DISC2 AWARDS

9/26/24

$22,455,687 **GWG RECOMMENDED**

$5,544,313 **REMAINING**

$28,000,000 **AMOUNT AVAILABLE**

$0 **BOARD APPROVED**

								Score Range		Number of GWG			
	APP #	TITLE	BUDGET REQ	FUND ?	SCORE (MEDIAN)	Mean	SD	Low	High	Y	N	Resubmission	Previous CIRM Funding
	DISC2-16738	Developing a universal CRISPR gene therapy approach to treat C9orf72 ALS	**$2,740,592**	**Y**	**95**	94	2	90	95	14	0	N	N
	DISC2-16715	Drug discovery for Charcot Marie Tooth Disease using hPSC-derived Schwann cells	**$2,471,664**	**Y**	**90**	90	1	88	92	13	0	N	N
	DISC2-16589	Genome editing of human Tregs to enable combinational tolerogenic therapy with T cell-targeted biologics for T1D	**$2,485,549**	**Y**	**90**	89	6	75	95	13	1	N	N
	DISC2-16638	Development of a Trifunctional Self-Renewing Memory CAR T Cell Therapy to Overcome the Heterogeneity and Suppressive Microenvironment of Glioblastoma	**$2,700,810**	**Y**	**90**	88	6	78	100	11	4	N	Y
	DISC2-16774	Therapeutic targeting of Glioblastoma Stem Cell survival and self-renewal signaling	**$2,567,379**	**Y**	**90**	88	6	80	95	11	4	N	N
	DISC2-16725	B cell receptor-mediated lentiviral expression of anti-HIV antibody	**$2,751,504**	**Y**	**89**	87	3	80	90	12	3	N	N
	DISC2-16562	Human induced pluripotent stem cells-derived glial enriched progenitors for the treatment of mild traumatic brain injury	**$2,255,189**	**Y**	**86**	87	3	80	92	12	1	Y	N
	DISC2-16590	Modulating cardiac myosin heavy chain isoform expression for treating cardiomyopathies	**$2,772,000**	**Y**	**85**	86	3	80	90	11	2	N	N
	DISC2-16772	Chemically engineered photoreceptors for vision restoration in retinal degeneration associated blindness.	**$1,711,000**	**Y**	**85**	85	2	80	90	13	1	Y	N
	DISC2-16704	In vivo engineering of immune cells for cancer therapy	**$2,721,797**	**N**	**83**	81	4	75	86	1	12	Y	N

	APP #	TITLE	BUDGET REQ	FUND ?	SCORE (MEDIAN)	Mean	SD	Low	High	Y	N	Resubmission	Previous CIRM Funding
	DISC2-16686	Development of iPSC-derived neural progenitors secreting GDNF for the treatment of ALS	**$2,677,445**	**N**	**81**	83	4	80	90	6	8	N	Y
	DISC2-16539	Development of a gene agnostic treatment for photoreceptor disease	**$1,225,975**	**N**	**80**	81	4	75	93	3	11	N	N
	DISC2-16799	Secreted Particle Information Transfer (SPIT) – A Cellular Platform for the Treatment of Systemic Genetic Disease, Starting with Fanconi Anemia	**$2,696,400**	**N**	**80**	81	3	80	90	1	11	N	N
	DISC2-16685	Transplantation of excitatory V2a interneurons to promote motor function recovery after spinal cord injury	**$2,771,998**	**N**	**80**	79	4	75	90	1	13	Y	Y
	DISC2-16652	Novel antisense therapy to treat neurodevelopmental and neurodegenerative genetic disease	**$2,088,264**	**N**	**80**	79	2	75	82	0	13	N	Y
	DISC2-16801	A small molecule therapeutic to differentiate cancer stem cells	**$2,772,002**	**N**	**80**	75	11	50	85	2	11	Y	N
	DISC2-16538	A Gene Therapy Approach to Cardiac troponin I Cardiomyopathy	**$2,772,000**	**N**	**80**	73	15	45	85	3	10	N	N
	DISC2-16569	Pioneering the next generation of MSH3 mRNA-silencing strategies to halt somatic expansion in Huntington's Disease	**$1,806,418**	**N**	**79**	77	2	74	80	0	14	N	N
	DISC2-16763	Development of a safe and effective transplantation strategy for engineered stem cell-derived islets for the treatment of diabetes	**$2,839,239**	**N**	**78**	78	2	75	80	0	14	N	Y
	DISC2-16784	Mechanism for the Curative Potential of Fucosylated Autologous Hematopoietic Stem Cell Transplantation in a Mouse EAE Model of Multiple Sclerosis	**$1,749,999**	**N**	**75**	77	3	72	85	1	14	N	N
	DISC2-16808	Gene corrected patient derived iPSC-based autologous platform for delivery of BDNF to treat Huntington's Disease	**$2,407,578**	**N**	**75**	77	2	75	80	0	15	Y	N
	DISC2-16581	Targeting protein-RNA interaction in cancer stem cells	**$2,727,716**	**N**	**75**	76	2	75	80	0	15	N	Y
	DISC2-16706	Cell-based, personalized gene therapy to precisely treat pulmonary hypertension	**$1,745,879**	**N**	**75**	76	4	70	80	0	15	N	N

	APP #	TITLE	BUDGET REQ	FUND ?	SCORE (MEDIAN)	Mean	SD	Low	High	Y	N	Resubmission	Previous CIRM Funding
	DISC2-16674	Developing a small molecule therapeutics for Parkinson's disease	**$2,661,248**	**N**	**75**	75	1	70	75	0	15	Y	Y
	DISC2-16660	HSC-inspired Metabolic Optical Biomarkers for Leukemia Stem Cells and Therapeutic Prediction	**$2,438,261**	**N**	**75**	74	2	70	75	0	15	N	N
	DISC2-16557	Providing a cure for pulmonary fibrosis using adeno-associated viral mediated SGPL1 gene therapy	**$2,806,731**	**N**	**75**	73	4	65	75	0	15	N	Y
	DISC2-16664	Pluripotent stem cell-derived liver organoids for treatment of liver disease	**$2,845,500**	**N**	**75**	73	3	70	75	0	15	Y	N
	DISC2-16680	Dual plasma protein and imaging biomarker of discogenic low back pain for stem cel therapy application	**$2,292,638**	**N**	**70**	71	4	60	80	0	15	N	Y
	DISC2-16624	Engineering tunable biomimetic adhesive hydrogel to deliver and enhance MSCs function for corneal regeneration	**$2,393,522**	**N**	**70**	69	4	60	79	0	15	Y	N
	DISC2-16549	Pluripotent Stem Cells for Osteochondral Repair and Regeneration	**$2,996,600**	**N**	**70**	66	6	50	70	0	15	N	Y
	DISC2-16730	Self-delivery of a liver-targeting CRISPR-Cas9 fusion protein for the treatment of acute intermittent porphyria	**$2,023,515**	**N**	**65**	68	4	65	75	0	15	Y	N
	DISC2-16640	Novel gene therapy vector using endogenous promoter to treat SLC6A1-related disorders	**$2,167,744**	**N**	**60**	60	8	50	70	0	12	Y	N
	DISC2-16752	Hyaluronidase-equipped NK-92 engineered with a chimeric antigen receptor as effective therapeutic candidate for pancreatic ductal adenocarcinoma	**$2,581,729**	**N**	**-**	**-**	6	**-**	**-**	0	15	N	N
	DISC2-16732	Dopaminergic regeneration of a novel nuclear Nurr1-positive neuronal progenitor derived from human embryonic stem cells by small molecule induction	**$2,804,000**	**N**	**-**	**-**	9	**-**	**-**	0	15	Y	N

Application #	DISC2-16738
Title (as written by the applicant)	Developing a universal CRISPR gene therapy approach to treat C9orf72 Amyotrophic Lateral Sclerosis (ALS)
Research Objective (as written by the applicant)	Development of a CRISPR genome editing therapy for Amyotrophic Lateral Sclerosis (ALS) caused by C9orf72 and the delivery vehicle required to bring this novel therapy to patients.
Impact (as written by the applicant)	A cure for neurodegenerative disease has never been achieved. CRISPR gene therapy for C9orf72 repeat expansion mutations could arrest or prevent C9-ALS/FTD.
Major Proposed Activities (as written by the applicant)	• Determine how to efficiently remove the C9orf72 repeat expansion from the genome of patient iPSCs using CRISPR • Determine the genomic off-target effects of CRISPR editing of the C9orf72 gene in patient iPSCs • Compare lipid nanoparticles (from the Clelland lab) and novel AAVs (from the Deverman lab) for the ability to delivery CRISPR reagents with broad coverage of the brain and spinal cord • Determine dosing of candidate therapeutic in the C9-BAC ALS model system in vivo • Determine biodistribution and biosafety of candidate therapeutic in C9-BAC ALS model system in vivo
Statement of Benefit to California (as written by the applicant)	Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative condition with a high cost to patients, their carers and the healthcare system. Arresting or preventing the onset of ALS in patients with mutations in C9orf72 would usher in the next phase of personalized and genomic medicine. We aim to change the course of ALS for patients, their loved ones and our healthcare system throughout California and worldwide.
Funds Requested	$2,740,592
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	94
Median	95
Standard Deviation	2
Highest	95
Lowest	90
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	14
(1-84): Not recommended for funding	0

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 14 **No:** 0	• Genome editing strategies are a good approach for familial Amyotrophic Lateral Sclerosis (ALS), a disease in high need of treatments and cures. • ALS is an incurable neurodegenerative disease leading to disability and death, with high health care costs. • The application will develop genome editing approach to correct a defect the C9orf72 gene that is frequently associated with familial ALS (about 10% of all ALS cases are familial) and autosomal dominant frontotemporal dementia (FTD). • Editing with CRISPR/Cas9 is expected to provide a therapy for most C9orf72 carriers. • The proposal will develop and eventually may produce a genetic therapy that will likely significantly improve patient care or provide a permanent cure. • Potentially curative for ALS with C9orf72 gene repeat expansions, which is a leading known genetic cause of ALS and FTD.
GWG Votes	**Is the rationale sound?**
Yes: 14 **No:** 0	• The project is based on a gene editing approach for C9orf72 repeat expansion mutation of relevance for ALS and FTD. • The excision of the C9orf72 repeat region while preserving all exons has been developed in patient-derived iPSCs. • The proposal aims and milestones are logical and will address critical bottleneck issues of in vivo gene correction in the brain. • A published *(PNAS)* paper describes the possibility of C9orf72 repeat excision in vitro in patient iPSCs. Preliminary results in the grant show that the applicant's process can achieve over 80% excision efficiency in patient iPSCs. • There isn't much description of the un-intended on-target edits. Straightforward application of DSBs has always been associated with frequent indels, large deletions and other structural rearrangements, particularly when two DSBs are induced at the target locus. • Yes. The applicant proposes a gene editing approach to remove a repeat expansion without exonic sequences. Two strong alternatives for delivery of therapy are proposed, and the project includes study in a mouse model to look at safety and efficacy. • Preliminary data show feasibility of editing strategy and provide some validation of delivery systems. Cellular models including patient cell lines, differentiation protocols, and functional readouts are established.
GWG Votes	**Is the project well planned and designed?**
Yes: 14 **No:** 0	• The project is well planned and makes use of appropriate human cell models and animal studies. The project is designed to be performed with high standards and scientific rigor. • The proposed plan and design is sound and there is high likelihood of producing a candidate gene therapy for ALS. • The screen for guides in iPSC and neurons is well conceived, as is screening for efficacy and toxicity *in vitro*. Sequencing assays to detect off-target events are well developed. Single molecule sequencing across the region of interest will be used to monitor

	excision. The proposed reporter mouse assay for delivery systems should be efficient and reliable. • The mouse model for the study of reversal of disease is the best available and complements the reporter assay for delivery of the CRISPR package. Biodistribution and safety will also be assessed in this study.
GWG Votes	**Is the project feasible?**
Yes: 14 **No:** 0	• Two delivery approaches (lipid particles and AAVs) will be tested, which increases the chance of success. The expected outcome for each aim is clearly listed and alternative strategies for each aim are described. • The team is well composed to carry out the project. • The planned studies can be accomplished within three (3) years. The PI and their collaborators have complementary expertise and skills to accomplish the proposed studies. • Yes. The PI has expertise in gene therapy and collaborators bring lipid nanoparticle and AAV delivery technology. These are leaders in their field. The PI chairs a relevant NIH consortium.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14 **No:** 0	• The gene editing strategy is universal. • Patient perspective is included in all stages. • This genetic form of ALS is prevalent in European populations. • The PI demonstrates strong commitment to DEI. The study will look at diverse genetic backgrounds in designing the targeting approach.

