucidate the mechanisms of how immune cells regulate cell reprogramming in cardiac regeneration and provide a basis to the development of novel therapeutics.
Impact (as written by the applicant)	This project will elucidate mechanisms of how macrophages regulate cell reprogramming, and help develop therapeutics to modulate immune cells to promote cell reprogramming and tissue regeneration.
Major Proposed Activities (as written by the applicant)	• Investigate how macrophage polarization regulates induced cardiomyocyte (iCM) reprogramming • Investigate how macrophage-secreted cytokines regulate iCM reprogramming • Investigate how macrophages and cytokines regulate intracellular signaling during cell reprogramming • Investigate how macrophages and cytokines regulate epigenetic state during cell reprogramming • Investigate how macrophages regulate iCM reprogramming and the improvement of cardiac issue function using multicellular 3D cardiac organoid model • Develop a drug delivery system and evaluate the therapeutic effects of immunomodulation on iCM reprogramming in 3D cardiac organoid model
Statement of Benefit to California (as written by the applicant)	Here we will use human cardiac cells derived from induced pluripotent stem cell lines or cardiac tissues from donors representing diverse genetic backgrounds, which will address the question whether the scientific findings can be applied to a diverse populations (age, gender, race). Our findings will also facilitate the development of immunomodulation therapy to improve cell reprogramming and tissue regeneration, which will help improve healthcare and benefit the State of California.
Funds Requested	$4,558,759
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	73
Median	75
Standard Deviation	5
Highest	75
Lowest	60
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	14

Key Questions and Comments

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

Does the project hold the necessary significance and potential for impact?
• This is a highly significant project. The goal is to investigate the mechanisms of the effects of macrophages in cellular reprogramming of human cardiac fibroblasts into cardiomyocytes and to optimize this process for future clinical applications. • Direct cardiac reprogramming offers a promising alternative to cell transplantation by bypassing challenges of engraftment and survival. However, the i*n vivo* efficiency of cell reprogramming remains low. Immune cells play an important role, but the effects of immune cell signaling on cell reprogramming are not well understood. • The study aims to elucidate how macrophages affect the process of reprogramming cardiac fibroblasts into cardiomyocytes,. The project plans to use human fibroblasts and macrophages derived from at least 20 different donors obtained through tissue collection and iPSC-derived, 3D multicellular, cardiac organoid models. • Accomplishment of this project can resolve how macrophages and their secreted cytokines regulate the reprogramming of cardiac fibroblasts into cardiomyocytes. It also has potential to lead to development of drug delivery methods to modulate the local immune environment to promote cell reprogramming and tissue regeneration. • The team includes expertise in biomaterials development, cell engineering, and bioengineering; organoid generation, heart development, and disease modeling; and multi-omics analysis. The proposed collaborations offer a unique synergy to investigate the mechanisms of how macrophages regulate cardiac cell reprogramming in human cells and in iPSC-derived, 3D cardiac organoid models.
Is the rationale sound?
• The project is based on a sound rationale. Reprogramming fibroblasts into induced cardiomyocytes (iCMs) using a combination of transcription factors—Gata4, Mef2c, and Tbx5 (GMT)—has been demonstrated previously. The proposed study will examine whether the efficiency of this reprogramming can be increased by anti-inflammatory M2 macrophages and/or their secreted factors. • *In vivo* direct cardiac reprogramming efficiency of cell reprogramming is low and how immune cells play a role is unclear. In the pilot studies, the team have discovered that immune cells play an important role in cardiac reprogramming and found that pro-inflammatory M1 macrophages can prevent cardiac reprogramming, while anti-inflammatory M2 macrophages can significantly boost cardiac reprogramming. The immunomodulatory cytokines can produce similar effects. These pilot studies were performed in the mouse cells. • Based on these results, the team hypothesizes that local modulation of macrophage phenotype can regulate the epigenetic state of cardiac fibroblasts to enhance direct cardiac reprogramming efficiency. Specifically, the applicants will reproduce their findings in reprograming human cardiac fibroblasts into cardiomyocytes, then test the immunomodulatory effects of macrophages on cardiac tissue function in an iPSC-derived, 3D cardiac organoid model.
Is the project well planned and designed?
• Overall, the research plan is reasonable. It consists of 6 milestones, which will address mechanisms of macrophage- and soluble factors- mediated reprogramming of GMT fibroblasts into iCMs. The experiments will be performed with human cells and will be carried out either in 2-D cell cultures or in 3D cultures of cardiac organoids. The cells will originate either from the donors' peripheral blood (monocytes), commercial sources (fibroblasts) or be derived from hiPSCs (fibroblasts). • The research plan is described in inconsistent detail. Some parts of the research plan are described in detail, such as Milestone 1.2. Others, however, lack sufficient detail, such as several sections in Milestone 2. • Milestone 2 does not describe how the concentrations of the macrophage-secreted cytokines and the time of their addition to fibroblasts will be selected, how inhibitors and blocking antibodies experiments will be conducted, and if and how the reprogramming efficiency of the cytokine-mediated and macrophage-mediated experiments will be compared. • The purpose of the work proposed for Milestone 3 within the framework of this project is not clear.

• The team will reproduce the findings in reprograming human cardiac fibroblasts into cardiomyocytes and test the immunomodulatory effects of macrophages on cardiac tissue function in iPSC-derived a 3D iPSC-derived cardiac organoid model. There is no information about human donor fibroblasts and macrophage isolation. Nor is there information on which and how many lines of iPSC-derived cardiac organoids will be used.
Is the project feasible?
• The team has the appropriate leadership and expertise to carry out the project and access to all needed resources and staff. The budget and timeline are appropriate. • The assays are standard. However, the pilot study was in mice. The team did not mention the plan details for human donor fibroblasts and macrophages isolation. In the iPSC-organoids system, it's not clear how many iPSC line they plan to test.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• The project will account for the influence of genetic and environmental factors by testing iPSC-derived fibroblasts and cardiomyocytes from diverse populations obtained from CIRM iPSC. • Findings from this project can potentially promote translation and therapeutic applications.

Application #	DISC0-17614
Title	A humanized organ-chip model to investigate neuroinflammation contributions in Frontotemporal Dementia
Project Objective (as written by the applicant)	The research will explore how C9orf72 mutations affect immune-neuronal interactions in FTD using stem cell-derived organ-chip models, identifying new therapeutic targets for regenerative therapies.
Impact (as written by the applicant)	The proposed studies would help in understanding neuroinflammation in FTD, particularly related to C9orf72 mutations, and help identify new therapeutic targets for neurodegenerative diseases.
Major Proposed Activities (as written by the applicant)	• A novel organ chip model to study neuroinflammation in neurodegeneration • Single nuclear RNA sequencing • Proteomics analysis of synapses • Role of Peripheral immune response in modulating CNS pathology
Statement of Benefit to California (as written by the applicant)	The proposed research will benefit California by advancing understanding of Frontotemporal Dementia, a growing public health concern, and identifying new therapeutic targets. By leveraging innovative organ-chip technology, the study could lead to the development of personalized treatments for neurodegenerative diseases, improving patient outcomes. Additionally, the research supports biotech innovation, positioning California as a leader in cutting-edge neurodegenerative disease research.
Funds Requested	$1,519,882
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	73
Median	70
Standard Deviation	4
Highest	80
Lowest	70
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	14

Key Questions and Comments

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

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

Does the project hold the necessary significance and potential for impact?
• There are no treatments available for neurodegenerative diseases such as frontotemporal dementia (FTD). Even in cases of FTD caused by known mutations in C9orf72, the mechanisms underlying neuronal loss, pathology and neuroinflammation remain poorly understood. This is in part due to challenges with existing animal and human models. As such, development of new models is required to better understand the pathogenic mechanisms. • The project aims to understand the pathogenesis of Frontotemporal Dementia (FTD), particularly in relation to the C9orf72 mutation which is already well studied. However this proposal focus more on the role of neuroinflammation which is understudied. Moreover, it will utilize an organ on chip technology to study microglial dysfunction and immune dysregulation, which is much needed identification of specific immune pathways that contribute to neurodegeneration in FTD will address a key knowledge gap. • The project seeks to establish a new organ on a chip model, and use it to better understand C6orf72 FTD in the context of neuroinflammation (microglia dysregulation and immune dysfunction). It proposes to address an important knowledge gap in this context, but perhaps one that is also an active area of investigation using human cellular and mouse models.
Is the rationale sound?
• The overarching premise is that iPSC-derived cells when cultured together will replicate some critical aspects of human disease that will allow novel insight into pathology and potentially, in the future, identify novel avenues for therapeutic intervention. • The role of inflammation in the disease process for neurodegenerative diseases including FTD is well established and the in corporation of cells related to this in the in vitro models is therefore well justified. There is limited evidence to date that immune cells derived from stem cells and cultured in vitro mimics the immune system in vivo or the similarity of microglia in vitro and in vivo. • The rationale for studying neuroinflammation and microglia dysfunction, and innate immunity in FTD is sound and well-justified. The need for the organ on a chip system and its superiority over multi-cell type 2D, organoid or assembloid systems, is less clear.
Is the project well planned and designed?
• This application focuses on the development of a novel organ-on-a-chip to model aspects of frontotemporal dementia. The application builds upon existing expertise in the development of a similar model focused on early onset ALS. • It is overall reasonably well designed, first establishing the system in one control cell line, then validating it in an additional 5 control cell lines and reprogramming and testing 6 patient cell lines, adding on perturbation (inflammation) and eventually treatment conditions, and multiple readout modalities. • There appears to be no measurement of repeat length in the six proposed patient-derived iPSC cases. Given the association of disease severity with repeat length this is an important omission. • The proposed studies lack clear criteria to determine whether the model sufficiently reflects human disease. • The project is planned in a stepwise manner. They will establish the model using control lines, then generate six patient derived lines, then use these lines for experiments. They will also investigate functional consequences. This is a massive undertaking and the level of ambition within the grant period is high.
Is the project feasible?
• The PI works within the *[name redacted]* laboratory. While the PI has a supportive letter, there is no evidence of financial commitment from the institution that would indicate the PI will transition to tenure-track faculty. • The project is ambitious and may be hard to complete in the given timeframe. • The team has expertise in cell models, neurodegeneration, lab-on-a-chip, and relevant analysis methods.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• Both male and female stem cell lines with C9orf72 mutations will be utilized. • FTD is among the most common neurodegenerative disease in patients under 65 years of age. Genetic cases of FTD account for 30% of cases, with mutations in C9orf72 accounting for a significant proportion of these.

- Yes, half male and half female lines are included.
- There are plans for outreach and educational activities including partnerships with patient organizations.

Application #	DISC0-18051
Title	Reversing Neuroinflammation and Alzheimer's Pathology via Therapeutic Blood Exchange, Regenerative EV Targeting, and Biomarker Discovery
Project Objective (as written by the applicant)	This project will establish circulating factors driving neuroinflammation and Alzheimer's, methods to remove and replace them with stem cell EVs, and biomarker based risk and regeneration prediction.
Impact (as written by the applicant)	This work will rapidly deliver clinically implementable strategies to predict, remove, and replace, metabolic and neuroinflammatory signatures driving Alzheimer's and most age associated disease.
Major Proposed Activities (as written by the applicant)	• We will identify circulating plasma components promoting neuroinflammation and Alzheimer's disease pathology in human stem cell derived organoids. • We will deliver a high throughput automated murine therapeutic blood exchange model to remove drivers of Alzheimer's pathology and improve cognition. • We will produce, profile, and human organoid screen clinical grade extracellular vesicles from promising and underexplored stem cell sources. • We will track enhanced deliver of therapeutic extracellular vesicles across the BBB with focused ultrasound technologies. • We will deliver a cross-tissue multi-omic based biomarker panel which predicts neurological disease, cognitive status, and regenerative responses.
Statement of Benefit to California (as written by the applicant)	This work address a critical need for accessible, regenerative therapies for Alzheimer's disease by predicting and removing systemic inflammation and blood-brain barrier dysfunction, relevant to a wide range of age associated cardiovascular, metabolic, and nervous system disorders. It offers scalable, cell-free interventions to mitigate aging and reduce California's projected $828.4B AD burden (2017-2030), and will benefit underserved populations disproportionately affected by neurodegeneration.
Funds Requested	$4,616,715
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	10
Highest	85
Lowest	55
Count	14

(85-100): Exceptional merit and warrants funding, if funds are available	2
(1-84): Not recommended for funding	12

Key Questions and Comments

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

Does the project hold the necessary significance and potential for impact?