Application #	DISC2-16715
Title (as written by the applicant)	Drug discovery for Charcot Marie Tooth Disease using hPSC-derived Schwann cells
Research Objective (as written by the applicant)	The therapeutic candidate we aim to discover under this award is clomipramine, an antidepressant that might help reduce a harmful protein in the nerve cells of patients with Charcot Marie Tooth Type 1a disease (CMT1A).
Impact (as written by the applicant)	This therapeutic candidate has the potential to treat debilitating neurological symptoms in patients with CMT1A.
Major Proposed Activities (as written by the applicant)	• Initial assessment of clomipramine in human stem cell derived CMT1A myelin cells • Extended Characterization of clomipramine in human stem cell derived CMT1A nerve models • Efficacy assessment in transplanted human stem cell derived CMT1A myelin cells • Efficacy studies of clomipramine in mouse models of CMT1A • Understanding the mechanism of action of clomipramine • Data analysis and reporting of findings
Statement of Benefit to California (as written by the applicant)	The proposed research will benefit the State of California by potentially providing a new therapy for a debilitating neurological disorder that affects many patients. This advancement could improve their quality of life, reducing healthcare costs associated with long-term care and disability. Additionally, the research will bolster the state's position as a leader in biomedical innovation, creating opportunities for local biotech companies and fostering growth in the life sciences sector.
Funds Requested	$2,471,664
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	92
Lowest	88
Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	13
(1-84): Not recommended for funding	0

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 13 **No:** 0	• This grant uses Schwann cells derived from PSCs as a cellular model of CMT. The deliverable would be a drug, but development of this drug would be greatly assisted by the stem cell model. • This group is the first to produce good Schwann cells from PSCs, and this provides a unique platform for Schwann cell drug screening in cell culture. This also alleviates a previous bottleneck comprised of the relative difficulty of obtaining Schwann cells from human cadavers and biopsies. • The candidate drug, clomipramine for CMT, is a clear deliverable that would be ready for human trials soon after completion of this study. • The unmet medical need is Charcot Marie Tooth disease that affects the peripheral nervous system. The genetic defect is amplification of the PMP22 gene, which encodes a structural myelin protein in Schwann Cells, leading to axonal demyelination. Schwann cells have been very difficult to generate from iPSC, but the applicant has overcome this obstacle and identified a candidate compound for treatment. • CMT1A has no approved therapies other than surgery or physical/occupational therapy. CMT1A is a remarkably common rare disease. The project directly addresses an unmet need. • The use of iPSCs for drug discovery in CMT1A allows for a number of patient cells to be screened, broadening the genetic and population base for any future therapy. Focusing upon the Schwan cells that are the disease-specific cell-type is particularly compelling for CMT1A and potential therapeutics. • A screen has been performed and has identified a FDA approved compound that could be repurposed. PMP22 expression is reduced in CMT1A cells and is a very justified therapeutic target.
GWG Votes	**Is the rationale sound?**
Yes: 13 **No:** 0	• Charcot-Marie tooth disease type 1A (CMT1A) is a genetic disorder caused by duplication of the PMP22 gene. This leads to myelination abnormalities. Clomipramine is FDA-approved. This grant seeks to ascertain if it can be repurposed to treat CMT1A. • This is the first group to produce quality Schwann Cells from PSCs, a unique reagent for the proposed work. See Fig 2 for this quality achievement. Having these cells in hand enables the entire remainder of this grant. • PMP22 is a structural myelin protein. Using Schwann cells from PSCs, this group performed a screen of 1443 FDA approved drugs, finding that clomipramine, an FDA approved psycho-pharmaceutical, reduces PMP22 protein levels by about 50%. Because CMT is caused by a duplication, reduction by 50% approximately would restore levels of PMP22 to normalcy. • There is evidence that the rat model is feasible based on preliminary data that shows the PSC-based Schwann cells can be engrafted successfully in to rat sciatic nerves. Further, these cells seem to be active in myelination of crush injuries. • The rationale is sound and has been previously successful in the applicants' hands in a prior screen for diabetic peripheral neuropathy. • The foundation of the project uses iPSC methods to generate Schwann cells and will enable translation of a new drug candidate for CMT1A. They are complemented well by the mouse model experiments in Aim 3 with the collaborators.

	• A new screen identifies clomipramine as a candidate that decreases PMP22 levels in Schwann cells. Known mechanisms of action for the drug block neurotransmitter uptake, but it is not clearly stated if these mechanisms are relevant to guide studies on Schwann cells and peripheral neurons. • For CMT1A, the genetic defect is clear: a duplication of the PMP22 gene/locus. Reducing PMP22 should be an effective therapy. Some genetic therapies, such as gene therapy, are challenging to titrate and therefore pose a concern as PMP22 deficiency leads to a distinct disease condition (Hereditary neuropathy with liability to pressure palsies, HNPP). • The development of Schwann cell iPSCs is a compelling aspect of this project, and the PI has recently accomplished this. Co-culturing of hPSC Schwann cells and hPSC neurons provides a compelling experimental context to examine molecular engagement, as well as functional activity in Schwann cells and myelination activity. • A drug screen of ~1400 compounds has been completed and a lead candidate has been identified that decreases PMP22 expression (Clomipramine) and one that appears to provide general protective activity (Bupropion). • The appropriate genetic context is best captured in a human cell model. Several "humanized" animal models exist for CMT1A, and they are very valuable as the human PMP22 gene is present. However, the ideal model does not currently exist.
GWG Votes	**Is the project well planned and designed?**
Yes: 13 **No:** 0	• This is a very well planned project with convincing preliminary data and a unique ability to make Schwann Cells from PSCs. There is good proof of concept data already in place. A reviewer remains very supportive of this grant but is not sure the research into MOAs and PMP22 regulatory genes is necessary to move clomipramine to the clinic for CMT. At least, the efficacy studies should be performed before MOA studies are performed. However, this is a very unique opportunity, and overall enthusiasm is quite high. • There is quite a bit of effort devoted to exploring and evaluating genes that are targets of clomipramine. Though this is laudable, I am not sure this is necessary to evaluate if clopmipramine can treat CMT1A. Since the CIRM call seeks to speedily develop a therapeutic, this endeavor may increase the time needed to arrive at a clinically relevant treatment. Perhaps this would be best left to a different grant. • It is proposed to use CMT patient-derived hiPSCs then make Schwann cells from these. This is a good approach, but the control would be normal iPSCs from other individuals. The approach would be improved by genetically engineering PMP22 duplications in hIPSC (or hESC lines) thus providing an isogenic control cell line. This would remove the factor of inter-individual variation. • The use of a rat model for CMT (in which human CMT1A Schwann cells are introduced) adds a good in vivo component to the research plan. This will aid in vivo assessment of clomipramine. • It is a high quality project with many strengths. A minor concern is that the preliminary result for the MEA assays in Aim1 only examines firing rate. Depending on the device available (the cited preprint indicates a specific redacted device), could axon tracking, and velocity be measured using a high density MEA system which would be relevant to a myelination phenotype? • Aim2 to define therapeutic target and mechanism of action has limited preliminary results identifying potential targets, although the applicant has an effective pipeline from previous studies. This aim seems open-ended with a proposed new genetic screen, and use of pharmacological agonists or antagonists. • The PI has a long-established track record of excellent work in closely related areas and leverages years of prior experience from cell transplantation/engraftment studies to Schwann cell hPSCs. The preliminary data are very strong and highlight the breadth of the team's research • The proposed experiments are well designed and appropriately controlled.

	• The inclusion of the CRISPR studies in which genetic pathway constituents will be identified/validated by CRISPR knockout is not well justified. While the clomipramine pathway may be partially elucidated, this does not move the compound closer to the clinic, and "hits" may fail for a variety of reasons. • It is not clear why the transplantation studies provide greater efficacy "power" compared to the C3 CMT1A mouse model. The engraftment comes with a number of caveats and the drug treatment may be impacted by the quality of the engrafted cells. Once a compound is identified, moving into a disease-relevant model, such as C3, seems appropriate. • There are some redundancies, but the timeline is appropriately aggressive.
GWG Votes	**Is the project feasible?**
Yes: 13 **No:** 0	• Yes, this is a very well constructed grant. Aims and timelines are achievable by this group. The milestones and timelines are realistic, not overly ambitious as is often seen. • This is a uniquely talented research team, and included individuals who have pioneered the production of Schwann cells from human PSCs. • The project is highly feasible as described with an outstanding team. • This 3 year project provides an aggressive, but achievable, timeline. Alternatives are included that are appropriate and well justified • The team is highly qualified and, in several instances, uniquely qualified for this project. • Yes – the team has worked in closely related fields and has all of the necessary resources. • There are no budget concerns.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13 **No:** 0	• Sex is addressed well. CMT is more prevalent in women, so female iPSCs will be made in sufficient quantity. Also, patient race will be addressed appropriately. • Yes-there are plans to specifically ensure that eventual treatments are tested and delivered to underserved racial and ethnic groups. • DEI is discussed mostly related to sex and potential for a diverse cohort of iPSC lines. • The plan includes DEI aspects for recruitment and employment. • The proposal includes the use of multiple cell donors from men, women and incorporates racial diversity in the genetic composition. Work from others has shown that the PMP22 duplication is not identical, and that using multiple models would be an important means to provide an effective therapeutic for a diverse population. • DEI principles are appropriately, although not extensively, addressed.

Application #	DISC2-16589
Title (as written by the applicant)	Genome editing of human Tregs to enable combinational tolerogenic therapy with T cell-targeted biologics for T1D
Research Objective (as written by the applicant)	Genome-edited autologous regulatory T cells for promoting immune tolerance in combination with T-cell-targeting therapies
Impact (as written by the applicant)	We focus on type 1 diabetes in this proof-of-concept study, but the therapeutic platform the study will develop can be applied to many autoimmune indications and transplantation.
Major Proposed Activities (as written by the applicant)	• Develop genome editing protocol to modify human regulatory T cells • Assess function of genome edited human Tregs in vitro • Assess the efficacy of combinational therapy of genome-edited human Tregs with T cell-targeted immunotherapies in models of graft versus host disease in a humanized mouse model • Establish a humanized mouse model of type 1 diabetes • Develop genome editing protocol to modify mouse regulatory T cells from humanized mouse • Assess the efficacy of combinational therapy of genome-edited mouse Tregs with T cell targeted immunotherapies in the humanized mouse models type 1 diabetes
Statement of Benefit to California (as written by the applicant)	Type 1 diabetes (T1D) affects 1.6 million in the US, including many Californians. Insulin is a lifesaving therapy for T1D by replacing this vital hormone that the patients cannot produce. A more effective way to treat T1D is to target the root cause of the disease - a faulty immune system. This project aims to develop an immune therapy by genetically modifying patients' own cells to restore immune self-control. This therapeutic platform may also biotech industry in California.
Funds Requested	$2,485,549
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	6
Highest	95
Lowest	75
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	13
(1-84): Not recommended for funding	1

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 13 **No:** 0	• Type 1 diabetes (T1D) is still an incurable disease and development of innovative therapy is needed. A FDA-approved immunotherapy for T1D is delaying disease progression, but not curative. The project would address a limitation of immunotherapy which depletes effector T cells but does not support and may inhibit protective function of Tregs. • This is a high significant project. It aims to develop a new therapeutic approach for overcoming autoimmunity-mediated pancreatic islets destruction responsible for Type 1 diabetes (T1D) using a combination immunotherapy that suppresses destructive effector T cells (Teffs) while sparing protective regulatory T cells (Tregs). If successful, the candidate has a strong potential to impact an unmet medical need. • The proposed study would generate expanded Tregs with gene editing that allows cell therapy with Tregs in combination with immunotherapy. If successful, the combination could be more effective than immunotherapy alone without significant side effects. • The applicant hypothesizes that a combination therapy of antibodies and autologous Tregs that are insensitive to them will favor protection of autoimmunity and delay onset of T1D. This approach will impact an unmet need and improve the life of people at risk of developing T1D and potentially those receiving islet transplants. • Initial work will be done using the FDA-approved immunotherapy, but the team will test a fully human antibody that should perform better in humans. • The proposed plan of investigation is overall outstanding and likely to deliver decisive results that have potential to change the treatment regimens for type 1 diabetes. • The team has a clear translational line of sight to the clinic and, hence, to improving the lives of people with type 1 diabetes. I would anticipate less than 10-year timeframe to patient care, if the project turned out overall positive with evidence of protected residual beta cells. • The antigen specificity of the clinical Treg candidate has not been fully considered. Tregs must be antigen-specific, e.g., engineered to express a TCR or a CAR specific for an antigen expressed in human islet cells.
GWG Votes	**Is the rationale sound?**
Yes: 13 **No:** 0	• This is an outstanding proposal aimed at editing Tregs in such a way that they would be insensitive to the immunotherapies recently approved to delay T1D onset. • There is sound rationale for the proposed editing in Tregs and allowing a combination therapy of Tregs with therapeutic antibodies. • The rationale for this product is that Tregs are also targeted by immunotherapies and their loss of activity has been shown to negatively impact T1D onset. By using gene editing to remove the immunotherapy binding site, the applicants plan to develop these drug-insensitive Tregs that would promote a more tolerant environment. • The project is based on a strong scientific rationale. The underlying hypothesis is that self-tolerance in T1D can be re-established by inactivating of autoreactive Teffs and boosting Tregs. A humanized mouse monoclonal antibody targets a T cell Receptor (TCR) complex and downmodulates activated Teffs. The antibody has been approved by FDA for T1D immunotherapy and has been shown to have a therapeutic effect in patients. However, the therapeutic effect of teplizumab is limited, because it downmodulates TCR on all T cells, including the protective Tregs. In the proposed project the investigators will engineer immunotherapy-resistant Tregs using a new

	editing technology to spare Tregs from immunotherapy downmodulation. They hypothesize that the protective effect of the engineered Tregs will synergize with the downmodulating effect of the immunotherapy on Teffs in restoring self-tolerance in T1D patients. • The project is highly innovative. To my knowledge no others have done or are currently attempting to address the same questions. • There is also good justification for using the proposed editing method with the goal of disrupting expression of the specific planned epitope without affecting functionality of the complex required for Treg function. • The team has shown the feasibility of using base editing to edit Tregs without affecting their function. Additionally, the important role of Tregs at promoting tolerance is very well known. • The project is enabling for the advancement of genetic therapies and could be enabling of stem cell-derived islet transplantation for patients that have lost all residual beta cell mass. • Clinical translation of Treg islet specificity remains uncertain. In the mouse model of T1D, the applicant would use Tregs from the TCR transgenic mouse. Such specificity cannot be used in the clinical product.
GWG Votes	**Is the project well planned and designed?**
Yes: 13 **No:** 0	• The project is well-planned, and it is likely to generate proof-of-concept data for further translational studies. The proposal includes a description of the specific steps to be taken for advancing the product into clinic. • The experimental plan is generally well developed with the use of the-state-of-the-art gene editing technology. • A xenogeneic GVHD model is already available for testing. There is also a clearly defined plan to obtain humanized T1D models. • In aim 2 they plan to use a mouse model to determine efficacy of the immunotherapy and gene edited Tregs in diabetes prevention and reversal. While this model might be challenging, the team has extensive expertise in this area and will be able to address potential pitfalls. • In aim 3, the team will test the feasibility of using a specified antibody that is entirely encoded by human genes. Luckily there is one in trial for multiple sclerosis. The team will test its efficacy in mouse models in comparison with immunotherapy. This alone could improve current treatment and eliminate the non-responders (who have been shown to develop antibodies). They will then create Tregs insensitive to foralumab and repeat experiments as in aim 2. • The potential pitfalls have been identified and clear contingency plans are in place. • The grant is extremely well-written.
GWG Votes	**Is the project feasible?**
Yes: 13 **No:** 0	• There is strong preliminary data showing that the applicant can successfully use a base editor platform to generate CD3EKO Tregs. • The project is feasible and likely to be achieved within the proposed timeline. • The proposed milestones are logical. The project is ambitious, and Aim 3, while interesting and reasonable, might be outside of the proposed timeline. • The PI is a recognized expert in T1D and has formed a synergistic collaboration with a leader in CRISPR genome engineering technologies. • The multidisciplinary nature and skills required for the project are unique and can only be accomplished by this team of investigators. • Yes, the team has the expertise, tools and state of the art equipment to perform the proposed activities.

	• The budget is appropriate.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13 **No:** 0	• The project plan and design adequately address and account for the influence of race, ethnicity, sex and gender diversity. • The therapy to be developed in this project targets T cells. The product will be autologous genetically modified Tregs. Thus, the therapy will be curative for a diverse population of people of different races, ethnicities, and genders. • This product will be available to everyone independently of race, sex, gender or ethnicity. • The plan does not adequately address and account for the influence of race, ethnicity, sex and gender diversity. • No plan for incorporating perspectives of the target population.

Application #	DISC2-16638
Title (as written by the applicant)	Development of a Trifunctional Self-Renewing Memory CAR T Cell Therapy to Overcome the Heterogeneity and Suppressive Microenvironment of Glioblastoma
Research Objective (as written by the applicant)	This personalized chimeric antigen receptor (CAR) T cell therapy will attack a wider range of cancer cells, and block cancer's defense mechanisms to empower the immune system to better fight cancer.
Impact (as written by the applicant)	This CAR T cell therapy will help patients with glioblastoma (GBM) and other high-grade gliomas, and it will advance the immune cell therapy field as a whole to treat solid tumors more effectively.
Major Proposed Activities (as written by the applicant)	• Develop a bispecific CAR that binds two different targets on tumor cells. This activity will test for anti-tumor function in several diverse glioma models both in vitro and in vivo. • Compare several approaches to multi-target CAR function, including pooling different CAR T cells, engineering CAR T cells to express two different CARs, or designing one CAR that binds to two targets. • Generate and optimize the anti-SPP1 blocking antibody (antigen binding portion, scFv) to be included in the human CAR T cell therapy. The anti-SPP1 will be secreted into the tumor microenvironment. • Evaluate in mice the anti-SPP1-secreting CAR T cells and compare to previous data of injecting anti-SPP1 systemically, which showed successful blocking of immunosuppressive tumor elements. • Optimize the CAR T cell therapeutic candidate that includes a bispecific CAR that targets two different tumor antigens and a secreted anti-SPP1 to block the immune-hostile tumor microenvironment. • Evaluate the CAR T cell therapeutic candidate in human GBM spheroid- and organoid-microglia models to support evaluation for the upcoming clinical trial.
Statement of Benefit to California (as written by the applicant)	Developing cellular immunotherapy both advances medical science and fosters local innovation, creating jobs and bolstering the state's reputation as a leader in cutting-edge healthcare. Through CIRM, California has pioneered cutting-edge immunotherapy treatments, maintaining its position as a global leader in biotech innovation, while providing hope and improved outcomes for patients statewide and across the world.
Funds Requested	$2,700,810
GWG Recommendation	***(85-100): Exceptional merit and warrants funding, if funds are available***
Process Vote	*All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."* *Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."*

SCORING DATA

Final Score: 90

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

Mean	88

Median	90
Standard Deviation	6
Highest	100
Lowest	78
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.

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 14 **No:** 0	• The project would lead to development of a next-generation chimeric antigen receptor (CAR-T) immunotherapy of glioblastoma (GBM), a highly aggressive brain tumor without curative therapy options. • Yes, the proposed technology is a bispecific engineered T-cell product that secretes an antibody to block secreted phosphoprotein 1 (SPP1), an immunosuppressive molecule found within the tumor microenvironment. The technology is likely to result in a candidate to treat GBM. • The new CAR-T product addresses key limitations of the current CAR-T cell therapies for GBM, including antigenic heterogeneity using bi-specific CAR design, suppressive tumor microenvironment (anti-SPP1 scFv), and persistence of the therapeutic cells (central memory cell manufacturing). • This is a highly translational project which is informed by results from the ongoing clinical trials in GBM patients. The applicant presents a logical progression from a successful candidate to the next clinical trial.
GWG Votes	**Is the rationale sound?**
Yes: 13 **No:** 1	• The scientific rationale for the project is very compelling. PI performed clinical testing of CAR-T cells targeting two different antigens and observed tumor escape when targeting a single antigen. This provides a justification for a dual-specific CAR. Moreover, the analysis of primary tumor samples showed that targeted genes have a reciprocal pattern of expression, and targeting both would cover > 90% of tumor cells. • The scientific rationale is sound. The targets of the cell therapy are validated through analysis of target antigen expression in patient tumors. The immunosuppressive target was identified as a factor that correlates with worse response from the previous version of this cell therapy. These data provide great rationale for the new design of this therapeutic. • SPP1 has also been discovered from the analysis of clinical samples of GBM patients treated with CAR-T cells. The results suggest that SPP1 serves as a major suppressive molecule produced by macrophages in the tumor microenvironment that inhibits T cell functionality and promotes tumor growth. This justifies adding anti-SPP1 single chain fragment variable (scFv) to the CAR-T cell product as a novel checkpoint inhibitor. • Yes, the preliminary data suggest reactivity of the CAR against two target antigens, and blockade of SPP1 synergizes with CAR T-cells in immunocompetent mouse models to eliminate the tumors.

	• Additional explanation is needed comparing the proposed product with the dual CAR T cells against target genes that have been reported recently from UPENN. This should include reasons for the lack of objective responses. • The rationale for the blockade of SPP1 is largely based on correlative evidence. The data presented comparing SPP1 blocking antibody with CAR T is confusing as the CAR-T has little activity. • Novelty of the proposal is the SSP1 blockade but there is no data provided as to the importance of that. • There is a feasibility weakness of not generating antibodies against SPP1 and it is not guaranteed to find candidates.
GWG Votes	**Is the project well planned and designed?**
Yes: 14 **No:** 0	• The project is exceptionally well planned and designed to achieve the expected outcome. PI proposes testing of various designs of dual CAR constructs and a stepwise testing using established in vitro and in vivo tumor models, including those with patient-derived xenografts. • Yes, the results of this study will provide sufficient data to advance this candidate towards a clinical trial, and the PI has adequately described the future steps. • Yes, the project is well-constructed. Aim 1 will evaluate the designs of bi-specific CAR proteins to eliminate heterogenous brain tumors, including mouse and human. Aim 2 will test the ability of anti-SPP1 secretion to synergize with the original CAR to eliminate GBM. Aim 3 will combine the leads of the previous aims to identify a lead candidate, which will be evaluated in patient-derived xenograft (PDX) models. • Anti-SPP1 scFv candidates will be appropriately tested in a syngeneic GBM model and the proposed experiments are supported by preliminary data in this model. • In the proposal there are innovative matrix-free spheroids models and patient-derived tumor organoid models with TME that will further inform selection of the lead construct for clinical testing. • There are no spatial transcriptomics proposed to determine the neighborhoods of SPP1+ cells and their interactions. • In the organoid study, single cell RNA sequencing (scRNA-seq) would be a better proposal than RNA-seq. Bulk transcriptomics of a mixed CAR-T, organoid, fibroblast, PBMC culture seems messy.
GWG Votes	**Is the project feasible?**
Yes: 14 **No:** 0	• The proposed milestones and expected project outcome are logical and likely to be achieved within the proposed timeline. • PI is a leader in the field of CAR-T immunotherapy in brain tumors. PI and the team are exceptionally well positioned to lead this project. • Yes, the investigators have great expertise and experience with CAR T-cells, pioneers in regional delivery of CAR-T cells. • City of Hope (COH) provides uniquely supportive environment for this project, including access to patient samples, established in vitro and in vivo experimental systems, statistical/bioinformatics support, proprietary reagents, and infrastructure for clinical translation with on-site cGMP facility and regulatory support.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**

Yes: 14 **No:** 0	• The project plan adequately addresses and accounts for the influence of race, ethnicity, sex and gender diversity.

Application #	DISC2-16774
Title (as written by the applicant)	Therapeutic targeting of glioblastoma stem cell (GSC) survival and self-renewal signaling
Research Objective (as written by the applicant)	This DISC2 tests a strategy to target survival and self-renewal in glioma stem cells (GSC) and will identify a therapeutic candidate for safety, dosing and efficacy testing.
Impact (as written by the applicant)	The glioma stem cell (GSC) population is resistant to radiation and DNA-targeted chemotherapy agents, driving tumor recurrence, and few therapeutic strategies have targeted GSC.
Major Proposed Activities (as written by the applicant)	• Investigate C3a-C3aR signaling in additional primary glioblastoma (GBM) cell lines in vitro, testing effects on proliferation, survival, self-renewal and metabolism. • Complete in silico design and screening of 100 RNAi candidates targeting C3aR expression with preliminary validation of sequences in HeLa cells, and 20 RNAi candidates for dose/cellular toxicity. • Complete testing of two different conjugate chemistries for GBM cell transfection and therapeutic candidate distribution after in vivo delivery. • Complete *in vivo* proof-of-concept testing for preliminary efficacy of 3 RNAi therapeutic candidates in combination with FLASH irradiation in an orthotopic GBM transplant model.
Statement of Benefit to California (as written by the applicant)	Glioblastoma (GBM) is the most common, aggressive, and lethal primary brain tumor. GBM has a median survival of 18-24 months. 70% of GBM patients exhibit progression/recurrence by 1 year after diagnosis and tumor resection, with less than 15% of patients surviving at 5 years. The glioma stem cell (GSC) population is associated with resistance to conventional therapy, contributing to tumor recurrence and emphasizing the need to target GSC to achieve effective treatment.
Funds Requested	$2,567,379
GWG Recommendation	***(85-100): Exceptional merit and warrants funding, if funds are available***
Process Vote	*All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."* *Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."*

SCORING DATA

Final Score: 90

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

Mean	88
Median	90
Standard Deviation	6
Highest	95
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.

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 14 **No:** 0	• Glioblastoma is a rapidly progressing, lethal cancer with very limited treatment options. 5-year relative survival is approximately 6.8%. Annually, about 10,000 individuals the U.S. die of this disease. The proposal has a good chance to result in a candidate that will inhibit the growth and likely kill glioblastoma cancer stem cells (CSCs). If so, one would predict a strong positive impact on survival. • The underlying mechanism is distinct from that of any current therapy, so there also is a good chance that a successful candidate would synergize with other established and potential treatment modalities, including radiation therapy, chemotherapy, and/or immunotherapy. • This may provide real hope for glioblastoma. • Glioblastoma is a devastating disease with short survival. Targeting the complement C3(C3) -complement C3a Receptor (C3aR) pathway is an interesting idea, and, if successful, would open an entirely new therapeutic approach. • There is no question that treatment of glioblastoma represents an enormous unmet medical need. The cancer is almost always fatal, with a short window for therapeutic options. • It should be considered that, even if the approach is not curative, an extension of life beyond 3 months (with perhaps far less severe side effects) would already be superior to what can currently be offered to newly diagnosed patients. • This application has great potential to improve patient outcomes.
GWG Votes	**Is the rationale sound?**
Yes: 14 **No:** 0	• The underlying assumption, that cancer stem cells (CSC) play a central role in the initiation and progression of malignant disease in general, and glioblastoma in particular, has been established over two decades of research in many laboratories. Furthermore, there is strong evidence that glioblastoma CSC derive from and phenotypically resemble non-transformed neural stem cells. • The applicant's laboratory made the innovative discovery that autocrine and paracrine signaling by complement protein C3a through its receptor C3aR is important for the survival and proliferation of neuronal stem cells. They asked if the same would be true of glioblastoma CSC and found strong evidence to support that hypothesis. This provides a sound basis to propose that blocking the C3A/C3aR axis could inhibit the growth and survival of glioblastoma CSC. • The preliminary data presented in the initial submission of the application (which is a resubmission) showed compellingly that an inhibitory RNA can prevent growth and induce programmed death of human glioblastoma cells in culture. They also showed convincingly that an inhibitory RNA can prevent tumor initiating cells (i.e., CSC) from starting new glioblastoma tumors in vivo, using an orthotopic PDX model. • The applicant discovered that GSC are "addicted" to C3a-C3aR signaling for survival and self-renewal. Consequently, blocking this signaling cascade using neutralizing antibodies increases cell death and limits self-renewal in tumor sphere formation assays.