- The applicant proposes to use a stem cell-derived model to discover potential aging and anti-aging factors. Stem cells will be used to produce extracellular vesicles (EVs) with possible therapeutic potential.
- Alzheimer's disease (AD) is a large public health burden in CA and the US (6.7M) and projected to double by 2050. There are no curative treatments, only those with limited disease-modifying activity - cholinesterases and antibodies - which are expensive.
- Plasmapheresis has a long history of being used in medicine to remove harmful antibodies. The PLASMA study testing whether fresh frozen plasma (FFP) from young donors could help in AD was inconclusive. In aged mice, administration of young mouse plasma and human cord plasma has been shown to reduce age-related deficits.
- The project addresses AD by applying therapeutic blood exchange (TBE) to modulate systemic factors - metabolic and inflammatory - that may contribute to neurodegeneration.
- Therapeutics and biomarkers to be developed may advance the field.
- The proposed collaborations leverage strong institutional and scientific synergies. The team's combined expertise in aging biology, AD models, EV biology, organoids, and clinical translation strengthens the project's impact potential.
- Applicants leverage two custom platforms for murine discovery work - blood plasma exchange, and focused ultrasound.
- The proposal leverages the CIRM-funded organoid core effectively.
- The team has expertise in AD, neuropathology, mouse AD models (primarily metabolism), AD organoids, and aging.

Is the rationale sound?

- Despite many reports demonstrating the ability of heterochronic plasma transfusions to reverse or accelerate the aging process in animal models, and identification of many anti-aging candidates, a promising anti-aging factor for clinical use has not emerged. Human trials of transfusion have been equivocal at best. The goals of the project are too broad, encompassing AD, metabolic dysfunction, and aging in general.
- The preliminary data focus on beneficial effects of therapeutic blood exchange (TBE) in the liver and on hindlimb perfusion post ischemia. There are no preliminary data on the blood brain barrier (BBB) or in AD models. It is unclear whether the applicant's TBE treatment dilutes aging factors or whether cellular components of the blood contribute. The mechanism is unclear, and the experiments seem inadequately controlled.
- The brain organoid model is claimed to have endothelial cells, but the source of these cells is unclear since the organoid seems to be derived from immortalized neural progenitors. If there are blood vessels present, they are likely to be abnormal.
- The scientific rationale is compelling, based on prior rodent studies showing that replacing aged plasma with saline-albumin can improve cognitive performance and reduce AD pathology.
- The approach is three-pronged: depletion (TBE), replacement (SC-EVs), and monitoring (biomarkers). Strong preliminary data showing TBE improves metabolic health, liver function, and blood flow. RNA-seq data demonstrate beneficial transcriptomic changes post-TBE.
- In Aim 1 the goal is to establish therapeutic efficacy and identify brain regions most responsive to TBE using a standard model of amyloid pathology, and potentially additional models.
- In Aim 2, the mechanism of action is explored with a multi-omics approach pre- and post-TBE. Aim 2 will also assess the role of EVs carry signaling molecules (proteins, RNAs, lipids) affecting neuroinflammation, synaptic function, and amyloid processing.

- Studies by [redacted specific lab] and others have implicated EVs in aging and neurodegeneration. TBE could alter EV concentration, source (cell of origin), or cargo content—leading to downstream effects on the brain.
- They will profile EVs (extracellular vesicles) isolated from mouse plasma before and after TBE using omics and explore correlations between EV changes and behavioral or neuropathological improvements following TBE.
- In Aim 3, some translational issues are addressed including dosage, frequency, and duration of TBE in animals, biomarker selection and a strategy for future IND submission, potentially in patients with mild cognitive impairment (MCI) or early-stage AD.

Is the project well planned and designed?

- Many prior studies have examined plasma from aging animals using multiomics technology. There are no preliminary data to indicate the specificity or sensitivity of the organoid assay for picking up factors that induce an aging phenotype, if indeed aging can be meaningfully measured in what is essentially a fetal model system. Levels of many plasma factors may change as a consequence of factors not directly related to aging.
- Effects of TBE may be the result of additive changes induced by a large number of factors.
- It is unlikely that specific factors will be identified in Aim 1.
- The preclinical design includes both behavioral and biochemical readouts in AD models, paired with rigorous TBE protocols.
- Potential challenges (e.g., volume tolerance in aged animals, interpretation of EV cargo) are acknowledged but mitigation strategies are only partly developed.
- For this project to translate successfully, the proposal must:
    - 1. Validate durable and meaningful cognitive benefit in animal models
    - 2. Identify mechanistically relevant plasma factors for biomarker development
    - 3. Differentiate TBE from plasma exchange or albumin infusion to justify a distinct regulatory pathway

Is the project feasible?

- The team's expertise in AD or biology of aging is unclear.
- The scope of the project is too large.
- They have access to necessary infrastructure, mouse models, and clinical data resources.
- The proposed budget and timeline appear reasonable for the scope of work, but additional contingency planning might improve feasibility.
- The project has a very ambitious scope across three major aims. The timeline may be challenging given the complexity.
- The proposal has heavy initial reliance on organoid screening.
- Some novel technical approaches may encounter unforeseen hurdles.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- Consideration is given to environmental exposures of communities at risk. Yes, the biobanks are built on samples from underserved communities. Potential therapeutics claimed to be less expensive than the MoAb currently in use for AD.
- The application discusses how systemic approaches like TBE could broadly impact age-related neurodegeneration and highlights their relevance to underserved populations with limited access to current AD therapies.
- Plans to investigate mechanisms using EVs may provide broadly applicable biomarkers or therapeutic targets.
- The proposal gives excellent attention to health disparities affecting underserved communities.
- It addresses cost barriers with potentially more accessible EV therapies.
- Diverse cell sources representing multiple ethnic backgrounds will be used.
- The biomarker panel could enable early detection.
- The proposal includes a comprehensive data sharing plan following FAIR principles.
- Outreach and educational efforts were minimally described and could be strengthened, particularly in relation to diversity and stakeholder engagement.

Application #	DISC0-18026
Title	Nucleoporin regulation of hematopoietic and leukemic stem cells.
Project Objective (as written by the applicant)	This project explores how the nuclear pore protein Nup210 controls hematopoietic and leukemia stem cells fate, to advance stem cell biology and reveal new therapeutic targets for blood cancers.
Impact (as written by the applicant)	This research will address a critical knowledge gap in our understanding of how nuclear pore components regulate blood stem cell function and contribute to leukemia.
Major Proposed Activities (as written by the applicant)	• Determine how Nup210 regulates the homeostasis and function of normal hematopoietic stem and progenitor cells using mouse models. • Investigate how Nup210 expression influences leukemia initiation and stem cell activity using murine models of AML driven by MLL fusion oncogenes. • Use CRISPR/Cas9 to modulate Nup210 in human AML cell lines and patient-derived leukemia cells to validate findings in humans. • Integrate multi-omics data from mouse models and patient samples to identify Nup210 regulated networks in leukemia and normal hematopoiesis.
Statement of Benefit to California (as written by the applicant)	This research will uncover how Nup210 supports leukemia stem cells in acute myeloid leukemia (AML), a cancer with high relapse rates that disproportionately affects older adults in California. By revealing how Nup210 contributes to blood development and leukemogenesis, we aim to identify new therapeutic targets. The project will also support scientific training, develop research tools, and foster collaborations, aligning with California's commitment to cancer and stem cell research innovation.
Funds Requested	$2,304,918
GWG Recommendation	**(1-84): Not recommended for funding**
Process Vote	All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG." Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."

Scoring Data

Final Score: 70

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

Mean	71
Median	70
Standard Deviation	10
Highest	85
Lowest	50
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	2
(1-84): Not recommended for funding	13

Key Questions and Comments

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

Does the project hold the necessary significance and potential for impact?

- Yes, the nucleoporin complex in normal and malignant hematology has not been well-studied or understood to date.
- Yes, the basic science, molecular capabilities, and patient-derived xenografts (PDX) and AML samples available are excellent and ideal for the proposed studies.
- This is a well-written proposal by strong team.
- The team is very strong on many fronts. They are leading experts on the role of nucleoporins in NL and leukemic cell biology.
- The collaborator offers expertise in AML, specifically in genetics/epigenetics and mouse models of AML. Another collaborator provides computational expertise.
- The proposal has a narrow focus on a specific gene (Nup210). The focus is based on the finding of its high expression and association with adverse prognosis in analysis of TCGA AML patient data by the PI and KO in mouse models. The significance and relevance to human AML is uncertain at this point.
- Nup210 is overexpressed in AML and correlates with poor outcome. If one can determine role of Nup210 then intelligent interdiction of this target may be possible in AML therapy which would be significant and impactful. Unfortunately XPO1 inhibitors have not been effective in AML

Is the rationale sound?

- The project is focused on a specific Nucleoporin (Nup210), based on its high expression in AML samples from (The Cancer Genome Atlas) TCGA across genetic groups, and the finding that KO in a mouse model reduces leukemia.
- The applicant presents strong and novel data linking Nup210 expression level to AML vs. normal blood cells, and a relationship to survival in AML patients. These preliminary findings are simple and clear, as shown in Figure 1, and a large cohort was used.
- The functional data using CRE-TAM KO of Nup210 is impressive, and is further expanded showing KO and OE modulate cell number in mouse and zebrafish.
- The molecular observation using GSEA in KO MLL model shown by RNA and ATAC that stemness or stem cell related genes are effected by Nup210 deletion provides a strong basis for the proposal's molecular work.
- Yes; the rationale is sound and evolving.
- The applicants overstate that Nup210 is required for leukemia. The data show leukemia is still present, is found in the blood, and colonies still detected. Thus leukemia is reduced, but not eliminated. Implications are related to clonal outputs that will be evident in human patient samples later on.
- Clonal populations and resistance are not studied in the PDX models, and thus it is difficult to relate homogenous mouse data to human heterogenous systems.
- There is a lost opportunity in using the drug, especially XPO1 (nuclear export inhibitor), in the cell lines or mouse transplants.
- The biggest concern is the patient samples, and how many and which will be picked. This is inadequately explained, and has a direct implication in providing evidence that mouse is a good predictor of Nup210 biology in the human.
- Molecular experiments are not well organized. The applicant does not describe how studies will be compared or data prioritized. Why do all of them need to be done? Full rationale is absent.
- It's unclear how mouse data and human molecular data will be compared and utilized.

Is the project well planned and designed?

- Yes. The mouse systems are likely very predictive, and well justified.
- Yes, the project is well planned. They will use OE, and KO models using hematopoietic CRE mice and look at quantity of LSK subpopulations and perform competitive transplants. All of these are straightforward.
- Potential pitfalls and alternative approaches were well defined in each section, which is a strength.

- It's unclear if AF9 vs. AF10 transduction will give different outcomes and how these will or will not be related to AML MLL mutant samples. This a leap.
- Heterogeneity and clonal resistance and clonal evolution in the human is not fully recognized. The mouse modeling and predictions are likely limited.
- The project extensively uses mouse models in more than half of the proposal. Of the other half, the majority focuses on leukemia cell lines. There is only limited use of primary human cells from patients with AML (in the last subaim).
- There is some discussion of experimental alternatives.

Is the project feasible?

- The expertise and infrastructure are in place.
- Yes. Resources are well established and AML patient samples are available, but unclear diversity and priority of use and competition for these rather unique samples.
- Yes, the budget and timeline are appropriate for the research proposed.
- Yes, expertise and resources are in place.
- Yes, the team has access to all needed resources.
- No concerns on budget and timing.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- Yes. This issue is acknowledged and AML samples will come from diverse ethnic backgrounds, and equally among male and female donors.
- Yes; this is addressed as well as possible via banked samples.
- The applicant proposes to use AML samples from patients of various ancestries and balance age and sex.
- The PI is involved in community workshops.
- Various outreach and educational activities are proposed and the PI has had previous roles in educational activities.

Application #	DISC0-17716
Title	Progression or Differentiation? What are the key factors that delineate Hypertensive Disorders of Pregnancy
Project Objective (as written by the applicant)	This project aims to elucidate the etiology of hypertensive disorders of pregnancy by using patient placenta-derived induced pluripotent stem cells to identify cellular mechanisms of disease.
Impact (as written by the applicant)	Identifying placental cell abnormalities in hypertensive disorders of pregnancy will improve early detection and lead to treatments for preeclampsia and hypertension, which currently do not exist.
Major Proposed Activities (as written by the applicant)	• Derive iPSC lines from four different subtypes of hypertensive disorders of pregnancy and perform cell-based assays and transcriptomic analyses. • Collect placenta samples and conduct RNA-seq analysis and molecular level data to identify hypertensive disorder subtype specific gene expression. • Perform maternal secretome profiling and integrate findings with clinical, molecular, transcriptomic, and histopathological results.
Statement of Benefit to California (as written by the applicant)	The proposed research will benefit California by advancing understanding of hypertensive disorders of pregnancy, which affects over 140,000 pregnancies in the state. The California Pregnancy-Associated Mortality Review determined that the majority of preeclampsia-related deaths are likely preventable if there are better early detection tools. Improved diagnostics and targeted therapies can lead to better maternal and fetal health outcomes, reducing healthcare costs and enhancing quality of life.
Funds Requested	$3,406,745
GWG Recommendation	**(1-84): Not recommended for funding**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

Scoring Data

Final Score: 70

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

Mean	71
Median	70
Standard Deviation	5
Highest	80
Lowest	65
Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	13

Key Questions and Comments

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

Does the project hold the necessary significance and potential for impact?

- The proposal focuses on an important and understudied disease, namely the various disease aspects associated with hypertension during pregnancy and the distinction or relationship to preeclampsia. The proposed studies utile stem cell derived cells and this approach is highly innovative. If successful, the proposal could add novel and important new insights into the underlying disease process, risk and pathophysiology.
- The thesis of this proposal is that preeclampsia is not on a continuum with chronic or gestational hypertension but is a separate disorder that develops during pregnancy secondary to abnormalities that occur early in pregnancy.
- Hypertension disorders of pregnancy are increasing now affecting up to 50% pregnancies. Preeclampsia remains a significant cause of maternal and fetal death.
- The application will use iPSC-derived models as well as in vivo samples to identify meaningful diagnostic avenues in part by better distinguishing hypertensive disorders of pregnancy (HDP) subtypes and their origins.
- This group has developed hiPSCs from preeclampsia affected placentas and more recently human trophoblast stem cells, which places them in a unique position to simulate trophoblast abnormalities. The behavior of a patient's cells can be correlated with clinical and histopathological outcomes.
- The 2 Aims will be addressed by 2 teams led by the PI and Co-PI respectively. The PI will lead the cell-based Aim 1 with the support of the Data Project Manager. This team led by the PI responsible for the clinical patient recruitment and the cell-based work to evaluate dysfunction in patient-derived hiPSCs. Collaboration with biobankers, a clinical research coordinator and science fellows and staff are key to this success. Aim 2 is led by a Co-I also with support from the Data Project manager and staff. This aim is also being bolstered by the proteomics guidance and support from a center for perinatal discovery.
- The two teams appear well integrated as evidenced by preliminary data gathering on both aims prior to submission.
- The team is highly qualified and the participating groups and infrastructure is highly supportive.
- Translation and analysis impact not well described.

Is the rationale sound?

- The proposal presents the current state of the field, areas of scientific interest and areas where expanding knowledge will add to the understanding of the underlying disease mechanisms. The proposal is timely and of significance as the disease areas will clearly benefit from further mechanistic insights into the disease processes.
- The applicant assumes a complete genetic basis that is also not leading to any clonal biases during the proposed generation of iPSCs then trophoblast stem cells (TSCs) and EVTs. It is unclear whether that holds true. In line with that, a major concern is the transformation of a complex organ-phenotype through multiple cell type conversions to a simplified cell culture model read-out. Aim 2 will add some context by profiling clinical samples.
- Preliminary data is mentioned but not shown despite plenty of space.
- Maternal vascular malperfusion (MVM) was identified as the hallmark pattern of placental injury in PE and siPE. Hovever MVM is also seen in some cases of gestational hypertension (gHTN) and in some non-hypertensive pregnancies. Thus the investigators are asking if there are specific differences in MVM unique to PE

Is the project well planned and designed?

- The project has a number of strengths. The use of stem cell derived models is justified and the proposed experiments conceptually are well supported and justified.
- The overall goal is to better understand the patient HDP pathologies and sources. A combination of clinical, pathology and cell culture model data should identify actionable differences and drivers. Design, numbers (few) and experimental conditions are well described. Data analysis and integration beyond the mention of available standard tools unclear.
- The study design is well justified. The inclusion criteria are well defined but do not include more detailed information which is necessary to evaluate whether the selection of the study participants in the different

groups is sufficiently stringent. For example, there are other well established works factors which influence disease risk (i.e. diabetes, the differences of the disease risk depending on the number of previous pregnancies, twins, etc).

- For analysis of maternal secretome (plasma) the major pitfall identified is sample heterogeneity. To address this, the applicants have designed the experiment to focus on cases with the same placental pathology and included non-hypertensive controls to allow detection of disease specific signals. The comparisons will ensure relevant clinical comparisons. If needed the n of samples for RNA-Seq will be increased.
- The study lacks a discussion how the treatment of the patients might affect the disease presentation, potential effects of the changes in the placenta as well as overall disease risk. With the limited number of participants, a very clear and stringent definition of the inclusion criteria for each group will be important for the overall power of the study.
- In Aim 1 the investigators will derive and characterize trophoblast stem cells from women with HDP. The goal is to conduct in depth cellular characterization in patient lines.
- Cell to cell line variability is discussed as a potential pitfall with which the team has previously satisfactorily dealt in prior studies. They will derive 4 lines each with at least 5 clones per cell line and select clones that pass QC then differentiate into trophoblast stem cells and verify by flow.
- 4 iPSC lines from 5 conditions = 20 lines. The banked patient cells have been collected for two conditions and current banking suggest enough cells will be banked from patients with two additional within year 1. A potential pitfall discussed is insufficient collection of cells for the fifth condition. This will shift the timeline for some deliverables. Although the applicant anticipate being able to shift prioritization for collection if enough samples are not collected in year 1 this will be expanded to year 2. This will shift the timeline for some deliverables early in the grant to a later quarter but not prevent deliverables by the end of grant period.
- A number of serum measurements will be collected. Again, it is not clear how the treatment and variability of the treatment (i.e. drugs) will affect these measurements.
- There are some concerns related to the fact that not all samples which might be required to achieve the proposed aims have been collected. While the investigators make an argument that the samples could be collected, the proposal would certainly be stronger if samples were available from the beginning.
- Limitations are well described and solutions are described. However either are concerns related to recruitment.
- The study lacks a detailed description on how the various data points will be analyzed. A more detailed description of the analysis approach would significantly improve the application.
- A significant pitfall is that proteomics are new areas of investigation for this team. To address this they have initiated consultations with Standard Biotools who is helping with experimental design and data analysis pipeline. They have also engaged with a center for perinatal discovery who have extensive experience in this arena.

Is the project feasible?

- The resources are very supportive and appropriate.
- The budget is appropriate.
- Through collaboration or consultation or via protocol the applicants have developed internally, it appears the team is well positioned to accomplish these aims.
- The budget for derivation of cells and molecular analyses seems appropriate.
- Technical aspects seem feasible. Expertise present, but concerns regarding the actual model system.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- The proposal can be extended to other populations and communities.
- These findings will be broadly applicable across pregnancy in all communities.
- All aspects are addressed appropriately.
- The data will include cells from pregnancies resulting in birth of both males and females. PE and maternal death from PE are significantly higher in the Black community, but the percentage of African Americans in the banked samples is low (5%). Presumably the data will be broadly applicable across PE irrespective of environmental or other external factors.
- As much as possible with reprogramming/stem cell derived models (depends on patients seen).
- Not fully representative for higher risk-populations.
- There are concerns that environmental factors are not well described and included.
- There are collaborative efforts in areas of weakness such as proteomics but specific educational activities other than weekly and monthly lab meetings are not specified.

Application #	DISC0-17622
Title	A Comprehensive Framework to Decipher and Leverage Human Skeletal Stem Cell Biology
Project Objective (as written by the applicant)	Elucidating human skeletal stem cell biology will provide the basis for a detailed mechanistic understanding of stem cell-based bone maintenance, aging and disease for new regenerative approaches.
Impact (as written by the applicant)	The lack of efficacious stem cell-based approaches urgently needed to treat musculoskeletal health conditions due to the study and use of heterogenous MSCs will be overcome.
Major Proposed Activities (as written by the applicant)	• Delineation and refinement of the human skeletal stem cell lineage tree • Single cell spatial in situ mapping of the human skeletal stem cell niche • Identification of differences in gene regulatory networks between freshly purified and cultured human skeletal stem cells • Optimization of in vitro conditions for the ex vivo expansion of bona fide human skeletal stem cells to generate therapeutic meaningful numbers • Testing of ex vivo expanded human skeletal stem cells for de novo tissue formation and regeneration in a xenograft setting in mice
Statement of Benefit to California (as written by the applicant)	California's population aged 65 years and over will approach the 10 million people mark by 2040. Age-related skeletal diseases encompass a wide spectrum of debilitating disorders. More than 6 million Californians suffer from osteoporosis or low bone density, leading to increased fracture risk and healthcare costs. Novel stem cell-based strategies will fuel the prevention and treatment of conditions related to skeletal degeneration improving quality of life and saving millions of tax dollars.
Funds Requested	$2,328,426
GWG Recommendation	**(1-84): Not recommended for funding**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

Scoring Data

Final Score: 70

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

Mean	70
Median	70
Standard Deviation	12
Highest	91
Lowest	50
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	2
(1-84): Not recommended for funding	13

Key Questions and Comments

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

Does the project hold the necessary significance and potential for impact?
• PI has presented extensive background data, mostly of work from their lab, on the characterization of human skeletal stem cell (hSSC). • The project will characterize skeletal stem cells. • The goal of the project is to define, spatially map, and functionally characterize hSSC lineages and their microenvironment. This is a continuation of pioneering work on hSSC isolation where the PI played a key role during their postdoctoral training. • Achieving these goals is likely to provide a new, effective platform for mechanistic studies of musculoskeletal disorders and may lead to the development of a new generation of therapeutic approaches to treat these disorders. As such, this work holds a significant potential to resolve major knowledge gaps in the biology and therapeutic applications of SSCs. • Considering the prevalence of osteoporosis and age-related bone disorders, functional characterization of human SSCs is relatively important and if successful, it would be impactful for possible therapeutic applications. However, considering major flaws in rationale and experimental design, the project will have limited impact.
Is the rationale sound?
• The project is based on a strong scientific rationale. During their postdoctoral training the PI played a major role in defining mouse SSCs (mSSCs), and in a partial characterization of human SSCs. The PI is now well-positioned to expand this initial work on hSSCs, and to comprehensively and functionally characterize subtypes and lineages of these cells. These advances will be necessary for clinical translation of hSSCs. • An age-related study of SSCs are important and well justified. • The aims are mostly exploratory in nature. • The rationale for further dissection of SSC lineage is not well justified, considering already known SSC subtypes and their functional phenotypes. • The rationale for Aim 2 (identification of targetable gene regulatory networks and compounds to enhance hSSC expansion) seems vague and exploratory.
Is the project well planned and designed?
• This is a well-designed project, and it is likely to generate meaningful results. • While the goals of the first aim are likely to be achieved within the timeframe of the project, the second aim is riskier. Nevertheless, even if the goals of this aim are not fully achieved, the proposed experiments are likely to generate useful results for future investigations. • Vague statements are made about the expansion in vitro of hSSCs, which will allow for the development of cells that could be used in regenerative therapies. • Overall, both aims are highly exploratory and heavily dependent on omics and screening studies, without a clear and focused data-driven hypothesis, and lack downstream focused prioritization plan. • Aim 1 outcome seems incremental. Also, the rationale for the addition of new antibodies for screening in Aim 1A is not clear. There is no clear justification for the need for and selection of new sets of antibodies. Additionally, experimental details about suggested in vitro and in vivo experiments are not well defined. • Aim 2 experiments for gene regulatory networks (GRN) and library screen are very exploratory, overambitious, and not focused enough. There is no clear plan to prioritization of top 100 GRNs and their subsequent validation. Same problem is also noted in library screen in Aim 2b. In addition, MTA assay alone is not enough to determine the stemness of the cells after expansion. • Sample size in some experiments seems not enough. For example, in Aim 1b, 3 donors per age group and 1 per sex seems too low for statistical analysis. Also, in the same aim for spatial transcriptomics of ageing human bones, young control group is missing.
Is the project feasible?
• The proposal builds on extensive preliminary data.