	• Interestingly, the applicant shows that increased C3a-C3aR-CD133 co-expression is negatively correlated with survival and susceptibility to blocking this pathway is positively correlated with the presence of CD133+ cells, suggesting an impact on cancer stem cells- the rational is intriguing and solid. • The applicant is pursuing the uses of neutralizing antibody as well as siRNA approaches; this could be more efficacious in duration and would allow for repeated injections. • It is not clear why the siRNA approach is being taken, as arguably the best evidence provided in the proposal is using the blocking mAb.
GWG Votes	**Is the project well planned and designed?**
Yes: 14 **No:** 0	• The project is well designed and planned to discover and validate a candidate RNA drug for translational studies. The applicant's team appears to have a sophisticated understanding of issues around the efficacy of such drugs, preferred chemistry platforms for clinical use, and specific challenges for delivery to the brain. Experiments are well designed to generate a strong candidate molecule. • The likelihood of developing a translation-ready product candidate seems strongly enhanced by the collaboration with a biotechnology company that specializes in self-delivering inhibitory RNA technology (sdRNAi). Per the company, the "technology is based on the deep chemical modification of siRNAs and allows efficient delivery of RNAi into all cell types ex-vivo and to certain organs in vivo. sdRNAi delivery does not require the use of any additional formulation or delivery techniques." • The proposal includes logical milestones: 1. Establish primary glioblastoma (GBM) line to test consistency of the response to autocrine C3aR blockade 2. Screen RNAi candidates targeting C3aR expression to optimize the product 3. Use three lead components for in vitro and in vivo testing of divalent vs. cholesterol RNAi conjugates. 4. Test the effect of paracrine C3 on primary glioblastoma (GBM) cell lines in vitro 5. Increase production of lead component 6. In vivo proof-of-concept in combination with temozolomide. • Excellent discussion of pitfalls is provided.
GWG Votes	**Is the project feasible?**
Yes: 14 **No:** 0	• The plan includes well thought-out milestones, with a logical progression from step to step. The timeline appears appropriate. • The proposed team is strong, and the application displays a sophisticated understanding and approach to the biology and medicine of glioblastoma. The two key leaders are experienced and appear highly qualified in the fields of normal neural stem cells and glioblastoma, respectively. They have not co-published in these fields. However, the quality of the application indicates that they have shared their expertise effectively, which bodes well for a strong collaboration. • The project is highly feasible. The applicant has pioneered this approach, and all aspects of the work have been either conducted in stem cells already and/or are supported by preliminary data • The response of the applicant to previous questions and concerns has been outstanding, and all issues have been addressed in an effective manner.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14 No: 0	• The application demonstrates awareness of, and sensitivity to, DEI issues. The available data on glioblastoma do not show significant variation among racial and ethnic groups. Clinical outcomes are poorer for uninsured individuals, suggesting an impact of economic disparity. • Glioblastoma is a scourge across the diverse California (and US) population. Successful project outcomes would serve an important unmet need across the entire population. • The applicant is not including consideration of race, ethnicity, or sex diversity into the proposed experiments. For example, the source of the cancer lines used is not clear (sex, ethnicity?). It is also not clear whether only one sex will be used for the *in vivo* studies. Having said this, there are no compelling data that would suggest one sex being more affected.

	• The applicant cites data suggesting that non-Hispanic whites have a higher incidence than Hispanic whites, Blacks, American Indians, Alaska Natives, Asians or Pacific Islanders. The project plan and design do not address this issue (i.e., choice of lines to be tested). • The applicant cites data showing that individuals with insurance had more than double the survival length of those that were uninsured. Also, patients on private insurance fare better than patients on Medicare, irrespective of receiving the standard of care. While the applicant cannot address the underlying reasons for this disparity, they acknowledge that equally access to clinical trials could at least in part address these disparities. • The applicant is aware of some reasons for the unequal participation in clinical trials and identifies communicating with patients in underserved and underrepresented communities and leveraging patient advocacy groups, building relationships, and connecting patients with helpful resources as a necessary step that will need to be done if and when the project moves forward.

Application #	DISC2-16725
Title (as written by the applicant)	B cell receptor-mediated lentiviral expression of anti-HIV antibody
Research Objective (as written by the applicant)	With a single administration to an individual, lentiviral vectors that selectively transduce B cells in vivo and express highly potent anti-HIV-1 proteins to suppress HIV-1 replication throughout life.
Impact (as written by the applicant)	HIV infection can lead to AIDS if not continuously managed with medication and care. We are developing gene therapy vectors that can suppress and possibly eradicate HIV-1 by a single administration.
Major Proposed Activities (as written by the applicant)	• Characterization of the properties of B cell specific lentiviral vectors and analyze innate and acquired immune responses against the vectors • Targeted transduction of B cells in humanized BLT mice • Optimize the vector structure and the amount of vector administration necessary for maximal production and antiviral effects of eCD4-Ig • Examine preventive effects of B cell targeting lentiviral vectors expressing eCD4-Ig on HIV-1 replication • Examine therapeutic effects of B cell targeting lentiviral vectors expressing eCD4-Ig on HIV-1 • Leverage BCR signaling in transduced B cells to enhance and prolong eCD4-Ig production
Statement of Benefit to California (as written by the applicant)	Currently, there are an estimated 150,900 people living with HIV (PLWH) in California. Each year, California sees approximately 4,444 new cases of HIV infection, which can lead to AIDS if not properly managed with medication and care. Treating PLWH in California typically costs approximately $19,912/year per person. We are developing gene therapy vectors that can produce anti-HIV-1 antibody/antibody-like molecules after a single intravenous injection throughout life.
Funds Requested	$2,751,504
GWG Recommendation	***(85-100): Exceptional merit and warrants funding, if funds are available***
Process Vote	*All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."* *Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."*

SCORING DATA

Final Score: 89

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

Mean	87
Median	89
Standard Deviation	3
Highest	90
Lowest	80
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	12

(1-84): Not recommended for funding	3

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 14 **No:** 0	• Yes. There are more than 150,000 PLWH in California and more than 40 million in the world. A therapeutic that could induce long term suppression of HIV replication would be of tremendous value to these individuals in terms of quality of life and would further decrease costs of HIV care and HIV transmission. • Vector-based therapies to achieve constitutive expression of anti-HIV proteins are an area of great interest in the field currently. • There are several novel features of the proposed studies that could accelerate the likelihood of developing a successful genetic therapy for HIV. First, targeting of B cells for transduction is a novel and innovative approach. Second, testing the ability of expanding transduced cells via BCR stimulation is important because, if successful, it could be applied not only in this case but widely in the field. • If this approach is successful, it could lead to a revolution in care for PLWH in terms of being able to live fulfilling lives without requiring medications. • HIV by and large remains incurable. Anti-retroviral therapy has important limitations. This proposal to develop an *in vivo* B cell transduction technology to introduce, in this case, an anti-HIV antibody-like molecule into endogenous B cells that would then become plasma cells and secrete the therapeutic addresses a major clinical need. • The project holds the necessary significance and potential for impact. • If successful, this may provide a major advancement to the field. • Interesting science - not clear that it has the highest unmet need and should have priority for CIRM. As pointed out, there are considerable efforts ongoing for HIV treatment options at the NIH.
GWG Votes	**Is the rationale sound?**
Yes: 14 **No:** 0	• There is very sound rationale provided to justify utilizing eCD4-HIV based on its potency and lack of evidence of resistant strains of virus. Strong preliminary data support the proposal, including the applicant team's ability to create vectors, the B cell targeting, the proliferation of cells, and the production of transgenic product in mice. • Plasma cells have evolved to be protein factories, and this proposal leverages them for in vivo production of a therapeutic. They can be long-lived and secrete antibodies at physiologically relevant levels. The difference(s) in specificity between their different vector options is unclear. It doesn't seem like there is a big difference in the % of B cells vs. non-B cells (NK, T, DC, mac) targeted by the vector (Fig 2c). Isn't this an issue? • The proposal includes strong preliminary data demonstrating vector localization to B cells. This substantiates the applicant's premise.
GWG Votes	**Is the project well planned and designed?**
Yes: 14 **No:** 0	• The project plan has a well-outlined series of experiments to optimize the vectors, test them in mice, and to establish the feasibility of BCR stimulation to expand transduced cells. • This is a very strong scientific team with expertise in vectors, humanized mice, and immunology. The application includes strong letters of support from collaborators.

	• The applicant proposes imaging using luciferase to determine location of modified cells, but this is not very sensitive. The final experiment (Aim 3) that seeks to boost engraftment/induce proliferation seems to propose flow cytometry to study B cell levels is tissue compartments. This should be performed in all in vivo experiments to complement imaging. • The project is well planned and designed, with strong milestones throughout.
GWG Votes	**Is the project feasible?**
Yes: 14 **No:** 0	• The proposed experiments all seem feasible. There are no concerns vis-a-vis execution of the project. • This is a strong team with excellent expertise in vector design and HIV modeling. • The data generated supports feasibility.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14 No: 0	• The proposal incorporates sex in animal models. A limitation of animal models, however, is that sex and ethnicity/race of fetal derived hematopoietic cells used in reconstituting humanized mice limits evaluation of these factors. • Black and LatinX communities are disproportionately affected by HIV in California. Furthermore, gender non-conforming populations and unhoused populations are also disproportionately affected. Many in these populations are underserved. A therapy that enables one injection to achieve virologic suppression would greatly benefit these populations. • The proposal includes allocation of resources towards attending conferences and seminars that address healthcare disparities, drug use, youth engagement, and intersection of HIV with marginalized communities, as well as HIV Grand Rounds seminars with opportunities to engage with experts and community organizations.

Application #	DISC2-16562
Title (as written by the applicant)	Human induced pluripotent stem cells-derived glial enriched progenitors for the treatment of mild traumatic brain injury
Research Objective (as written by the applicant)	Allogeneic human induced pluripotent stem cell-derived glial enriched progenitor cell therapy to treat mild traumatic brain injury
Impact (as written by the applicant)	Prior to this study, glial cell-based therapies have never been tested as a therapeutic candidate for the treatment of mild traumatic brain injury.
Major Proposed Activities (as written by the applicant)	• Milestone 1- Determine hiPSC-GEPs efficacy • Milestone 2- Characterize hiPSC-GEPs-induced mechanism of repair Milestone 3- Determine plasticity and neural network connectivity changes
Statement of Benefit to California (as written by the applicant)	Mild traumatic brain injury is a devastating disease with no treatment. It is estimated that 100 to 300 per 100,000 people seek medical attention for mTBI annually worldwide. Nearly 32,900 people diagnosed with a non-fatal TBI are hospitalized in California each year. There is a crucial need for better therapeutic treatments towards enhancing recovery and rehabilitative mechanisms after mTBI.
Funds Requested	$2,255,189
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	92
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.

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 14 **No:** 0	• The proposed technology uses brief deferoxamine (DFX) treatment of iPSC to commit them to become glial-enriched progenitor cells (GEP). This technology is proposed for stem cell regenerative therapy of traumatic brain injury. The technology could have a strong impact on unmet medical needs for TBI. • Here they have an interesting cell product, a hiPSC derived Glial Enriched Progenitor (GEP) cell, which they are either testing or planning to test in several different indications. In this proposal, it will be used for the treatment of mild traumatic brain injury (mTBI). • This an intriguing product for this indication. Although mild concussions often resolve on their own, it is clear that longer term damage particularly from repeated mild concussion occurs in many individuals. Currently there is no effective treatment; thus, this would be a breakthrough, could be very impactful for this indication and may have positive effects on other disease states. • Mild repetitive TBI is a major health concern with no cure. The patient population is large and long term impacts are enormous. • Stem cell therapies have shown great potential and are at the forefront of therapeutics. • The applicant will test multiple eligible donor lines: two donor eligible cell lines from the UCLA GMP hiPS cell core and two hiPSC lines from a vendor as part of their partnership with CIRM. • Criteria for the safety profile are defined, CMC and manufacturing is addressed, and CIRM funded prior work in this area. • The dosing regimen is defined as 2-4 million hiPSC-GEPs per patient, and the delivery system is FDA approved. • The future target population are patients who experienced sustained concussions or repetitive head impacts (RHI) and experience post-concussive symptoms for at least two months after their injury. What are these symptoms? Is testing the therapy in a mouse model after 30 days post injury relevant for the proposed patient cohort? This is not discussed.
GWG Votes	**Is the rationale sound?**
Yes: 14 **No:** 0	• The rationale for using the GEP cells is strong. They are more quickly made than other stem cell types and have appropriate regenerative properties for the cell type. Preliminary results indicate their utility for mTBI treatment, and other applications to white matter stroke (WMS) and vascular dementia are underway by the Co-PI. • Recipients are expected to undergo immune suppression to prevent rejection of the GEP cells generated from a single iPSC line. The potential for using hypoimmune iPSC is not discussed and would help address DEI issues. • The previous submission was lacking preliminary data for this specific indication and so was a leap from white matter stroke where they had preliminary data. They have now added preliminary data in mTBI showing functional improvements. • The new preliminary data has added to an already strong proposal. They have done an excellent job of addressing previous reviewer concerns. • The rationale is logical. This is an astrocytic transplant, and mTBI shares many commonalities with WMS, where hiPSC-GEPs therapy has proven efficacy. • The applicant established a mouse model of repetitive mTBI that, to a certain degree, mimics human mTBI patients and is associated with long term cognitive impairments, damage, astrogliosis and white matter damage consistent with the human injury. • Previously reviewers asked for efficacy data. Applicants now show that transplantation days post last mTBI and analysis a few weeks post mTBI show reversal of behavioral phenotypes and high graft survival.

	• Applicants also characterized the cell post transplantation. Months post-transplant, hiPSC-GEPs expressed human GFAP, but not markers of neurons or oligodendrocyte precursor cells.
GWG Votes	**Is the project well planned and designed?**
Yes: 14 **No:** 0	• The project is well planned and designed to examine behavioral effects and dosing on treated mice. Applicants will study the mechanism of repair using proliferation/pathology and cell marker studies as well as snRNASeq, in vivo neuronal calcium imaging and brain slice electrophysiology to explore neuronal network function in damage and repair. • Potential pitfalls are discussed well. A limitation is the need for immune suppression in recipients. Hypoimmune iPSC could be considered to help overcome this limitation. • Here they will investigate acute and chronic mTBI in young and aged mice using behavior defects as an endpoint. They will also investigate dosing. • The applicants also plan to determine the cellular and molecular mechanisms of hiPSC-GEPs-mediated repair after mTBI. • They also plan to investigate the effects of hiPSC-GEPs on neuronal network function in mTBI and measure electrophysiological determinants of neuronal function in hippocampal slices. All of these milestones are important and should add to the overall body of knowledge in this arena. • The plan to measure behavioral deficits, vascular damage, and cellular and molecular changes using very cutting edge and innovative techniques including Motion Sequencing (MoSeq) analysis, Quantitative Cortical Mapping, brain slice electrophysiology, single nuclei RNA-sequencing and others. • The establishment of the MoSeq assay provides feasibility of testing a large number of animals in an unbiased manner. • The project is well designed, and endpoints are established. • Safety issues are addressed. The applicant shows a reasonable safety profile. They tested tumorigenic potential and found that the product (hiPSCGEPs) contained a rate of hiPSC below 0.1%, which is considered to be safe. • Dose ranging is included and will only be conducted with the most promising line. • Groups are defined, and the number of animals needed is clear. Using hiPSC-NPCs as a control is an additional strength. • A good pitfall discussion is provided. • One concern that was raised previously was the limitation of using NSG mice (as opposed to immunosupressive drugs) that do not represent a clinical model and might not allow for potential problems of graft survival and induction of inflammation. This issue has not been addressed, and the applicant is still focusing the entire study on NSG animals, despite acknowledging that immunosupression via drugs is common state of the art. The limitation thus remains a restriction upon the impact.
GWG Votes	**Is the project feasible?**
Yes: 14 **No:** 0	• The experimental plan has no drawbacks as written and fills some previous gaps by incorporating new suggestions made by previous reviewers. The milestones are logical and achievable. • The proposed team leadership is highly qualified for this project. Other key personnel are not described, making it hard to judge this aspect of staffing. • They do mention some pitfalls, but note all techniques employed have been used previously. Additional letters of support for core labs and collaborators would strengthen the proposal. • The entire team is not in place; this could result in slippage of the timeline. • The hiPSC-GEPs already have safety, identity, purity, activity, and stability qualification assays completed. This will help to remove additional work and risk. • This is a highly experienced team.

GWG Votes	Does the project uphold the principles of diversity, equity and inclusion (DEI)?
Yes: 14 No: 0	• DEI for the proposed project focusses mainly on sex and age, and the potential to examine more diverse iPSC lines in the future. The impact of mTBI on underserved communities is discussed. • They do address sex and age in their animal models, but they cannot address ethnicity/race at this point. • This would appear to be a universal therapy, but testing for this aspect can wait until more proof of concept information is obtained here. • The team appears to understand the perspective of many different groups and has incorporated that into the research plan appropriate for this stage. • Pre-clinical efficacy modeling will occur in both male and female mice. Multiple donor lines will already be tested. • The PI is active in the TBI community service and patient education, and advocates for TBI awareness and funding to promote diversity and equality in TBI research. The co-investigator is a mentor for a community college outreach program, a science small groups program and is also a mentor for the host university-Historically Black Medical College (HBMC) Research Program.

Application #	DISC2-16590
Title (as written by the applicant)	Modulating cardiac myosin heavy chain isoform expression for treating cardiomyopathies
Research Objective (as written by the applicant)	To discover CRISPR-based genetic strategies that will directly and therapeutically modify cardiac myosin heavy chain isoform expression to treat heart failure (HF) and cardiomyopathies.
Impact (as written by the applicant)	Heart Failure and Cardiomyopathies
Major Proposed Activities (as written by the applicant)	• Validate CRISPR-based MYH6/MYH7 gene regulation and switching strategy as a genetic therapy for cardiomyopathy in human ventricular cardiomyocytes • Identify key sequences/nucleotides that control MYH6/MYH7 expression and switching using CRISPR-based base editing (BE) screening strategy • Investigate whether regulating/switching MYH6/MYH7 expression using dCasMINI-based effectors (CRISPR activator, interference, BE) can therapeutically modify heart disease in hPSC cardiomyopathy models • Investigate and validate that MYH6/MYH7 gene regulation therapeutic strategies are broadly applicable across a genetically diverse population • Test AAV dCasMINI effector MYH6/MYH7 genetic therapy dosing and safety in in vivo mouse models • Test AAV dCasMINI effector MYH6/MYH7 genetic therapy efficacy in vivo in a mouse model of cardiomyopathy
Statement of Benefit to California (as written by the applicant)	Heart failure is a growing global epidemic and one of the major causes of morbidity and mortality worldwide, and its prevalence among U.S. adults ranges from ~1.9-2.6%. California also has the highest overall age-adjusted mortality rate associated with hypertrophic cardiomyopathy. Our proposed research will deliver a genetic-based therapy that will directly increase cardiac function to treat heart failure and cardiomyopathy patients, thus benefitting the State of California and its citizens.
Funds Requested	$2,772,000
GWG Recommendation	***(85-100): Exceptional merit and warrants funding, if funds are available***
Process Vote	*All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."* *Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."*

SCORING DATA

Final Score: 85

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

Mean	86
Median	85
Standard Deviation	3
Highest	90
Lowest	80

Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	11
(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.

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 13 **No:** 0	• Heart failure (HF) affects nearly 7 million Americans and mutations in MYH6 and MYH7 genes are common causes in HF. The proposed technology of reverting expression of MYH7 to MYH6 using CRISPR technology to increase cardiac function could impact this unmet medical need. • Heart failure is a growing global epidemic and one of the major causes of morbidity and mortality worldwide. The proposal aims to develop new genetic strategies that manipulate the expression ratio of cardiac myosin heavy chain genes (MYH6 and MYH7) for treating heart failure and cardiomyopathies. • The project aims to develop a CRISPR-based genetic therapy to regulate cardiac gene expression, which could pave the way for similar genetic therapies for other conditions. • The proposed approach will apply CRISPR/Cas9-based genetic strategies (CRIPSRi, CRISPRa, base editing), delivered by an AAV targeting the promoters of MYH6 and MYH7 genes. The applicant will test the approach in hiPSC-derived cardiomyocytes (CMs) and mouse models. • Overall, the proposal is well-constructed. CRISPRi/a targeting to MYH6/MYH7 to manipulate the gene expression is a convincing approach. However, the therapeutic ratio of MYH6/MYH7 is unclear. Would there be any side effects of decreasing the MYH7 level? • No preliminary data are provided for the potential of AAV-dCasMINI-based effectors and their delivery. • Using base editing to change one single base to achieve the desired MYH6/MYH7 gene expression ratio is not a convincing approach. More evidence that this will work would be great. • The proposed therapy aims to address heart failure and cardiomyopathy by targeting MYH6 and MYH7 gene regulation, which are crucial for cardiac contractility. Given the rising prevalence of heart failure, this therapeutic approach could significantly impact patient outcomes by improving cardiac function. • The innovative use of CRISPR-based technologies to modulate MYH6/MYH7 expression represents a novel approach that could address the current lack of therapies targeting myocardial contractility. • By focusing on a genetic mechanism underlying heart failure, this project could potentially offer a new treatment modality for a condition with limited current options.
GWG Votes	**Is the rationale sound?**
Yes: 12 **No:**	• The project is based on a very sound scientific rationale that follows the precedent of success in manipulating the beta-globin LCR to treat globinopathies in blood.

1	• MYH6 and MYH7 serve as the primary myosin heavy chain proteins and are antithetically expressed at significantly different ratios according to cardiomyocyte cell type and state (development and disease). Hearts expressing higher levels of MYH6 with lower levels of MYH7 display improved responses to HF/cardiac stress. Thus, discovering strategies to shift the ratios of these MYH isoforms during cardiac disease may significantly improve heart function and lead to new approaches for treating HF. The proposed approach will apply CRISPR/Cas9-based genetic strategies (CRIPSRi, CRISPRa, and base editing) to do so in hiPSC derived CMs and mouse models. • The preliminary results show that dCas9-based CRISPRi inhibition of the MYH7 promoter can rescue some cardiac defect phenotypes. Preliminary base editing screens produced some hit sgRNAs, suggesting that targeting the MYH7-promoter may affect the MYH6/MYH7 ratio in CMs, supporting the rationale. However, no validation data has been shown yet. • No preliminary data were shown for the feasibility of packaging dCasMINI-based effectors into AAVs, but the applicant has a Letter of Support related to this. • The scientific rationale is based on the established roles of MYH6 and MYH7 in cardiac function and the potential benefits of modulating their expression to improve heart contractility. The project's focus on addressing a critical aspect of heart failure pathophysiology demonstrates a well-grounded scientific basis. • Preliminary data show that modulating MYH6 and MYH7 expression can improve contractility in cardiomyocyte models, supporting the project's feasibility. Early CRISPR experiments indicate successful gene editing and functional improvements in cellular models. Alignment of preliminary findings with the proposed therapeutic outcomes strengthens the project's viability. • This is actually a very exciting proposal. There is a major weakness in that the THERAPEUTIC rationale and explanation of how the CANDIDATE would work and be tested, is not given. The application simply states that the candidate is intended for patients with heart failure and/or genetic and acquired cardiomyopathies, including hypertrophic and dilated cardiomyopathies (HCM and DCM). Explanation is needed! • The applicant should consider discussing the relevance of myosin inhibitors to the target patients. These have been tested in iPSC models of MYH7 variants. • While not relevant for the translational aspect of this proposal, the CRE3 element is conserved, and forms open chromatin as seen by ATACSeq. While it is not an enhancer, it may be important for publishing the LCR findings to examine what role it might play in shutting down MYH6 expression in adult CMs. Unlike globin switching, the cardiac LCR interacts with the more distant promoter first.
GWG Votes	**Is the project well planned and designed?**
Yes: 13 **No:** 0	• This is a high quality project that is well constructed. The project has two major aims and seven milestones to develop and then validate CRISPR-based MYH6/MYH7 gene regulation in wildtype and disease models. The applicant will test the dCasMINI-based effectors using two major models: human stem cell derived vCMs and a mouse model to test the dCasMINI-based effectors. • The project plan includes well-defined milestones and success criteria to track progress towards developing the therapeutic candidate. • The use of human stem cells and rigorous preclinical models ensure that the project is designed to generate robust proof-of-concept data. • Ethical considerations and compliance with donor eligibility requirements are addressed to ensure the suitability of the cell sources for translational studies. • The project plan includes well-defined milestones and success criteria to track progress towards developing the therapeutic candidate. • The use of human stem cells and rigorous preclinical models ensures that the project is designed to generate robust proof-of-concept data. • This is a detailed and structured project plan, with specific tasks and milestones.

	• The inclusion of experienced researchers and established methodologies further supports the quality of the project design. • Continuous evaluation and adaptation strategies enhance the project's overall robustness and likelihood of success. • Different CRISPR techniques and backup strategies are presented to ensure project continuity in case of potential problems. • This project's timeline is reasonable.
GWG Votes	**Is the project feasible?**
Yes: 13 **No:** 0	• The project is feasible and builds on a tremendous amount of high calibre preliminary results. • Mostly achievable. There is a concern for the effectives of AAV-dCasMINI-based effectors and its delivery. • The PI is a cardiovascular physician-scientist and experience in cardiovascular development, disease and regeneration research; and clinical/translational cardiology background. • There is competition in this space from other myosin modulators. It might be worth some time to test this product against those products. • The timeline is structured to allow for the sequential completion of key tasks. • The project plan includes sufficient detail to assess the feasibility of each milestone. • This project will include experts in cardiovascular research, gene therapy, and CRISPR technology, ensuring the necessary expertise to execute the project. • The inclusion of experienced researchers and support staff indicates adequate staffing to meet the project's demands. • Collaboration with external advisors and institutions provides additional support and expertise, enhancing the project's feasibility. • The project utilizes top-tier facilities and the latest technologies. • This proposal has appropriate budget justification.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13 **No: 0**	• There is an excellent DEI description outlining underserved communities and associated heart failure (HF) risk factors. Diverse perspectives are well incorporated, e.g., the applicant will form a DEI advisory committee for the project. • The research plan incorporates strategies to involve diverse populations. • The therapy aims to address heart failure, a condition with significant prevalence in diverse and underserved populations, particularly within California. • The development of a gene-agnostic therapy ensures that the benefits can extend to various demographic groups without genetic specificity limitations. • The project plan actively involves patient advocacy groups.