- The PI has presented extensive background data, mostly of work from their lab, on the characterization of hSSC.
- The PI is an assistant professor who started his lab in 2023. He has an excellent training track record and has appropriate expertise in leading the proposed project. He appropriately described a leadership plan as it applies to his laboratory personnel.
- The project is too vague and exploratory. There is a high dependence on omics without a clear prioritization plan.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- California's population includes individuals and communities that are disproportionately affected by the musculoskeletal diseases, including aged, low-income, and minority groups. Skeletal stem cells research holds a significant potential to result in new effective regenerative medicine therapies to treat musculoskeletal diseases thus benefiting a full spectrum of California's diverse population.
- The project accounts for the possible influence of genetic and environmental factors as well as patients' age.
- The proposal includes general statements about how the sex of the sample will be known and no sample will be excluded based on age, sex, race, medication/drug use, and co-morbidities.
- A very general statement is provided; the proposal states that older people will benefit.
- Considering major shortfalls in the application, the outcomes will have a limited applicability.

Application #	DISC0-17351
Title	Hematopoietic stem cell response to trained immunity
Project Objective (as written by the applicant)	Successful completion of the proposed studies will fundamentally advance our understanding of the hematopoietic stem cell response to trained immunity during the aging process.
Impact (as written by the applicant)	Because trained immunity is actively investigated as an intervention in the clinic, the proposed studies could lead to a novel therapeutic target for the treatment of diseases.
Major Proposed Activities (as written by the applicant)	• Determine whether aged HSCs respond differentially to trained immunity compared to young HSCs. • Determine a role of CD38 in HSC response to trained immunity at young age. • Determine a role of CD38 in HSC response to trained immunity at old age.
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	$1,994,415
GWG Recommendation	**(1-84): Not recommended for funding**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

Scoring Data

Final Score: 70

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

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

Key Questions and Comments

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

Does the project hold the necessary significance and potential for impact?

- The potential role of the CD38 NAD+ (nicotinamide adenine dinucleotide) metabolic enzyme in HSCs (hematopoietic stem cells) for 'trained immunity' responses is being explored using a CD38 KO mouse and small molecule inhibitor, with implications for blunted trained immunity responses in the elderly with high CD38 expression.
- Very unique proposal on innate immune training of HSC and progenitors will application to aged population for vaccination to cancer therapy.
- Well detailed and established.
- The ultimate goals would have great impact - the ability for the elderly to better respond to vaccination would be a distinct advantage and promote the healthy life course.

Is the rationale sound?

- Strong preliminary data to support CD38 is implicated in trained HSC immune response, but not in homeostatic situations e.g., KO CD38 has no differences in cell number or proliferation in KTLS or SLAM enriched HSCs using young mice, but KO HSC are compromised in CRU assays.
- Information regarding origin and details of 78c and its specificity as an inhibitor of CD38 is lacking.
- Given the molecular capacity of the team, it is unclear why CRISPR KO was not directly examined with and without 78c treatment in human HSC or progenitors. The proposal relies on genetic causal data from mouse and extrapolation to human.
- Unclear why polymorphisms in CD38 are critical and if multiomic or separated RNA and ATAC analysis will be performed e.g., Aim 2.2.
- Cytokine stimulation and effects on TCA reduced ATP levels in CD38 KO suggesting a link of these processes e.g., CD38 biology and mitochondrial metabolism. Unclear if this was related or correlated to trained HSC.
- One of the issues highlighted was the disconnect in CD38 levels in young and aged mice and the central hypothesis around its link to trained immunity.
- Trained immunity needs to be explained and contextualized to the proposal. References are provided, but little description or detail is given to appreciate the goals of the proposal and background literature.
- Concern: Figure 10 shows that CD38 expression promotes HSPC expansion in response to trained immunity. This seems contradictory to their hypothesis regarding the effect of high CD38 expression on elderly HSCs.

Is the project well planned and designed?

- The project is well planned and designed. The sample sizes of human patients could be too small to see effects - especially if the level of non-response is high. It would be difficult to observe differences with comparative techniques.
- Data on CD38 deficiency in young HSCs show a modest reduction in ki67 staining (Fig 2D), suggesting additional roles (beyond proliferation; e.g., self-renewal, differentiation) for CD38 in regulation of HSC biology. The data in Fig 5 with old (24 month) HSCs would be stronger if a young comparison group were included and if the ribosomal gene expression data were described more thoroughly. The description of Aim 1.2 also lists 30-40% for non-responders regarding trained immunity from BCG vaccine.
- Mouse studies using KO systems of CD38 are well designed, but molecular results expected are poorly linked and/or detailed in relation to subsequent human studies of normal HSCs or HSC from BCG immunized patients.
- It is unclear how HSC vs. HSC from BCG will be compared in Aim 2.2.
- Is it possible that second CRU of KO CD38 by providing insights in rate of regeneration or frequency if done at LDA? KO HSC are compromised, but kinetics may provide additional insights in HSC biology and metabolism.
- Unclear, but the majority would at the level of molecular work up but unclear how clinical groups with BCG patient samples will be working with basic scientists on HSC measure and in vitro immune biology that will be assessed.
- Expected results are detailed. Alternatives superficially discussed.

Is the project feasible?

- Yes; the team is well-positioned for proposed studies.
- The budget and timeline are appropriate for the research proposed.
- Everything is in place including experts in the area. Clinical sample details seem less clear, but the sample are likely available.

• The overall concept is interesting; however, relatively little preliminary data are provided to support their hypothesis and consider alternatives. Unclear if elderly HSCs are not intrinsically defective in trained immunity responses via CD38 mechanisms.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• Yes, collection of HSC samples and BCG donors from a diverse cohort of donors is in place to assure broad ethnic backgrounds are represented. • Yes, the project outcomes extend or validate the applicability of regenerative medicine discoveries to additional affected populations, patients or communities. • Yes. The applicant team is participating in several outreach, partnership, or educational activities to inform population impact. • The applicant considers different age groups, and accounts for both sexes, but sample sizes are potentially limited for omicron comparisons.

Application #	DISC0-17940
Title	Lnc-ing myelin and oligodendroglial dynamics to autism spectrum disorders
Project Objective (as written by the applicant)	We aim to determine how long non-coding RNAs (lncRNA) regulate myelin-forming glia and contribute to region-specific myelin imbalances in autism through the use of iPSCs from individuals with autism.
Impact (as written by the applicant)	As lncRNAs vary between species, studying how they impact myelin dynamics specifically in human cells is imperative to understanding their contributions to normal and aberrant myelin development.
Major Proposed Activities (as written by the applicant)	• Elucidate the molecular mechanisms by which CHASERR (and other lncRNAs) regulate myelin, addressing a foundational knowledge gap in neurodevelopment • Establish CHASERR iPSCs as a novel and indispensable tool for studying lncRNA function in human neurodevelopment • Provide a scalable platform for testing RNA-based therapeutics, with potential to restore balanced myelination and improve neural connectivity in ASD • Promote inclusive, human-relevant research, ensuring that the benefits of regenerative medicine reach diverse populations
Statement of Benefit to California (as written by the applicant)	California is home to more children living with ASDs than any other state in the US. Given that different communities may exhibit distinct genetic and environmental factors contributing to ASDs, understanding how this diversity influences myelin dynamics in ASDs is vital to enhance therapeutic interventions. This project is particularly relevant in addressing the unmet medical needs of this diverse patient population as we will employ the use of iPSCs from varied genetic backgrounds.
Funds Requested	$1,386,620
GWG Recommendation	**(1-84): Not recommended for funding**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

Scoring Data

Final Score: 70

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

Mean	70
Median	70
Standard Deviation	3
Highest	80
Lowest	65
Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	13

Key Questions and Comments

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

Does the project hold the necessary significance and potential for impact?
• The contribution of myelin abnormalities to autism spectrum disorder (ASD) pathophysiology remains a critical gap in our understanding. Evidence for a mosaic of both hypermyelination and hypomyelination across and within individuals points to the importance of myelin regulation and dysregulation in ASD. • While patients with an extremely rare CHASERR mutation exhibit hypomyelination and ASD-like neurological phenotypes, the mechanisms by which CHASERR regulates myelination are not known. However, the proposed model that CHASERR exerts its regulation of myelination via CHD2 will not be directly tested. Given the extremely rare nature of CHASERR mutations the impact of these studies is substantially reduced. • Only four ASD cell lines will be used. As such, the role of genetic diversity on myelination dysfunction will likely not be adequately captured. Better selection and justification of individual ASD lines would improve the impact of these studies. • The project uses patient iPSC to study the role of lncRNA in myelination and is relevant to ASD. • The focus on myelination in ASD has merit. • The project has the potential to bridge a knowledge gap in the role of lncRNAs/CHASERR in myelination, and the contributions of oligodendrocytes versus neurons to this function. The implications for human disease and regenerative medicine are less clear.
Is the rationale sound?
• CHASSER mutations are associated with hypomyelination. Why study a rare mutation in lncRNA if target is known and relatively well studied? • Ultra rare disease model, however patient derived lines could be recreated on a different genetic background using gene editing. Myelination defects in ASD are common and brain region specific. Brain regions (specificity of myelination defects) are not addressed in the model. • Direct evidence that lncRNA in general target myelination genes in ASD is lacking. • Significance of gene expression changes in Fig 1a are unclear. Figs 2 and 3 provide only indirect evidence to support regulation of myelination by lncRNA. • Unlike CHD7/8, CHD2 mutations are not associated with deficits in white matter development but rather regulation of neurogenesis. The rationale for a role of CHD2 on oligodendrocyte differentiation is not clearly supported by the literature. This brings into question the scientific premise for this application. • The rationale for exploring the role of CHASERR in regulating myelination in oligodendrocytes is robust, but gaps remain in the connection to ASD. 1) The lines with CHASERR mutations are advertised as a unique resource, but it is unclear what clinical diagnoses these patients have? 2) The ASD lines seem to be from idiopathic cases (no mention of specific mutations). Patient lines with CHD2 mutations would have been a better fit, since CHASERR regulates CHD2, a high-confidence risk gene for ASD.
Is the project well planned and designed?
• The applicant describes a detailed analysis of iPSC-derived oligodendroglia at various stages of differentiation alone in Aim 1 and with neuronal co-culture Aim 3. However, these experiments are technically challenging and preliminary data demonstrating feasibility in the lab are not provided. • Preliminary data by reanalysis of the existing spatial transcriptomic data sets described in Aim 1 (page 14) should be used to further support the premise and rationale of the proposed experiments. If the existing published data do not support the rationale, this would further reduce enthusiasm. • Aim 1 will address brain regions specific expression of lncRNA ASD is a complex clinical disorder. How are ASD cell lines chosen? • It's not clear how well myelination will proceed in this system even in control cells; myelination is difficult to model in organoids. • If existing data are available as an alternative to Aim1c plans, why are they not analyzed and included as preliminary data here? • The main concern is the expected heterogeneity in the cellular phenotypes using the listed resources. The team describes heterogeneous phenotypes in ASD as far as 1) either hypo or hyper myelination; and 2) in males vs females. Given that non-genetically defined ASD patient lines (male and female) are used,

and only a small number, there is a good chance that the phenotypes might be too heterogeneous in this cohort.
Is the project feasible?
• Overall, the team has the necessary expertise, with one exception being perhaps expertise in models of neurodevelopmental disorders and autism. • The PI is an Assistant Professor at an academic institution in California who has published high impact studies on activity-dependent myelination while a postdoc and subsequently as a senior author examining oligodendrocyte lineage cells. The PI is also PI on one active NIH R01 and has substantial funding active funding from other societies and foundations. • Neither team lead has expertise with hiPSC-based derivation of OPCs and oligodendrocytes. • The PI is a very capable neuroscientist and white matter expert. • Brain tissue-availability of high-quality material is not clear.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• The project initially focuses on the pathobiology of mutations in an ultrarare form of ASD. Additionally, iPSCs from various genetic backgrounds will be examined to account for the influence of genetics. • The PI has participated in outreach; other responses in the population impact section are unspecific and generic. • Yes, the project proposes the use of male and female lines and lines from donors of diverse ancestries.