Application #	DISC2-16772
Title (as written by the applicant)	Chemically engineered photoreceptors for vision restoration in retinal degeneration associated blindness.
Research Objective (as written by the applicant)	This proposal will develop a cell-based therapy that can restore vision in retinal degeneration associated blindness such as Stargardt disease and age-related macular degeneration.
Impact (as written by the applicant)	Chemically induced method will overcome inefficient differentiation techniques, potential insertional mutagenesis and time intensive quality assessment associated with pluripotent stem cells.
Major Proposed Activities (as written by the applicant)	• Human skin fibroblasts will be chemically induced to candidate photoreceptor cells. Chemically induced cells will be isolated and assessed for gene expression signature by single cell RNA sequencing. • Skin fibroblasts derived isolated chemically induced photoreceptor cells will be assessed for chromatin signature by single cell chromatin sequencing methods. • Function of chemically induced photoreceptor cells will be assessed by microscopic evaluation of calcium influx/efflux upon light stimulation and chemical treatments. • Isolated chemically induced photoreceptor cells will be injected into the retina of the rodent eyes to examine their potential for integration and survival inside the retina. • Chemically induced photoreceptors will be injected into the eyes of blind mice and rats followed by assessment of vision restoration by retinal electrophysiological & visual behavior tests. • Toxicity and mechanism of vision restoration after chemically induced photoreceptor injection will be assessed by microscopic analysis of transplanted retinal tissues.
Statement of Benefit to California (as written by the applicant)	Photoreceptor-loss induced retinal degenerations affects diverse human racial and ethnic groups from all over the world including California. The proposed research will include fibroblast from diverse human ethnic groups for the generation of candidate photoreceptor cells. Successful generation of candidate cells followed by vision restoration in preclinical animal models would pave the way for the application of this approach in a diverse human population including citizens of California.
Funds Requested	$1,711,000
GWG Recommendation	***(85-100): Exceptional merit and warrants funding, if funds are available***
Process Vote	*All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."* *Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."*

SCORING DATA

Final Score: 85

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

Mean	85
Median	85

Standard Deviation	2
Highest	90
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.

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 13 **No:** 0	• This work holds the potential to help a large unmet need in patients with retinal degeneration. • If successful, this work could accelerate development. However, it is very early with questions remaining around the ability to convert the fibroblasts to chemically induced photoreceptor cells (CiPCs), the dosing, the translatability and the durability of these cells. • This proposal is innovative and does hold promise over pluripotent stem cells as a product. • The allogeneic cell-based therapy approach may be less likely to require long term immunotherapy. • The proposed strategy could avoid the lengthy cell manufacturing, and the viral vectors commonly used in alternative strategies. • The product has the potential to impact irreversible vision loss that enormously affects quality of life in both pediatric and adult populations. • Current stem cell derived photoreceptor replacement therapies have severe limitations and face challenges that have not yet been overcome. • The proposed reprogramming from fibroblasts provides an alternative approach and addresses an important bottleneck. Transplantation of CiPCs could provide a long-term solution for photoreceptor replacement.
GWG Votes	**Is the rationale sound?**
Yes: 13 **No:** 0	• The cell therapy candidate will be generated by direct cell reprogramming using a set of 5 small molecules and a patient's own fibroblasts. • The applicant has already provided proof of principle and can show that GFP+ mouse CiPCs are able to restore vision when transplanted into rodent retinal degeneration model. • The CiPCs in blind mice "restored" visual function as assessed by pupil and ERG in the majority of tested mice. • The rationale and advances over alternative strategies are well considered. • The applicant identified a chemical conversion protocol that allows generation of CiPCs from both mouse and human fibroblasts.

	• The applicant provided a thoughtful discussion of previous critiques and tried to address all the points that were raised.
GWG Votes	**Is the project well planned and designed?**
Yes: 13 **No:** 0	• This is a well-designed proposal supported by preliminary data. • The applicant presents an excellent discussion of pitfalls and approaches. • The team should be able to get to progress to a translatable program. • The additional attention to CMC and iPSC-PR controls significantly helps the plan. • Extension of the in vivo experiments to include human CiPCs that will be produced in a manner (HLA- typed) similar to what may be used in patients is a critical step towards translation. • The additional attention to CMC and iPSC-PR controls significantly helps the plan. • The project is well planned and designed, but there are several challenges that are not discussed: 1) the method of delivery is subretinal, yet there are no safety concerns discussed and/or mentioned as a risk 2) the applicant does not discuss the need and/or ability to perform repeat injections 3) CMC and the ability to scale and test the 5 chemicals causing the conversion are not fully discussed from a regulatory perspective. • The durability of reprogrammed cells after engraftment has not been demonstrated. • No pitfalls or alternative approaches are mentioned, and no risk analysis plan or possible adversities are identified in the project plan.
GWG Votes	**Is the project feasible?**
Yes: 12 **No:** 1	• The prior reviewers had concerns on timelines, resources and number of preclinical models being used. The authors have responded and addressed these concerns appropriately, • Tight timelines, limited cell lines and doses remain. A reviewer wondered why the project is so limited in scope. • The project is feasible, but there are several challenges that are not discussed. • The number of cells that integrate may be very low, given the preliminary data, and this information is essential to determine in the proposed activities. • Milestones are likely achievable, and the experiments are logical. Highly qualified investigators on the team have experience in the experiments proposed.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 12 **No:** 1	• The application gives a brief overview and addresses the DEI issues of retinal degenerative disease. The team mention recruiting individuals of diverse ethnicity, race and levels of training to participate in the lab and the project. • The applicants can use patients' own fibroblasts to accommodate race, gender, sex, and age diversity. • The project sufficiently upholds the principles of DEI, but the team could add more activities to strengthen their work in this direction. • The product would be suitable for all and would not exclude any population based on gender age or ethnicity. • The sex of the animals that will receive the transplants are not disclosed and seem not to include male as well as female recipients. It is also not clear whether regeneration or rescue is different in male or females in the model. • DEI is not specifically addressed. The applicant states that "As a faculty member, I will reach out to this committee to make partnerships with various patient organizations to collect research materials (such as skin biopsy) for the proposed research".

Application #	DISC2-16704
Title (as written by the applicant)	In vivo engineering of immune cells for cancer therapy
Research Objective (as written by the applicant)	These studies will advance our ability to use a targeted gene therapy approach to engineer or reprogram patients' immune cells to better target and kill refractory malignancies.
Impact (as written by the applicant)	These studies aim to provide better, cheaper and more accessible therapies for ovarian cancer, hepatocellular carcinoma and potentially other refractory malignancies.
Major Proposed Activities (as written by the applicant)	• Utilize our novel virus-like particles (VLPs), termed Enveloped NanoBodies (ENaBs) targeted to different immune cells antigens to efficiently express CARs in T cells, NK cells and macrophages in vitro • Utilize targeted ENaBs to knock-down expression of TGFBR2 in engineered immune cells to improve anti-tumor activity. • Utilize targeted ENaBs to express immune-stimulating cytokines in engineered immune cells to improve anti-tumor activity. • Direct in vivo engineering of immune cells to test for anti-cancer activity using immune competent mouse models and murine tumor cells • Direct in vivo engineering of immune cells to test for anti-cancer activity using humanized immunodeficient mice with human immune cells and human tumor cells.
Statement of Benefit to California (as written by the applicant)	These studies will develop a novel gene therapy approach to better treat ovarian cancer and hepatocellular carcinoma - malignancies with few good treatment options. This approach will potentially be more accessible, cheaper and more effective than current regimens. Therefore, these studies can reduce the cancer burden for California residents. Also, since these malignancies disproportionately impact medically underserved populations, advances for these patients will be especially valuable.
Funds Requested	$2,721,797
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	81
Median	83
Standard Deviation	4
Highest	86
Lowest	75
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.

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 12 **No:** 1	• This project aims to develop a non-integrating, non-viral, genetic vector to express chimeric antigen receptors (CARs) and other genes of interest in targeted immune cells for in vivo cancer immunotherapy. • The initial therapeutic candidates would target mesothelin or glypican 3 (GPC3) expressed in ovarian cancer or hepatocellular carcinoma. Both are major types of cancer with poor outcomes for the majority of patients. Therefore, the project addresses an unmet medical need. • The current CAR-redirected immunotherapy is based on ex vivo manufactured autologous or allogeneic T, NK and other immune effectors that are largely ineffective in solid tumors. The proposed product is designed to generate CAR-redirected cells in vivo. • Yes. The proposed technology is a virus-like particle (VLP) termed Enveloped Nanobodies (ENaB) that can deliver mRNA and convert immune cells into CAR+ cells for the treatment of several types of cancers. • The project holds significance in overcoming challenges of cell therapy. However, unclear how the approach will overcome grand challenges In CAR T trafficking persistence against overwhelmingly immunosuppressive microenvironments. • However, it remains unclear how these in vivo generated CAR-T, CAR-NK or CAR-M cells would overcome critical barriers responsible for the therapeutic failure of their ex vivo generated counterparts, including tumor escape via antigen loss, exhaustion, immune checkpoints, metabolic competition, tumor-protective stroma, etc.
GWG Votes	**Is the rationale sound?**
Yes: 12 **No:** 1	• The technological part of the project is sound and there is a strong scientific rationale for the development of VLP-based delivery of CAR mRNA to selected immune effector cells in vivo. • There is also a solid rationale based on literature and preliminary data for targeting transforming growth factor beta receptor 2 (TGFBR2) in the immune effector cells. • Yes. The rationale for this approach is sound. Other approaches for in vivo gene editing, including non-viral, are in the public domain and soon to be clinically investigated. • Yes. The preliminary data suggest that this approach can target and introduce genetic material into T-cells, NK cells, and macrophages. • Preliminary evaluation of this approach in an immunodeficient mouse model found that NK cells and macrophages demonstrate the best activity, even in comparison to PBMCs. • The choice of cytokines for co-delivery with CAR mRNA is also well supported by literature. However, there is still a conceptual disconnect. There is no explanation how the in vivo generated CAR effector cells would overcome the mechanisms of tumor

	resistance/escape responsible for the failure of ex-vivo generated effector cells, expressing the same/similar CARs, cytokines, and/or TGFBR2 constructs. • The rationale is sound, but proof of concept data is missing. The efficacy data in Figure 7 is not well controlled and effects could be due to nonspecific effects of VLP. Control VLPs should be used. Efficacy data presented appears only to be with a n=3 with marginal effect despite treatment within 48h of tumor inoculation. • Preliminary data are limited.
GWG Votes	**Is the project well planned and designed?**
Yes: 11 **No:** 2	• The project is well planned and developed. The revised version is more focused and largely responsive to the prior reviewers' critiques related to the technical aspects of the proposal. • The project is well-constructed and planned. • The versatility of the approach in transfecting multiple myeloid cells and T cells is very attractive and experimental approach is reasonable. • The feasibility of identifying targets for ENaB engineering of specific immune cells requires the target receptor to endocytose upon binding. The PI lists markers of immune cells but no reference to evidence that these are targetable receptors for endocytosis of payloads. • Aim 2 will investigate in vivo engineering. Determination of the dosage and off-target targeting of these ENaBs does not appear to be proposed.
GWG Votes	**Is the project feasible?**
Yes: 13 **No:** 0	• There is a proof of concept preliminary data showing in vivo CAR delivery to primary human immune cells that mediate antitumor activity in a xenogeneic mouse model. • The PI and team are outstanding. • The team is led by an expert in immune cell engineering and pioneer in NK cell therapies. • The approach appears feasible.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13 **No:** 0	• The project plan adequately addresses and accounts for the influence of race, ethnicity, sex and gender diversity. • The project upholds the principles of diversity, equity and inclusion (DEI)

Application #	DISC2-16686
Title (as written by the applicant)	Development of iPSC-derived neural progenitors secreting GDNF for the treatment of Amyotrophic Lateral Sclerosis (ALS)
Research Objective (as written by the applicant)	An off-the-shelf induced pluripotent stem cell-derived neural progenitor cell for the treatment of Amyotrophic Lateral Sclerosis (ALS)
Impact (as written by the applicant)	Amyotrophic Lateral Sclerosis (ALS), Parkinson's Disease, Retinitis Pigmentosa, Stroke
Major Proposed Activities (as written by the applicant)	• Generation translatable iPSC derived neural progenitors and selection of the best cell line • Selection of the GDNF transgene insertion method and best promotor • Expand, cryopreserve and characterize the lead therapeutic candidate (initial purity, identity and potency assays) • Demonstrate delayed motor neuron degeneration in the lumbar spinal cord • Demonstrate delay in motor neuron degeneration in the cervical spinal cord • Demonstrate extended survival and/or delayed onset after motor cortex and/or triple site transplantation
Statement of Benefit to California (as written by the applicant)	Amyotrophic Lateral Sclerosis (ALS) is a devastating disease that carries a large burden emotional and financial burden on the patient, their families, and the state's healthcare system (up to $300,000 annually/per patient). This therapy has the potential to lower the costs of care, but more importantly decrease suffering of Californians with ALS. All research activities will be performed in California and therefore increase state revenue and supporting employment of Californians. Future trials would also be in California.
Funds Requested	$2,677,445
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: 81

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	81
Standard Deviation	4
Highest	90
Lowest	80
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	6*

(1-84): Not recommended for funding	8

* See Minority Report below

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 13 **No:** 0	• This project impacts an unmet medical need as there is currently no effective treatment for amyotrophic lateral sclerosis (ALS). The proposal is to generate an allogeneic iPSC-derived neural progenitor cell line genetically modified to secrete GDNF (CNS-iNPC-GDNF) and evaluate the therapeutic potential in rat disease model to to treat ALS. • There are no cures or effective treatments for ALS, so any development in this disease area will have large impact on unmet clinical need. The proposed therapy targets both genetic and sporadic patient groups which increases the potential impact. • The expected cell line will increase the likelihood of successfully developing a stem cell-based and genetic therapy. The team also expect the cell product will provide long-term efficacy in patients. • There are no successful therapies for sporadic ALS (85% of cases). The likelihood of success depends upon whether the overall concept proves meritorious in the ongoing clinical trial of a product derived from a different starting material. • The path for clinical development is carefully considered and described in detail in the proposal. The development follows clinical trials using a similar product but based on a different NPC source. • A positive outcome would provide a renewable source of cells for use as NPC therapeutic for ALS. However, this project does not really provide a new strategy, just an alternative cell source for an existing strategy. Arguably, the applicants could have foreseen this difficulty with current cell source and addressed it earlier. • The innovation is marginal.
GWG Votes	**Is the rationale sound?**
Yes: 13 **No:** 0	• The project is based on sound scientific rationale. Previous studies showed that human neural progenitor cells (NPCs) derived from iPSCs can be genetically engineered to stably release the growth factor GDNF using lentivirus infection, and delivery of this cell and gene therapy to the ALS rat spinal cord was shown to be safe with no signs of tumors or toxic effects and to protect dying motor neurons. • The team currently has ongoing clinical trials using NPCs releasing GDNF. The new candidate will be derived from NPCs derived from iPSCs, and with engineered version of GDNF, providing a combined stem cell and gene therapy product for ALS. • Preliminary data show iNPCs differentiation and stable GDNF expression via lentivirus. This iPSC derived NPC-GDNF perform as well as NPC-GDNF in animal studies, which is supportive. • Similar strategies have been developed from the same team using a different starting material. To switch from this source to iPSC, as is done in this proposal, is a logical step and a necessary development towards a clinically relevant therapy. • The strategy of delivering a GDNF-producing cell is based on two complementary principles. The first is that the product is expected to result in astrocytes with long term survival that may restore the microenvironment through release of protective factors,

	uptake of toxic molecules and/or physical interaction with motor neuron processes. The second is the added delivery of the well known neuroprotective factor GDNF. • Preliminary data published in *Nature Medicine* several years ago show that the cell therapy is tolerated and that grafts persist. An update on this trial would have been helpful and may have made this application more compelling. • The merit of this project is dependent on the degree of success of an ongoing project. • To make a combination cell/gene product is more challenging than a cell-only product, and the extra benefit of GDNF is not clearly documented.
GWG Votes	**Is the project well planned and designed?**
Yes: 13 **No:** 0	• The project plans to make NPCs from three clinically translatable iPSC lines, evaluate three promoters for driving GDNF transgene expression, and then knock-in the most effective cassette to the viral transgene. Investigators plan to evaluate engraftment, proliferation, migration and GDNF secretion in the spinal cord of rats. The project is appropriately planned. • The pitfall of GDNF expression level is identified. It is not clear exactly how much is required to achieve a clinical effect. The applicant will evaluate this in a rat model of ALS. • The project is well designed and clearly described with stepwise progression towards the generation and genetic engineering of the cells as well as the documentation of their safety and efficacy in relevant animal model. • It might be wise to do WGS or long read sequencing of the candidate cell line up front as well as after editing, otherwise there may be a halt midway through the project. This type of transgene may be subject to silencing. What criteria are in place to ensure absence of undifferentiated cells from the product and purity? • NPC derived from hPSC may carry additional safety risks such as more proliferative capacity and/or presence of undifferentiated or off target cells. • The proposal has a limited safety discussion.
GWG Votes	**Is the project feasible?**
Yes: 13 **No:** 0	• The proposed milestones are highly achievable. Based on the team's experience with previous clinical trials, this product cell line is likely to be translated to the clinic. • The PI and the team have strong backgrounds in neuron disease and cell therapy. • This is a challenging project with several pivotal steps that needs to be achieved. This is recognized in the application. The proposal also includes well-developed and detailed contingency plans. • The preliminary experimental data are in general strong and support the project. However, a clearer documentation GDNF benefit would further strengthen the proposal. • The project follows logical and stepwise development with clear milestones and decision points. It is ambitious, but possible, to achieve given the expertise of the team and key resources and cell lines already in place. • The applicants have extensive experience in this approach.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13 **No:** 0	• In this proposal, the team plans to test three iPSC-derived cell lines. With the developed cell line, the team expects to deliver a "one-time" therapy that will serve a benefit for all populations, especially underserved communities that have less access to healthcare and are often lost to follow-up. • The proposal contains rationale and plans for making the product broadly available. • Patient involvement and perspectives are included in the project design and implementation. • The prior clinical trial showed promise in diverse populations. • The DEI statement is generic.

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.

Application DISC2-16686, titled "Development of iPSC-derived neural progenitors secreting GDNF for the treatment of ALS", received an overall (median) score of 81, with a scoring range of 80 to 90. Six (6) Grants Working Group (GWG) panelists scored the application 85 or higher, thus recommending funding. The remaining eight (8) scoring panelists score the application from 80 to 82, indicating that the applicant may revise, resubmit, and bypass positive selection in the next DISC2 review. The primary reviewer for the application gave the highest score.

Reviewers supporting funding described the proposal as ambitious and well planned, particularly noting a logical, stepwise project plan, a strong discussion of project pitfalls with contingency plans, and the qualifications and experience of the team. With regard to diversity, equity, and inclusion, the supportive minority appreciated the overview goals of a one-time therapy, future plans for making the product broadly available, and the diverse participant cohort in a prior/ongoing trial of a predecessor version of the proposed product.

All reviewers agreed that the team is qualified, and the project has significance and potential for impact due to the unmet medical need associated with ALS. While the majority thought that additional data from the ongoing trial are needed for evaluation of project merit, supportive reviewers focused on favorable preliminary data *in vitro,* and *in vivo* in a rat model, and early results of the related trial. Supportive reviewers' comments did not directly address majority comments indicating that the innovation of the project is marginal.

Application #	DISC2-16539
Title (as written by the applicant)	Development of a gene agnostic treatment for photoreceptor disease
Research Objective (as written by the applicant)	This CIRM project will discover a therapeutic candidate that will be used to prolong photoreceptor health in blinding conditions regardless of the gene mutation associated with the diagnosis.
Impact (as written by the applicant)	This discovery will positively impact the metabolic cascade of several neurodegenerative and blinding disorders, but this study focuses on demonstrating benefit in retinitis pigmentosa.
Major Proposed Activities (as written by the applicant)	• Evaluate novel mutants for disrupted interactions to trigger a metabolic fuel-switch in photoreceptors being starved of glucose. • Design and screen gene therapy vectors to target photoreceptors to provide our therapeutic candidate. • Demonstrate the gene agnostic therapeutic benefit of the candidate using distinct mouse models of photoreceptor degeneration. • Evaluate the therapeutic benefit in human cells using patient-derived retinal organoids. • Restoration of photoreceptor structure and function after administration of the therapeutic candidate in clinically relevant rat model of retinitis pigmentosa. • Prepare the translational plan to advance our therapeutic to clinical studies. This includes California patient advocacy group engagement to ensure the therapeutic benefits its diverse population.
Statement of Benefit to California (as written by the applicant)	California has over 10,000 people affected by retinitis pigmentosa (RP), a debilitating progressive condition that leads to blindness. Presently, treatment options are limited, focusing on small genetic subsets of patients. A gene-agnostic therapy is needed to benefit a broader range of individuals within the RP community. Developing such a treatment not only promises to enhance quality of life for affected individuals but also alleviates strain on healthcare resources throughout California.
Funds Requested	$1,225,975
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	93

Lowest	75
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	3
(1-84): Not recommended for funding	11

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 13 **No:** 0	• Innovative approach with the promise of providing a gene agnostic protein via Adeno-Associated Virus (AAV) to enable alternative energy stores to dying rod photoreceptors in Retinitis Pigmentosa (RP). • This is a very innovative proposal that uses a highly translatable AAV platform. • This project has significant potential for impact, especially as it is an innovative approach that is gene agnostic to the RP mutations.
GWG Votes	**Is the rationale sound?**
Yes: 11 **No:** 2	• The rationale is based on a finding of the role of the target gene in hibernating rodents. The gene underpins these rodents' ability to restore and rejuvenate photoreceptors in a dormant state. • There is concern that effects of proposed gene may not translate to dying and sick rod photoreceptors, and this approach may not be enough to provide an alternative fuel source. • The proposal includes a streamlined plan that is commensurate with the urgency for CIRM therapeutic development. • Further preliminary data in a relevant disease model would help to demonstrate feasibility.
GWG Votes	**Is the project well planned and designed?**
Yes: 11 **No:** 2	• The plan would answer key questions about preservation, dose, and rescue in relevant models. This data would be foundational for future IND-enabling studies. • The milestones are reasonable; the models are as well. • The proposal is well designed in a stepwise approach. • One critique: The applicant would benefit from assessing more AAV serotypes to help benchmark their ultimate selection.
GWG Votes	**Is the project feasible?**
Yes: 13 **No:** 0	• The team is well positioned to quickly translate the approach as an AAV gene therapy in the subretinal space. The project is significantly de-risked by prior INDs and approved products. • Although the timeline for proposed activities is tight, it is doable. However, there is risk if something goes astray.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13 **No:**	• Yes, the project sufficiently upholds the principles of DEI.

0	• This application does not include a strong DEI proposal. There is minimal mention and/or incorporation regarding a DEI plan and no mention of sex of the animals. The proposed DEI plan could be more robust.

Application #	DISC2-16799
Title (as written by the applicant)	Secreted Particle Information Transfer (SPIT) – A Cellular Platform for the Treatment of Systemic Genetic Disease, Starting with Fanconi Anemia
Research Objective (as written by the applicant)	An autologous hematopoietic stem cell (HSC) therapy that will utilize engineered HSCs as vectors for the systemic delivery of genetic engineering enzymes for the treatment of Fanconi Anemia.
Impact (as written by the applicant)	The therapeutic developed will be directly applied for the treatment of Fanconi Anemia and will serve as a flexible platform for the treatment of systemic genetic diseases generally.
Major Proposed Activities (as written by the applicant)	• Identify cell-penetrating peptides (CPPs), endosomal escape peptides (EEPs) and protease cleavage sequences that can enhance cell-cell engineering by SPIT • Integrate CPPs, EEPs and protease cleavage sequences into a single vector to synergistically enhance cell-cell engineering by SPIT • Develop a chemically regulatable humanized GAG-adenine base editor (ABE) variant to improve the safety profile of our therapeutic • Incorporate all optimizations of GAG-ABE into a single clinically translatable vector and demonstrate disease modifying activity in patient cell lines • Assess the ability of current and optimized SPIT vectors for systemic gene editing in a fluorescent reporter mouse model • Demonstrate systemic *in vivo* genetic correction of Fanconi Anemia in a mouse model of the disease
Statement of Benefit to California (as written by the applicant)	The proposed gene therapy for Fanconi Anemia (FA) will significantly benefit California by enhancing patient quality of life and developing a transformative platform that positions the state as a leader in advanced gene therapies. This research may attract biotech investments and stimulate job creation, while delivering crucial data on innovative treatments for systemic genetic diseases, influencing the broader field of cell and gene therapy.
Funds Requested	$2,696,400
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	3
Highest	90
Lowest	80

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.

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 12 **No:** 0	• This is a high risk, high reward application; the project could revolutionize gene therapy for many diseases. The track record of the applicant is utterly amazing. If anyone can make this work, this Principal Investigator (PI) can. • Yes, while other approaches in the area of gene therapy exist, the proposed technology addresses the unmet need in diseases that require affordable, effective, and truly systemic treatment. • The secreted particle information transfer (SPIT), if shown to be effective, has potential to treat any manner of gene therapy-treatable disease. This approach specifically addresses bottlenecks in vector production and cost that limit other approaches. • The applicant demonstrates concern for clinical obstacles in the pitfalls section, correctly noting that efficiency and immunogenicity at clinical scale could limit translation. Alternative approaches to enhancing efficiency as well as decreasing immunogenicity showed a thoughtful approach to translational issues. • Yes, the proposal may generate an HSC-based therapy to treat Fanconi anemia. • In the long term, yes, depending on key results of the experiments proposed. • The proposal is very detailed and thoughtful.
GWG Votes	**Is the rationale sound?**
Yes: 12 **No:** 0	• Yes, the proposal advances recent successes in gene therapy and genetic engineering of HSCs, as well as the development of virus-like particles, to generate a new product. • The preliminary data are compelling. The EGFP model provides a useful way to evaluate this technology. • Yes, however, In the absence of knowing the threshold of correction required for clinical impact in FA patients, the rationale remains untested. The justification for using SPIT, and for using HSCs vs. progenitors, remains unclear. • Yes, the rationale depends on long-term persistence of modified cells. The targeting of HSCs uniquely enables this. • This is an excellent display of molecular biology and vector engineering. The applicant's methods for activating minimal GAG fusion toward clinical use are creative and innovative. • Yes, the unique capacity of this approach to deliver systemic enzyme therapy in a potentially continuous fashion via engineered cells is scientifically valid. One caveat, is the *ex vivo* HSC modification approach specifically necessary to achieve the aims? For example, delivery of an HSC targeted gene therapy vector could modify HSCs *in vivo*. Alternatively, if systemic gene therapy did not provide necessary continuous enzyme, repeated dosing could be an alternative strategy.