Application #	DISC0-17893
Title	Exploring the potential of RNase H1 inhibition and forced accumulation of R-loops as new therapeutic strategies for myeloid malignancies
Project Objective (as written by the applicant)	The present proposal will explore whether exacerbating R-loop accumulation in myeloid cancer cells will impair the proliferation of malignant cells, representing a new therapeutic opportunity.
Impact (as written by the applicant)	Myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), since R-loops-directed therapies could complement and enhance standard chemotherapy and cancer immunotherapy approaches in MDS and AML.
Major Proposed Activities (as written by the applicant)	• Characterize the effects of ramping up R-loop accumulation in human models of myeloid cancers, through genetic use of RNase H1 enzyme. • Test if pharmacological inhibition of RNase H1 to exacerbate R-loop accumulation will impair the proliferation of human myeloid cancer cells • Identify additional R-loop regulators whose silencing will stop myeloid cancer cell proliferation, which could be targeted using FDA-approved drugs.
Statement of Benefit to California (as written by the applicant)	Myelodysplastic syndrome (MDS) is a malignancy commonly diagnosed in older individuals, characterized by clonal proliferation of hematopoietic stem cells. People with MDS are at risk of the disease progressing to Acute Myeloid Leukemia (AML), the most common type of acute leukemia in adults, including Californians. The goal of this proposal is to test novel therapeutic targets against these diseases, which would benefit the health of Californians.
Funds Requested	$2,727,360
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	5
Highest	75
Lowest	55
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

Key Questions and Comments

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

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

Does the project hold the necessary significance and potential for impact?
• Yes. Currently, mechanisms of MDS and AML disease are unknown, and drugs are largely ineffective to prevent relapse or AML onset. • The project is potentially impactful, if R-loop targeting and use of H1 inhibitors proves valid in primary MDS and AML samples. • Proposed collaborations are well organized. • The project aims to test the hypothesis that formation of R loops is a synthetic lethality and that inhibition of RNase H1 (the enzyme that degrades the RNA in R loops) may be a therapeutic target for MDS, AML and potentially other cancers with excess R loops. However the role and even the presence of R loops in MDS and AML is controversial. • The project holds the potential to resolve a key knowledge gap and proposes an innovative line of investigation around RNAase. • The proposed collaborations offer a unique synergy or advantage that augments the potential impact of the project.
Is the rationale sound?
• It is unclear what form of MDS or AML, if any, the mouse model is mimicking. Pediatric or adult MDS? Morphological and molecular comparison is required. Also, consideration of key clinical indicators of MDS to AML transition should be utilized to better contextualize the model, and later the findings, appropriately. • The proposal does not address why H1 knock out (KO) and TET deletion are both required for increasing dsDNA breaks, whereas neither TET or H1 KO alone has similar effects. This is not addressed at the molecular or biochemical level in the proposed studies. • It's unclear if the KO effect would differ if done initially in hematopoietic progenitor cells (HSPCs) vs. downstream cells. This is important to better understand the context-specific effects that may associate with R-loop and double stranded DNA (dsDNA) break targeting. • The proposal includes strong preliminary data to support targeting of R-loops. • KO of H1 increases DNA breaks, but only in TET inducible KO cells and not in wild type cells. Thus H1 seems specific to either TET or leukemia context or both. • The rationale is rather weak. It is based mainly on findings from the PI in a less relevant model, namely mouse cells with profound triple TET KO, whereas human MDS and AML harbors mutations in TET2 that typically result in haploinsufficiency. Additionally, the rationale for induction of immune responses by R loops is inconsistent with the clinical observations that MDS has not exhibited responses to immune checkpoint blockade. • The rationale appears sound but could use a greater amount of preliminary data to bolster the premise.
Is the project well planned and designed?
• This is a clear and concise proposal with strong data to support pursuit. • Team communications and management of all aspects of the project are well organized. • The proposal is clearly written and everything in place. The N's and n's are not provided, so it is difficult to fully assess feasibility as written. • It's unclear how subtypes of resistance will be addressed, and whether this relates to secondary AML vs. AML in the absence of pre-diagnosed MDS. This is a major concern for interpretation of results from this proposal. • The compensatory or redundant effects of H2 are not considered in the application. This is especially concerning as R-loops are correlative at best. There are no causal experiments proposed to determine if R-loop can be targeted, versus H1 having other biological rolls and effects (e.g., mitochondrial DNA and removal of RNA primers for DNA replication). • Existing drugs for AML and MDS should be combined with drugs screens and CRISPR screen proposed, with and without alpha-hydroxytropolones. • The efficiency of CRISPR and single guide RNA (sgRNA) in human samples, and how these will be selected and validated has not been described. Technical challenges are likely in terms of efficiency and specific sgRNAs. • Candidate DNA breaks will be found in genes from Aim 1, but how and if these will be validated or pursued is not mentioned. This lowers the value proposition of this work. • Team communications and management of all aspects of the project are well organized.

- There are several feasibility issues mainly pertaining to the genetic modification of human HSPCS. Homology directed repair to introduce hotspot mutations in the two target genes as the PI proposes may not be efficient enough to support the proposed studies without some type of selection. Delivery of Cas9 in human HSPCs is not considered. It's unknown if the proposed compounds in Aim 2 are cell-permeable; they have only been tested in recombinant RNAse H1.
- The project is appropriately planned and designed but dense in many places.
- Pitfalls are described.

Is the project feasible?

- The team has appropriate leadership and expertise to carry out the proposed activities.
- Yes. Experts for all aspects required have been gathered for this project.
- The budget and timeline are appropriate for the research proposed.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- Yes. The applicant acknowledges that more males get MDS and will look for this in results and use of samples. They also will attempt to use samples representing three ethnic groups for MDS patient samples.
- The applicant mentions that HSPCs from donors of different sexes and ancestries will be used if possible, but provide no specific description of how the latter will be accomplished.
- The PI proposes that the findings may extend to other cancers with abundant R loops, but this is highly uncertain.
- The project plan and design adequately address and account for the influence of genetic, environmental and/or other external factors.
- The applicant describes efforts for outreach.

Application #	DISC0-17853
Title	Oncogenic drivers of mixed phenotype acute leukemia and its genetic instability
Project Objective (as written by the applicant)	Mixed phenotype acute leukemia (MPAL) is a highly lethal cancer derived from a hematopoietic stem/progenitor cell. This study will determine molecular mechanisms of MPAL initiation and progression.
Impact (as written by the applicant)	We will identify key genes for driving MPAL development, a bottleneck in understanding MPAL biology, and uncover a major mechanism for promoting genetic mutations and drug resistance in MPAL.
Major Proposed Activities (as written by the applicant)	• Determine the roles of two novel genes we identified for their cooperation to promote MPAL initiation and progression. • Determine the roles of an aberrant DNA repair system in promoting acquisition of genetic mutations and drug resistance of MPAL. • Validate novel genetic and epigenetic alterations of the genes we identified in human MPAL patient cohorts.
Statement of Benefit to California (as written by the applicant)	MPAL has a dismal prognosis with the median survival in adults less than one year. The consensus to treat MPAL is lacking. There is an unmet medical need to improve our understanding of the disease and discover new therapies. California has the highest leukemia deaths and 2nd highest leukemia incidences in the United States. The proposed studies will uncover new MPAL disease mechanisms, which will inform the development of new therapies for MPAL patients in California and beyond.
Funds Requested	$3,152,434
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	67
Median	70
Standard Deviation	4
Highest	71
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.

Does the project hold the necessary significance and potential for impact?
• This project addresses a therapeutic void in mixed-phenotype acute leukemia, which has the worst survival of any adult acute leukemia subtype, by introducing druggable targets that have not yet been explored. • MPAL survival is the worst in leukemias and very little is known about resistance or refractory disease. • Yes, of all leukemias, stemness (e.g.,hematopoietic stem cells) is known to be involved in sustaining or allowing MPAL initiation. • The proposed study is based on findings pointing to the role of two specific gene alterations and of a pathway deriving from a mouse model of spontaneous MPAL (mixed phenotype leukemia), whose relevance to human disease is uncertain. While some data are presented to suggest potential genetic (rare) or epigenetic changes in expression of these genes in human MPAL, the evidence for a prominent role in the disease is limited. • The PI has expertise in basic research and mouse models; the co-I is a hematologist. Other team members provide expertise in bioinformatics, DNA repair and pathology. • This is a Team Track application that includes synergy of a basic science PI, a leukemia clinician scientist, and a bioinfomatician. This synergy offers the opportunity to explore mechanism, multi-omics analyses, and validation. • Strong team between the PI and Co-I. • A clear path to a patient-ready product is not well articulated.
Is the rationale sound?
• Mutations in the proposed genes occur in mouse MPAL samples and are mirrored in human datasets, providing coherence of a mechanistic hypothesis. • Aged mice and use of aged HSPC throughout are not addressed or acknowledged in the context of experiments proposed. What is the pre-disposed landscape that accounts for the need to model this in aged cells? • Aside from error prone instability, the basis of this in relation to drug resistance as set out initially is not experimentally addressed or questioned. The absence of drug combinations for therapy are notably missing from proposal. For example, relapsed cells, the nature of resistance clones, etc... are all aspects studied and proven to be important in other leukemias. In addition, the proposal makes no distinction in the model or in the use of a bank of refractory vs. relapsed disease MPAL cells. This is an essential question. • The origin of cells lines and when cell lines vs. mouse vs. human cells are being used progressively becomes unclear throughout the grant, making it difficult to appreciate how and when validation is required and the overall feasibility given the number of experiments proposed. • The chemical inhibitors and rationale for choice, dose, duration is not always detailed. It is not acknowledged that primary cells, mouse MPAL model and cell lines likely have very different tolerances to inhibitors and IC50, etc... • Epigenetic and transcriptional regulation of targets are shown, but it is unclear if this happens in the same cells or samples given the vagueness of informatics used to define these. Does this relate to patient variant allele frequency? • It is unclear how and why “PDX models” from St. Jude's are being purchased and how these relate to, or differ from, the proposed three growth factor mice recipients in MPAL transplants. • The number and combinations of knockout and overexpression mutants is impressive, but it is unclear why they are generated, how they will be compared or related to the hypothesis and when these will be used with drugs. • The relationship between Aims 1.1 to 1.2 and Aims 1.3 and 1.4 is questionable. • The basis of the preliminary data for Aim 2 is unclear, and the rationale to provide this data in this proposal is unclear. Comparisons are done with CD34+ HSPCs, but it is unclear if these are all sorted and if MPAL cells are sorted. This would make gene cluster and differential gene expression difficult. • Very strong and detailed rationale section. Since the preliminary data is extended in the experimental plan, it's difficult to discern what is proposed vs. already completed. • Knockout of one gene increases apoptosis of MPAL cells lines and this is increased further by inhibition of a receptor. This shows the gene is functionally important and collaborates with the receptor.

- Impressive use of MPAL mouse model. Very unique and the phenotype is generated and shows increase in indels and enrichment of C > T mutations via RNA-seq and bisulfite sequencing.
- Gain of function of one gene and loss of function of a second gene is likely, given the strong data shown in Figures 8 and 9 using a MPAL model.
- The degree to which findings from phenotypic mouse models translate to human disease has historically not been high, and the track record of these approaches is not great.

Is the project well planned and designed?

- The design is focused on two areas: 1) determining the cooperation between the proposed genes in MPAL; and 2) determining the roles of the proposed pathway in MPAL genetic instability.
- There is a well-constructed plan for team communications and management of all aspects of the project.
- The proposed human studies are mostly limited to genomics. Functional studies proposed mainly involve mouse models and some primary MPAL cells. There are feasibility concerns pertaining to genetic modification of primary human blasts which is technically challenging due to viability issues and limited *ex vivo* growth of these cells.
- There is not much discussion of obvious pitfalls pertaining to relevance and links to known genetic drivers of MPAL.
- The applicants have a done an excellent job in defining pitfalls and describing next experiments is required, e.g. plan b and even plan c in many cases.
- Contingencies are included, including inducible gene expression if overexpression is toxic, serial bone marrow transfers if HSPC transformation stalls, and alternative chromatin assays.

Is the project feasible?

- The team is composed of experts that have worked in this area for years.
- Skills and expertise are in place. Excellent resources, especially the basic and clinical partnership of the PI and Co-I. The institution's bank, with greater than 250 MPAL samples, with genetic and clinical annotation is very unique internationally.
- Patient samples and technologies are in place.
- Applicant institution has all the resources necessary for this project.
- The budget and timeline are appropriate.
- The budget is within DISC0 limits.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- Yes, the analysis shows enrichment of MPAL cases in the Hispanic population despite WHO suggesting different composition.
- The applicant institution cohort is enriched for Hispanic and Asian patients and the sample selection will reflect California census demographics.
- The findings could generalize to any leukemia with error-prone repair.
- Genetic factors are discussed.
- Yes, strongly, and detailed activities and philosophy related to inclusion.
- Educational and outreach activities described.
- Yes, the PI has and continues to participate in CIRM Bridges programs.
- Uncertain.

Application #	DISC0-17365
Title	Targeting Aberrant Basaloid and Basal Cell Transdifferentiation for Therapeutic Intervention in Pulmonary Fibrosis
Project Objective (as written by the applicant)	Pulmonary fibrosis is a fatal disorder of lung stem cells in older adults: Explore a mechanism connecting age with disease. A drug screening tool to fuel reversal of dysfunctional stem cell state.
Impact (as written by the applicant)	Mechanistic understanding of IPF cytopathology with age will change future IPF drug development. In part this will be powered by a new drug screening tool aimed at reversing the cytopathology.
Major Proposed Activities (as written by the applicant)	• Studies linking age to epigenetic risk for IPF stem cell dysfunction and disease • Studies expanding the role of small airway lining stem cells in cytopathology of IPF • Small molecule image-based screen for compounds that reverse alveolar type II (AT2) stem cells to airway basal cell transdifferentiation in IPF.
Statement of Benefit to California (as written by the applicant)	An aging population will confront the State of California and its citizens for decades to come, underscoring the importance of effective therapies against progression of age-related chronic diseases such as pulmonary fibrosis, an epithelial stem cell (AT2) disorder un-improved by current treatments. Our new data link epigenetic AT2 aging with disease risk. We will further define underlying mechanisms and screen small molecules to identify drug candidates reversing the fibrotic cytopathology.
Funds Requested	$2,483,451
GWG Recommendation	**(1-84): Not recommended for funding**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

Scoring Data

Final Score: 65

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

Mean	65
Median	65
Standard Deviation	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.

Does the project hold the necessary significance and potential for impact?
• The growing aging population is a global problem. Pulmonary fibrosis, affecting >200K patients in the US yearly, is a strongly age-dependent disorder without effective treatment. This application aims to dissect a causal mechanism connecting older age with the appearance of idiopathic pulmonary fibrosis (IPF) cytopathology and disease onset and find a therapeutic approach to reverse the pathological basaloid/basal cell accumulation in IPF patients. • This study is developed to apply a small molecule screening approach (Aim 3) aimed at restoring AT2 (alveolar type 2 cells) stemness and AT1 differentiation capacity in pathological basaloid cells, cell states closely associated with the initiation and progression of Idiopathic Pulmonary Fibrosis (IPF), a debilitating and treatment-refractory lung disease. The project has potential to address a critical knowledge gap in our understanding of how aging and epigenetic dysregulation impair alveolar regeneration. • This proposal aims to understand mechanisms driving the transdifferentiation of AT2 lung cells into basal stem cells and then to identify small molecule that could limit this process or reverse it. The goal will be to limit tissue damage during IPF, is a major health care challenge. Thus this project is timely and important. • The project is embedded within a strong institutional environment, with access to relevant expertise in stem cell biology, lung regeneration, and single-cell transcriptomics. • The proposal integrates diverse methodologies, such as small molecule screening, scRNA-seq, and epigenetic profiling, that reflect a multidisciplinary approach, albeit under the direction of a single laboratory. • This is a single PI application and the main collaboration is with a small molecule discovery center. This collaboration is essential and could allow the identification of small molecules reverting basal stem cells into AT2 cells. This collaboration definitely increases the impact of the project. • The screening platform relies on iPSC-derived AT2 cells, which differ in differentiation potential and epigenetic landscape from primary AT2 cells, raising concerns about translational relevance when epigenetics is a priority in the application. While primary AT2s will be used to validate the screen, it is unclear how donors will be selected and there are concerns hits may be missed. • It is unclear how well *in vitro* induced basaloid states recapitulate the in vivo disease state, particularly in the absence of key physiological cues such as air-liquid interface. • Much of the preliminary data is correlative with limited protein-level or in situ validation. • The aims and rationale for the project are sound and hold a strong potential for impact, however the specific research plans are difficult to follow and only partially would contribute to the delivery of the aims.
Is the rationale sound?
• Yes, the project is based on sound scientific rationale. The overarching goal, restoring AT2 stemness and AT1 differentiation capacity in pathological basaloid cells associated with IPF, is well-justified. • The hypothesis that age-associated epigenetic changes bias AT2 cells toward a dysfunctional, basaloid-like state is both plausible and biologically relevant, aligning with established literature and preliminary single-cell RNA-seq data. • The rationale in general is sound. • The proposed small molecule screening to reverse this cell state transition is a logical strategy that leverages the PI's prior work. • The rationale is partially supported by the available data, though there are key concerns regarding the interpretation and robustness of some preliminary findings. For instance, the RNA-seq data presented in Fig. 2 shows increases in basal markers (KRT5, TP63) and a modest decrease in surfactant protein C (SFTPC) expression with age; however, the magnitude of change does not clearly support a full loss of alveolar identity or complete transition to a basaloid phenotype. • While the observation of a predominant basaloid phenotype in donors over 60 is intriguing, it is unclear whether these donors had IPF, which complicates interpretation of age versus disease effects. • Other research groups have successfully maintained AT2 cells from aged donors in culture for longer durations than reported in this study, raising concerns about whether specific conditions in the current model may artificially promote basaloid differentiation. Without clarification on these methodological differences, it remains uncertain how well the in vitro findings reflect in vivo biology or pathogenesis of IPF. • The concept is strong, additional mechanistic validation and comparison with established models would strengthen the rationale further.

- AT2 cells play a key role in lung regeneration. So, the possibility to maintain their number upon injury could help to protect tissue and promoting repair. So, the rationale of the application is sound but the overall project is high risk.

Is the project well planned and designed?

- The use of in vitro organoid is very useful as the inclusion of xenograft approach in Aim 2.
- Aim 2 complements the project by broadening the scope of pathological features studied in vivo, it seems to shift the focus from epigenetic regulation and small molecule screening to ECM degradation and basal cell-mediated tissue remodeling. Clearer clarification of how the epigenetic mechanisms uncovered in Aim 1 and the therapeutic targets identified in Aim 3 relate mechanistically to the microcyst formation and MMP1 activity studied in Aim 2. The in vitro models do not generate microcysts.
- The validation in mouse models in aim 2 is justified, despite the known differences in murine airway responses and will give a relevant in vivo context to Aim 2. However, the integration of Aim 2 with Aims 1 and 3 could be strengthened.
- The project is logically planned in general, however at a closer look it is difficult to follow and there are flaws in the design which makes the proposal unfocused, especially in Aim 2.
- It's not clear how Aim 1 and Aim 2 would contribute to or inform studies in Aim 3.
- The application is well-written but some parts are difficult to follow. For example the link between Aim 1 and Aim 2 especially the focus on MMP1 is relatively unclear.
- The overall design of the project is conceptually sound and aims to address an important question in stem cell biology and lung disease. However, there are several concerns that limit confidence in the plan's ability to yield robust and translatable results.
- The use of iAT2 cells as the primary platform for small molecule screening is not fully justified given their known differences in differentiation behavior and epigenetic state compared to primary human AT2 cells. These differences could influence both the baseline phenotype and response to therapeutic candidates, potentially skewing results.
- While iAT2s offer scalability and real time reporting, the proposal does not address the potential limitations of the reprogrammed iPSC epigenetic state and how this may differ significantly from an aged state. It is not clear how the iAT2 will be validated and purity ensured.
- The design does not adequately address donor variability, particularly for primary cells isolated from IPF lungs, where cell quality and viability are often limiting. The absence of information on how many donors will be used or how variability will be controlled raises questions about the reproducibility of the findings.
- There is limited integration air-liquid interfacing cultures, critical for the alveolar cell function, which could more faithfully recapitulate physiological cell behavior. The absence of such models may limit the biological relevance of the in vitro findings.
- Much of the preliminary data is correlative and lacks protein-level or functional validation, weakening the mechanistic underpinnings of the hypotheses being tested.
- There is limited discussion of how well the in vitro phenotypes, including the basaloid state, align with in vivo observations. The absence of an air-liquid interface, which is known to influence alveolar cell behavior, is a notable omission not addressed.
- The project management plan lacks sufficient detail about how milestones will be tracked, how roles and responsibilities are distributed among personnel, and how challenges such as data reproducibility or experimental failure will be addressed.
- Critical controls (e.g., for viral transduction, IL-1β treatment, or donor cell variability) are not clearly described, which reflects a lack of attention to experimental design detail that could affect project execution.
- While the project proposes IL-1β as a driver of the pathological phenotype, the evidence is limited and largely correlative. There is no clear plan to test alternative inflammatory mediators or pathways if IL-1β blockade proves ineffective or non-specific.
- The role of fibroblast seems to be ignored while these cells are likely to play a key role in vivo and in their culture system.
- There is little information about the type of injury that can induce the transdifferentiation of AT2 cells into basal stem cells. Is it infection, smoking, etc.
- The application includes back up plan mainly for technical problems. However, conceptual challenges could also arise. Indeed, epigenetic regulation especially chromatin organisation might be affected by culture conditions. In addition JmJ3 might not be the only regulator involved. It is unclear what happens then.

Is the project feasible?

- The lead applicant seems to have access to all the resource necessary to achieve this project.

- The timeline and budget seems appropriate for such complex project. It is not clear who will be performing the experiments described in the proposal.
- The research team has extensive experience and strengths in nearly all aspects of the project. There are no concerns over their ability to perform and successfully interpret the proposed studies. The only potential gap is in iPSC technology and iPSC-derived cells. Including an iPSC expert could strengthen the project and ensure quality control in the aims involving iPSC-derived cells.
- Too many studies are proposed without clear vision of how the outcomes would contribute to delivery of the main aims.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- If successful, the project will identify new small molecule that could help to limit injury in lungs during aging. So, it could result in new treatment for a broad diversity of patients.
- Sex differences will be considered. However, environmental impact and genetic diversity are not mentioned in the application. This aspect could be included even in vitro.
- The applicants have a track record in mentoring and educational activity. However, the information provided is very broad and could have been more detailed.
- The support of summer interns (college student and medical interns) is described and presentation of the data at scientific meetings. The description of this outreach could have provided additional detail, but outreach initiatives appear to be in place.
- The proposal mentions the importance of finding new treatments to improve access to lower income patients, however does not mention the influence of gender or ethnicity and how these variates will be incorporated in the research plan.
- While young and old are stated it is not clear what age ranges will be used for the studies and how sex will be considered as a variable. This is particularly important for IPF which has a substantial male predominance.
- Environmental and other external factors influencing epigenetic and phenotypic changes are not extensively addressed.
- While the project focuses on aging and disease-specific mechanisms in alveolar epithelial cells, the limited clarity on how well in vitro findings replicate in vivo human tissue changes could restrict the broader applicability.

Application #	DISC0-17677
Title	Cell-specific effects of APOE4 gene editing in Alzheimer's disease neurodegeneration
Project Objective (as written by the applicant)	Define how APOE4 disrupts lipid metabolism and drives Alzheimer's pathology in human iPSC-derived neurons and astrocytes to guide cell-specific CRISPR gene therapies for Alzheimer's disease.
Impact (as written by the applicant)	This research will clarify cell-specific drivers of APOE4 toxicity in Alzheimer's Disease, overcoming a major hurdle to developing precise and safe gene therapy cures.
Major Proposed Activities (as written by the applicant)	• APOE4 gene editing using CRISPR in human iPSC-derived neurons and astrocytes to assess effects on lipid metabolism and AD-relevant phenotypes • Analyze safety, efficacy, and off-target effects of APOE4 editing to inform future therapeutic development • Perform cell-type specific gene editing in humanized late onset Alzheimer's Disease mouse models and evaluate translationally relevant outcomes
Statement of Benefit to California (as written by the applicant)	As the largest state in the US, California and Californians are greatly impacted by Alzheimer's Disease. Alzheimer's disease has devastating consequences for those affected and their caretakers. Alzheimer's disease also represents one of the state's largest healthcare expenditures. Our project will benefit Californians by directly employing Californians and attracting additional investments as these new discoveries in CRISPR-based treatments move towards clinical trials for Alzheimer's Disease.
Funds Requested	$4,838,321
GWG Recommendation	**(1-84): Not recommended for funding**
Process Vote	All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG." Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."

Scoring Data

Final Score: 65

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

Mean	64
Median	65
Standard Deviation	6
Highest	75
Lowest	50
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.

Does the project hold the necessary significance and potential for impact?
• The APOE4 (apolipoprotein E) gene is the major genetic risk factor for Alzheimer's disease (AD). The field lacks clarity on which cell type should be the target for gene therapy, specifically whether neurons, astrocytes, liver cells, or combinations are the key drivers of APOE4-mediated Alzheimer's pathology. • The study aims to systematically dissect the contributions of APOE4 in neurons and astrocytes, and peripherally in the liver, and clarify the precise targets for effective APOE4 gene editing, addressing a knowledge gap in the field of neurodegenerative disease research, and a critical bottleneck for developing targeted CRISPR-based APOE4 gene therapy for AD. • APOE4 is the most common genetic risk factor for AD, increasing the risk 10-fold in homozygous carriers. A major barrier to clinical translation of gene therapy targeting of APOE4 is the lack of clarity on which cell types and organs must be targeted for effective gene therapy. • The project involves novel pairing of a neuroscientist and a gene therapy scientist. • The project is of high clinical relevance: APOE4 is the strongest genetic risk factor for sporadic AD and impacts 40–65% of patients, highlighting a significant unmet need. A successful approach would have broad applicability. • The proposal's value lies in determining which approach (liver vs. CNS) offers a therapeutic benefit—this foundational knowledge is crucial regardless of delivery challenges. If liver targeting proves successful, that pathway becomes much more clinically feasible. • Most APOE is produced by the liver, and liver-derived APOE is linked to BBB (blood–brain barrier) breakdown, systemic inflammation, and microglial activation. • This collaboration provides strong synergy with clear benefits over individual efforts. Both investigators actively treat the target patient population, which is rare and offers real-world validation of research directions. The co-investigator's direct AD/APOE4 patient experience combined with the PI's expertise in acute neurological care creates a comprehensive clinical perspective. Patient engagement efforts are strengthened by both investigators' clinical credibility. • This collaboration between clinically active physician scientists in CRISPR/iPSCs technologies and mouse models of neurologic disease offer a unique synergy. However, it's not clear which lab will be responsible for neuron and astrocyte differentiation, and collecting phenotypes.
Is the rationale sound?
• The research is posed to address the possibility that APOE gene correction in the liver (rather than brain) could be a therapeutic avenue for treatment of Alzheimer's disease (AD). • The proposal is complex, testing gene therapy in APOE3 and APOE4 iPSC neurons and astrocytes, humanized APOE3 and APOE4-KI mouse models, and tissue-specific in vivo CRISPR delivery to the liver and brain. • Are there easier approaches? Perhaps cell-type-specific ectopic APOE4 expression in wildtype animals would be sufficient to study which sources of APOE are sufficient to cause or ameliorate APOE4-related phenotypes in neurons. • APOE is highly expressed by astrocytes, where it is responsible for synthesizing and transporting cholesterol to neurons. In AD, neurons also upregulate APOE and are the key cell type lost in disease. The liver is the main APOE producer, and animal models suggest that liver-produced APOE4 may contribute to AD pathogenesis, despite not crossing the BBB. • The team has a panel of APOE/AD patient and isogenic human iPSC lines differentiated to neurons and astrocytes. However, no data have been provided regarding neuron and astrocyte differentiation quality. Quality data across the 14 clones would be key. • The hypothesis is based on a strong body of genetic and mechanistic literature supporting APOE4 as a toxic gain-of-function allele.
Is the project well planned and designed?
• Given preliminary data included, there are major concerns about the ability to resolve APOE4 vs APOE3 phenotypes, or the extent to which CRISPR editing can rescue effects. • The discussion of potential pitfalls is poor.

- The differentiation quality and variation checking across the panel of the cell lines is missing, which would be the foundation for Aim 1. The potential problems of completely excising APOE from APOE4/4 cell types or mice are not discussed.
- Functional rescue is well defined as a phenotypic shift of E3/– cells or edited mice toward E3/E3 baselines and away from E3/E4 or E4/E4 phenotypes, without converting to APOE–/–-like profiles.
- The project scope is broad, covering multiple cell types, delivery vehicles, and in vitro/in vivo models, which could risk diluting focus. Behavioral rescue is the main therapeutic goal, but initial behavioral deficits are not shown in their mouse models. This gap reduces the clarity of Aim 2.

Is the project feasible?

- The PI is responsible for characterization of all APOE phenotypes but has an unclear track record in APOE hiPSC or APOE animal models. The Co-I is focused on new CRISPR therapeutics and will lead all in vivo CRISPR editing.
- This is a high-risk project with an uncertain timeline.
- The Co-I has extensive experience in administering gene editing vectors in vivo and assessing neurovascular, behavioral, and histologic response to therapies in mice. The PI is trailblazing the development of CRISPR gene therapies for neurologic diseases. However, it's not clear which lab will be responsible for neuron and astrocyte differentiation, and collect phenotypes.
- The institutional infrastructure includes iPSC core facilities, behavioral and histological testing, next-gen AAV tools, and omics pipelines.
- The multi-arm behavioral study design, coupled with histological and lipidomic profiling, may be difficult to complete within the grant period.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- The project may help to create CRISPR based gene therapy for patients carry APOE4.
- APOE4 is highly penetrant across ancestries, but with a small number hiPSC donors at this stage, the work does not yet represent outcomes from a diverse cohort.
- APOE has large effects in all ancestries.
- All mandatory DEI training has been completed.

Application #	DISC0-17499
Title	Engineering universal hPSCs for skeletal muscle
Project Objective (as written by the applicant)	This work will develop a universal human pluripotent stem cell strategy that will be safe and effective with potential to lower costs and treat more patients in immediate need of a cell replacement.
Impact (as written by the applicant)	Current universal strategies do not work for all diseases and are not safe. This work will identify identifying the most essential immune shielding factors able to restore function in vivo.
Major Proposed Activities (as written by the applicant)	• Evaluate two universal strategies in skeletal muscle in vitro • Generate and evaluate universal strategies in humanized mouse models in vivo • Identify essential immune shielding factors to enhance a universal cell strategy in skeletal muscle • Proteomics identification of immune shielding interacting protein partners • Development of an in vitro screen for immune shielding and validation of novel immune shielding factors • Evaluate immune recognition/shielding in vivo in humanized mice with muscle loss as well as restored function
Statement of Benefit to California (as written by the applicant)	Incidences in CA of fracture associated volumetric muscle loss is ~ 30.7 per 100,000 persons/year and many more occur in devastating muscle injuries. Generation of a universal cell strategy would enable generation of off-the-shelf cells that could treat patients with immediate needs and no access/financial support for treatments. Further this work will be informative for determining the effectiveness of universal cell therapies across a range of patient diseases amendable to cell therapy in CA.
Funds Requested	$4,225,803
GWG Recommendation	**(1-84): Not recommended for funding**
Process Vote	All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.” Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”

Scoring Data

Final Score: 60

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

Mean	60
Median	60
Standard Deviation	8
Highest	80
Lowest	50
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.

Does the project hold the necessary significance and potential for impact?

- The proposal aims to develop an off-the-shelf universal cell strategy that will work for skeletal muscle. Skeletal muscle presents a barrier to universal cell use since muscle is multi-nucleated, regions of rejection occur due to RNA sharing between nuclei including transfer of the MHC complexes.
- The proposal aims to identify novel immune shielding factors identified from the human placenta using proteomics and mass spec to engineer improved universal hPSCs, which hold the potential to resolve a knowledge gap for both understanding of the immune shielding and application of stem cell transplantation therapy.
- The development of universal strategies has the potential to treat all patients.
- The goal is to develop an off-the-shelf universal cell strategy that will work for skeletal muscle. This treatment will need to avoid the immune response.
- The collaborations offer a unique synergy including expertise in skeletal muscle differentiation and universal hPSCs *[(redacted PI name]),* immunology and humanized mouse models *[(redacted Co-I name])* and *in vivo* mouse models of VML and muscle engraftment from hPSCs *([redacted Co-I name]).*
- Volumetric muscle loss (VML) injuries are among the most common types of muscle injuries with high complications and any cell therapy for VML would be significant. In addition, developing a universal cell therapy capable of escaping immune response will be important.
- Considering the actual size of VML injuries in human and the need for a vascularized and innervated 3D bioengineered muscle construct, current approach of using only one cell type (muscle) in a very small tibialis anterior (TA) VML model in mouse will not lead to a significant therapeutic outcome.
- VML injuries are mostly chronic in nature, due to the need for tissue debridement in acute phase and its remodeling during initial healing. Acute VML model in an immunodeficient or partially humanized mouse model does not properly recapitulate the human VML scenario.

Is the rationale sound?

- Current strategies for generating universal hPSCs may not work for skeletal muscle as skeletal muscle is multinucleated.
- Another strategy - overexpression of eight (8) factors - has not been evaluated in skeletal muscle. The proposal has a sound scientific rationale, aiming to compare current strategies and identify novel immune-shielding factors derived from the human placenta.
- The team has robust protocols for skeletal muscle differentiation and engraftment *in vivo.* Preliminary data show the universal cell line iACT hPSCs can differentiate well, and can engraft and generate large regions of human myofibers in humanized BLT mice *in vivo.* Additionally, T cell–mediated activation can be detected in WT but not in iACT universal hPSCs, suggesting the presence of a functional immune detection platform.
- There is concern that there are no relevant preliminary data or any background research about identifying new immune shielding candidate factors from human syncytiotrophoblast cells from human placenta. There is no information about the cell source and how they are going to identify new factors.
- Generation of a universal hPSC line to escape immune reaction is a great idea. However, considering the need to overexpress several genes to achieve this goal using OE viral vectors brings significant safety concerns (such as genome instability, tumorigenesis). This has not been considered in rationalizing this approach.
- Considering the chronic nature of VML (as most cases are due to combat injuries and the wound needs initial debridement and recovery, before any muscle replacement therapy), using patient derived iPSCs might be a better approach without the need for immune shielding or the risk of tumogenesis due to several gene OE.
- Aim 3 will look at the cells characterized in Aim 1 and Aim 2 (Aim 2 is not needed, see below). An issue is that the VML model is not developed (Aim 3), so it is unclear if the team will be able to test the characterized cells from Aim 1.

Is the project well planned and designed?

- Aim 1 experiments are well defined and clear.

- It does not appear that the team has a way to test whether the cells can aid in volumetric muscle loss (VML), as Aim 3 appears to be preliminary.
- Aim 2 appears not to be well linked to the goals in Aims 1 and 3.
- Aims 1 and 3 is well planned and will likely generate meaningful results. Aim 2 the identification of novel immune shielding candidate factors from human placental needs a clearer and more detailed experimental plan.
- Potential pitfalls for mice model and alternative approaches presented. Failed of defining immune shielding factors in the human placenta is not identified. The source of the syncytiotrophoblasts from human placentas is not mentioned.
- Aim 2 is very hypothetical, underdeveloped, and vague. It is highly exploratory, without a clear data driven hypothesis. Proteomic screening, prioritization, and CRISPR a I or lentiviral screens are also underdeveloped and seems vague.
- In vitro screens for immune shielding and analysis are not clearly explained, or lack important experimental details (such as the need of cell irradiation, inclusion of pooled CRISPRa or CRISPRi or lenti OE screens, cell viability readout, etc.).
- The suggested VML model in TA is not a true representation of the human VML. Larger animal VML models and chronic VML models are more appropriate.
- Aim 3 experiments need longer time course studies to evaluate safety.

Is the project feasible?

- This is an excellent team. The proposal needs to provide more background for part of using syncytiotrophoblasts from human placentas to identify immune shielding factors.
- Overall, yes. However, there are a few feasibility concerns related to suggested experiments, such as smRNA-FISH on cryosections, CRISPR screens and in vitro immune shielding experiments which will benefit from adding convincing data.
- It's unclear how immune shielding “candidates” from the placenta will be identified in Aim 2.
- Aim 3 requires a VML model to test the cells that has not been developed, which makes it difficult to test any of the cells that are being characterized.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?

- Team states that they have been in discussion with muscle foundations regarding outreach and funding for this work.
- Development of a universal stem cell therapy for muscle in this project has important societal, biology and transplantation therapy impacts.
- Yes. A larger VML model is needed to deliver more impact for affected patient populations.

Application #	DISC0-17929
Title	Engineering synthetic gene oscillators to slow hematopoietic stem cell aging
Project Objective (as written by the applicant)	This research will apply synthetic biology to reprogram stem cell aging, advancing the knowledge about the biology of stem cell aging and the applications toward regenerative medicine.
Impact (as written by the applicant)	The proposed study could address the knowledge of stem cell aging and overcome challenges in stem cell-based therapies by improving the effectiveness of stem cells derived from aged patients.
Major Proposed Activities (as written by the applicant)	• Characterizing synthetic gene oscillator components in human leukemia cell line and primary human HSPC cells. • Constructing and optimizing gene oscillators to regulate mitochondrial biogenesis and rDNA stability in human HSCs. • Evaluating in vivo translational potential of engineered human HSCs with optimized synthetic gene circuits.
Statement of Benefit to California (as written by the applicant)	The proposed research will benefit California by advancing regenerative medicine, particularly in aging-related diseases. By developing synthetic gene circuits to regulate stem cell aging, the research can improve treatments for many age-related chronic diseases. Enabled personalized therapies could address diverse genetic backgrounds, enhancing healthcare access and outcomes, while positioning California as a leader in innovative biotech and healthcare solutions.
Funds Requested	$2,310,000
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: --

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

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

Key Questions and Comments

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

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

Does the project hold the necessary significance and potential for impact?
• Aging in hematopoietic stem cells (HSCs) is incompletely understood but is believed to occur via two distinct pathways: genomic instability and mitochondrial dysfunction. The Principal Investigator (PI) has previously demonstrated that synthetic gene oscillators could delay aging in yeast, which, according to the investigators, bears similarity to HSC aging. They now plan to apply the same principle to interrogate HSC aging. This is a fundamental gap in knowledge. • This is an individual PI grant, which would be strengthened if submitted as a Team Track project. The PI is clearly an expert in synthetic biology, computational biology, and aging, but additional team members with hematopoietic, HSC, and mammalian cell or whole animal modeling experience would be helpful. Some of the proposed collaborators could fit this role. • The ultimate goal is to develop cell therapies to restore HSC function that could be used in the context of transplantation cell and gene therapies, particularly for aging-related diseases, but at present the relevance of this approach to human aging is uncertain. • A key collaborator brings expertise in HSC biology. • The knowledge gap is aging in HSCs.
Is the rationale sound?
• The rationale is based on a synthetic genetic oscillator that the PI developed and showed to extend the lifespan of yeast cells. However, there have been no preliminary studies conducted to date to establish the feasibility of this approach in any mammalian cells, including primary human HSCs. This is a major limitation of this proposal. • The investigators are encouraged to apply again with more preliminary data supporting the application of these synthetic oscillator constructs in mammalian cells. • The biology of HSC aging is poorly characterized compared to that of yeast cells. A lot more preliminary work would be needed to establish strong rationale that this approach could work in mammalian cells and in particular in human HSCs. • This is an interesting idea, but the proposal needs preliminary data in HSCs including evidence of the ability to engineer synthetic gene oscillators for HSCs. • Preliminary data do not fully support this proposal.
Is the project well planned and designed?
• The PI and co-investigators have not discussed the genetic modification of the primary human HSCs in detail. • Some studies seem to be superficially planned. Specifically: integration of these constructs in human cell lines using lentiviral transduction is suboptimal due to variable expression from integration sites and silencing. Knockin in AAVS1 or another defined genomic site seems more appropriate. Issues of efficiency and silencing upon transduction of human HSCs are also not considered. There have been no preliminary studies to identify candidate elements as a starting point. • Some potential problems pertaining to efficient and reliable genetic modification of HSCs for the proposed studies are not adequately addressed. • The preliminary data do not support that this will work in mammalian cells, nor demonstrate that the group has adequate experience in mammalian cells, HSCs, genome engineering, or in vivo modeling. • This is a neat idea, but currently has weak support.
Is the project feasible?
• Access to resources is appropriate. • This is an individual PI grant, which would be strengthened if submitted as a Team Track project. The PI is an expert in synthetic biology, but additional team members with hematopoietic stem cell and genome editing expertise would be helpful. • There are no preliminary data in primary human cells or mammalian cells provided in the proposal. Without that, it is impossible to judge feasibility. • This is a risky project as planned and could yield no meaningful results. • Additional team members needed with HSPC, hematology, genome editing, and in vivo modeling experience are needed. • No. This is too big of a stretch for an individual PI grant. This should be a team grant or have stronger preliminary data in mammalian cells and ideally HSCs.

Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• The study incorporates use of HSCs from genetically diverse individuals and diverse sex and ancestries. • Yes, they plan to use a wide variety of genetically diverse HSPCs. • Yes, everyone needs HSPCs and reducing aging in HSPCs would potentially impact regenerative medicine across diverse populations. • Yes, there are plans for educational activities aiming at providing high school and undergraduate students hands-on experience on engineering and education on advances in stem cell biology and regenerative medicine. Also, K-12 outreach activities and planned collaborations with patient advocacy organizations and clinical networks. • Somewhat. The team had a program from 2015-2019 for two weeks in the summer for high school and undergraduate students. Outside of that, they say they will or plan to have outreach activities including working renewing their summer course, and partnering with the academic enrichment programs at the host institution. Also, they mention collaborating with patient advocacy organizations, but give no specific examples. • If successful, these data could be very helpful for the HSC biology and HSC gene therapy field, but that application is limited by the potential for successful implementation.

Application #	DISC0-17442
Title	Generation of a human synthetic limb-inducing cell
Project Objective (as written by the applicant)	Humans are not able to regenerate missing limbs. We will test a new method to turn stem cells into cells capable of stimulating a person's own cells to regrow limb tissue after injury or limb loss.
Impact (as written by the applicant)	No current therapy exists to regrow injured or missing limbs by implanting a universal cell type capable of mobilizing the patient's own native tissue to regrow the missing tissue.
Major Proposed Activities (as written by the applicant)	• Test the ability of engineered neural crest cells to contribute to limb development in zebrafish • Generate human synthetic limb organizing cells • Compare human synthetic limb organizing cells to limb organizing cells from the zebrafish model • Test the ability of human synthetic limb organizing cells to induce limb formation in chick models • Test the ability of human synthetic limb organizing cells to regenerate injured limbs in chick models
Statement of Benefit to California (as written by the applicant)	Each year, over 15,000 Californians suffer full or partial limb loss due to diabetes, military trauma, and other accidents. The ability of some vertebrates to regrow limbs holds promise for human therapies, yet progress in this field has remained slow. Here, we take a wholly new approach by generating synthetic limb inducing cells from human pluripotent stem cells. Our long-term goal is to develop an off-the-shelf, universal human cell product capable of augmenting limb regrowth in patients.
Funds Requested	$3,323,200
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: --

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

Mean	--
Median	--
Standard Deviation	8
Highest	--
Lowest	--
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

Key Questions and Comments

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

Does the project hold the necessary significance and potential for impact?
• Approximately one in 28 newborns per year is born with some form of birth defect, and a substance that could regenerate limbs would be life-changing. • 5,000 Californians suffer full or partial limb loss, and a substance that could regenerate limbs would be life-changing. • The project will test the capacity of a fusion transgene to lock neural crest cells (NCCs) in a limb-inducing state. • At present the project has very little potential for impact.
Is the rationale sound?
• The scientific rationale is weak; it is not supported by strong preliminary data to suggest that the proposed approach would be effective in regenerating limbs in non-regenerating species. • The transgenic NCCs induce ectopic limbs. This is novel, but there are a ton of genes that induce ectopic limbs in vivo in embryos. • The rationale for the project is rooted in the idea that the proposed human gene expressing NCC-like cells can serve as a potent source of biological activity for limb regeneration in non-regenerating species and ultimately, in humans. The scientific rationale in support of this hypothesis is weak. • Also, with an eye for future clinical applications, safety of the exposure of human tissues to the proposed transgene - even transiently - is questionable.
Is the project well planned and designed?
• The research plan and aims are logical in overview. • The design of the project is logical. • Some potential pitfalls are identified and alternatives are presented. However, the investigators do not present viable alternatives if they cannot induce measurable limb-inducing activity in a chick model. • The team is missing a limb biology expert. • The fusion transgene reprograms NCCs to a mesodermal fate, leading to de novo limb formation in zebrafish. However, that has no relevance to limb regeneration. • The applicant should review the work of John Saunders (1960s) and then Cheryl Tickle in the 1970s and 1980s (her 2004 paper is referenced, but there is important older literature). The applicant should also read the work of Cliff Tabin in the 1990's. These prior limb biologists all showed that cells/genes that can turn on the limb pathway can generate ectopic limbs and/or regrow limbs that have been truncated in various ways.
Is the project feasible?
• The team has the appropriate expertise to carry out the project. However, additional expertise in mammalian regeneration would be valuable. • The proposed experiments have all been performed decades ago (some in the 1980s, even before Shh was discovered) with cells/genes that can induce limb formation. It is unclear how the proposed experiments are different. • The team has the appropriate expertise to carry out the project. However, additional expertise in mammalian regeneration would be valuable. While mammalian regeneration experiments are not proposed in the Research Plan, the ultimate goal is to induce limb regeneration in mammals, and the expert's perspective on how the experimental design can be optimized for mammalian regeneration would be valuable.
Does the project include considerations for maximizing the impact of successful outcomes across affected populations?
• The long-term goal of the project is to develop a universally effective therapy for limb regeneration. Since limb loss occurs across all racial and ethnic groups, sexes, genders and age groups, the project's outcome will be applicable to a broad population.