	• Yes, both primary human cell work and mouse models demonstrate technical expertise in the area. It's still unclear if the planned alterations and modifications to the product will be successful, but prior experience supports the currently proposed project.
GWG Votes	**Is the project well planned and designed?**
Yes: 10 **No:** 2	• Assays in Milestone 1 do not directly measure increases in cell penetration, endosomal escape, or protease activity. Instead, they determine the end result: change in EGFP expression by base editing. The new protein in Activity 1 of Aim 2 has high risk of not working. There is an alternative approach presented for this pitfall, but it is not convincing. • The applicant plans to use healthy donor HSCs, which is appropriate given limited numbers of FA patients. However, this could limit generalizability and translation. • Yes, though subsequent aims are highly dependent on early aims. For example, if SPIT is too inefficient, and alternatives are too immunogenic, then there is no point to optimizing HSCs for SPIT vectors. This places particular importance on establishing the vector design. • Numerous alternative approaches are identified throughout. • The timeline is appropriate for the proposed project. • Yes, it's well planned and designed. However, the proposed studies of the biology of human HSCs are weaker, and the majority of the project is on mouse studies, vector engineering, and optimization. • There are several weaknesses, and the proposal heavily weighs on vector engineering without a clear idea of why this engineering is required. • Effective methods for HSC targeted gene therapy exist; progression to more complex and sophisticated engineering and targeting on non-hematopoietic cells is seemingly necessary. The rationale for these efforts was unclear. • Non-hematopoietic cells, the additional basis of SPIT, were not examined. The leaves the rationale for the proposal beyond HSC targeting unclear. The applicant is missing an opportunity. • Non-HSC targeting should be considered, as long-lived progenitors may provide sufficient therapeutic impact. No progenitor assays were proposed in mouse or human, and the focus on HSC alone may be unnecessary. In the absence of knowing the threshold of correction required for clinical impact in FA patients, this remains untested. • The project lacks proof of principle testing in human cells, and it does not include patient derived xenotransplant models or determination of function after lentiviral engineering of putative human HSCs. This is reserved mostly for the mouse. There is a concern that mouse effectiveness is unlikely to represent numbers and frequency, or even safety issues required for human. This could be tested in human cells *in vitro* and *in vivo*. • In the context of human, it's unclear how the engineering of GAG and removal fusion proteins to reduce immunogenicity will be tested aside for changing the vector. *In vitro* assays? Syngeneic mouse models?
GWG Votes	**Is the project feasible?**
Yes: 12 **No:** 0	• Feasibility is very dependent on whether the goal is translation vs. proof of principle in designing a vector for ABE delivery. • The background and commitment of this group, including collaborators that pioneered ABE is a major strength of this application. • The applicant team has extensive experience in this area as well as more broadly in translational research. • The applicant is well-equipped with resources for the conduct of the project. • Adequate resources are available to conduct the studies described. • The milestones are feasible as described.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**

Yes: 12 **No:** 0	• Considerations of the influence of race, ethnicity, sex and gender diversity are well integrated. • If applied broadly, the project outcomes inform the development of a product serving the unmet medical needs of the diverse California population. • The applicant's efforts to incorporate experience from the patient population in project implementation are well described.

Application #	DISC2-16685
Title (as written by the applicant)	Transplantation of excitatory V2a interneurons to promote motor function recovery after spinal cord injury
Research Objective (as written by the applicant)	The objective is to generate enriched human spinal cord V2a excitatory interneurons that can enhance descending neuronal relay formation and motor functional recovery after spinal cord injury.
Impact (as written by the applicant)	As there are currently no effective therapies for spinal cord injury (SCI), the proposed studies will develop a novel and more specific cell product that will improve motor function for SCI patients.
Major Proposed Activities (as written by the applicant)	• Verification and optimization of our current protocol to generate spinalized or spinal cord V2a (scV2a) interneurons in vitro • Single cell sequencing to characterize scV2a interneurons derived from H9 ESC line at molecular level • Generate a reporter cell line that depends on Vsx2 expression for monitoring scV2a neuron differentiation, maturation, and integration • Optimization of parameters of scV2a interneuron transplantation in vivo after spinal cord injury • Investigate whether transplanted scV2a excitatory interneurons successfully form descending neuronal relays • Motor behavioral tests
Statement of Benefit to California (as written by the applicant)	SCI affects approximately 300,000 people in the U.S., with more than 20,000 new injuries per year. People with SCI often endure decades of severe disability, with staggering physical, emotional, and financial costs. The first year of treatment alone is $1 million for a quadriplegic patient. Better treatments are needed, and even a modest increase in functional capacity (1-2 spinal levels) can produce meaningful improvement in quality of life and cost savings for California.
Funds Requested	$2,771,998
GWG Recommendation	***(1-84): Not recommended for funding***
Process Vote	*All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."* *Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."*

SCORING DATA

Final Score: 80

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

Mean	79
Median	80
Standard Deviation	4
Highest	90
Lowest	75
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	1

(1-84): Not recommended for funding	13

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 11 **No:** 2	• The candidate is a combination product: human embryonic stem cell (hESC)-derived scV2a progenitors combined with a biological sealant and growth factors for treatment of spinal cord injury. This would be an advance over current standard of care for SCI treatments. • Knowledge gained from these studies will continue to advance the field. However, this is a very complicated product, potentially making FDA approval arduous. • This resubmitted proposal is focused on generation of a human interneuron population from a FDA approved stem cell line for transplantation into spinal cord injury. It will be a one time treatment. • The applicant makes a good case for how their approach differs from other existing approaches. This generates confidence that this work will not simply copy other efforts but will represent an independent, different track for a potential cell therapy. • The proposal is focused on transplantation two weeks post injury, which represents the subacute state. Thus, it does not address the majority of spinal cord injuries, which are chronic. • The applicant provides a clear candidate profile and addresses important milestones that are required for clinical translation. The applicant has the advantage that the parental cell line from which the new product is generated is already in advanced stages of FDA-regulated clinical translation. • SCI represents a significant unmet medical need. The study is similar to previous work funded by CIRM with some significant modifications to the overall strategy.
GWG Votes	**Is the rationale sound?**
Yes: 13 **No:** 0	• The resubmission is responsive to the previous critiques, and progress has been made to optimize the differentiation process. • The proposal is well supported by published data from the applicant's and others' previous work. • The importance of V2a interneurons as a target for cellular therapies for SCI is clearly rationalized. • The concept of transplanting scV2a inhibitory interneurons might lead to better restoration of connections between descending neurons and motor neurons. It is logical to derive these from the proposed progenitors, but it is unclear that this will provide significant advantage. • The rationale for transplanting spinalized V2a interneurons is based on observation of highly successful recovery of spinal cord injury using spinalized neural stem cells. The applicant proposes that the recovery could be further enhanced by enriching the graft population to a range of 25-50%. • As NSCs are not limited to generating interneurons and potentially produce other cells as well, it is possible that the effect of these transplants is due not to interneurons, but to

	the presence of other cells. It is a stretch to ascribe the recovery to the few interneurons that are present in the graft. • The composition of the graft population is poorly defined. What other cells are present, and how consistent is the entire population? These issues are not sufficiently addressed.
GWG Votes	**Is the project well planned and designed?**
Yes: 9 **No:** 4	• The project is well designed overall, and preliminary data support the approaches. All techniques used have been established by the applicant and endpoints and sample numbers are defined. • The transplantation window is limited to a two week post injury; this limits the impact of the research and excludes chronic patients. • The applicant is using only immune compromised rats as models. It is not clear why at least a small cohort of rats that receive immune suppression (which is the clinically relevant situation) is not proposed. This is a remaining limitation for potential translatability. • In this submission, the applicants have included both male and female animals and have added additional cells lines for testing. • The method to optimize the number of growth factors proposed will be time consuming and costly. They will need to investigate which factors to include and their concentrations. An in vitro method of optimization would be better deployed for this step, followed by advancing a subset of conditions to the animal model. • What is the cell product, a mixture of cell types or pure V2A neurons? If the former, what are the criteria for purity? The progenitor preparation differs from NPC. How are V2A progenitors defined? • How will components of complex trophic support mixture be assessed for activity in vivo? These are complicated experiments given the potential number of factors involved. • The initial progenitor induction and final differentiation pathway are not adequately characterized. This is a good choice of precursor cell but undifferentiated progenitors in the cell therapy prep could give rise to a number of off target derivatives, including muscle, bone and cartilage.
GWG Votes	**Is the project feasible?**
Yes: 10 **No:** 3	• Overall, the project is feasible; the team is highly experienced. • The proposed studies are quite complicated, and many will require several iterations to optimize outcomes, making this difficult to complete in three years. • This is a good team, very passionate about the field. For most key techniques, the applicants have provided proof-of-concept data to suggest technical feasibility. • How fragile are mature cV2a neurons? Are they indeed transplantable? How are they purified? What is the progenitor that would be used if the cells are too fragile to survive transplantation on their own? This cell type is not defined in the application. • It will not be possible to test all permutations and combinations of trophic factors as the applicants propose. This is not realistic.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13 **No:** 0	• Experiments will use stem cells from both genders, and male and female animals will be used. • The proposed allogenic cell product will be equally suited for treatment of all SCI patients independent of race, ethnicity, gender, sexual orientation, age or any other factor reflecting the diversity of California's population. • The team appears to be well-balanced, including two members that have sustained spinal cord injury. • The DEI statement is adequate. • The proposed allogenic cell product will be suited for treatment of all SCI patients. The proposal is not considering age, but both sexes will be used.

	• The initial parental cell line is generated for a female donor, but future experiments will include male lines. • SCI disproportionately affects African Americans (almost 50% greater chance of injury compared to non-African Americans) and the affected age group has aged. At the same time rehabilitation has declined and is, for many patients, not affordable. A single time treatment would make a real difference to under-served communities, although the eventual costs are not discussed. • The proposal is informed by the input from individuals with SCI; two members of the team are affected by SCI.

Application #	DISC2-16652
Title (as written by the applicant)	Novel antisense therapy to treat neurodevelopmental and neurodegenerative genetic disease
Research Objective (as written by the applicant)	We will help develop new drugs for four different monogenetic neurodevelopmental/neurodegenerative pediatric diseases where treatments are lacking.
Impact (as written by the applicant)	1. Developmental PACS2 encephalopathy, 2. Bryant-Li-Bhoj H3F3A Neurodevelopmental syndrome, 3. Neurodevelopmental EZH1 syndrome, 4. Neurodegenerative NARS1 syndrome.
Major Proposed Activities (as written by the applicant)	• Secure and propagate IPSCs and Neural Progenitor Cells (NPC) from 8 patients representing four NNDs, each with a defined genetic mutation, and matching control lines. • Assess each line for altered cellular transcription, signaling and health as predicted from the gene mutation. • Phase each mutation for precise ASO targeting to the mutant haplotype. • Design, synthesize and apply targeted ASOs to each NPC line to assess target engagement and correction of genetic lesion. • Apply targeted ASO to each stem cell line to assess restoration of cellular transcription, signaling and health. • Incorporate knowledge into new [redacted] FDA applications to support administration of drug to patients in the clinic at [redacted] Children's Hospital.
Statement of Benefit to California (as written by the applicant)	Neurodevelopmental and neurodegenerative disease (NNDs) impacts 1:50 California children, with conditions like severe epilepsy, autism, and intellectual disability. In prior CIRM-funded efforts we generated a library of stem cells from patients, and in parallel we identified their genetic mutations. Now the stage is set to test if correction of the genetic mutation through ASO gene therapy shows disease-modifying activity in patient cells. Results will set the stage future clinical trials.
Funds Requested	$2,088,264
GWG Recommendation	***(1-84): Not recommended for funding***
Process Vote	*All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."* *Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."*

SCORING DATA

Final Score: 80

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

Mean	79
Median	80

Standard Deviation	2
Highest	82
Lowest	75
Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	13

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 12 **No:** 1	• The antisense oligonucleotide (ASO) approach is likely to reduce expression of the toxic gain of function (TGOF) allele and this should rescue the expression profiles as a step towards the unmet medical needs of NND patients. • The application is proposing to evaluate effect of antisense oligonucleotide (ASOs) candidates designed for four different gene mutations (PACS2, EZH1, NARS1 and H3F3A) implicated in pediatric neurodevelopmental and neurodegenerative disorders (NNDs) to "restore" gene expression profiles in patient NPCs derived from iPSCs. • Toxic GOF is said to account for half of disease-causing mutations in NDD. Applicants will work with a new foundation with a significant track record in the field to support ASO development. This project should enhance pipeline for ASO development. • The genetic mechanism of these mutations is suggested to be as TGOF; however the presented arguments do not exclude possibility of haploinsufficiency or dominant negative mechanisms. Therefore, the choice of ASOs that downregulate mutant mRNA transcripts may not be safe or therapeutically effective. • No details of the mutation type and specific position for the candidate genes is presented, making it difficult to evaluate if the proposal will have desired impact. • Off-target effects often present challenges by binding and downregulating the non-mutant transcripts. The off-target effects of the candidate ASOs will not be tested in the proposal. • Moreover, even if some candidate ASOs can make it to approved therapies, life-long dependence of patients in repeat treatments is a big limitation. For example, Eteplirsen must be administered intrathecally on a weekly basis for the remainder of the patient's life. • Gene correction by gene editing is likely a far better lifelong curative therapy than ASOs for rare conditions with little or no chance of commercialization. • Four single gene NDD will be targeted with ASOs. Toxic gain of function in these disorders cannot be fixed with viral gene therapy.
GWG Votes	**Is the rationale sound?**
Yes: 12 **No:** 1	• Preliminary data is very strong showing knockdowns using ASOs, correction of an patient differential gene expression (DGE) signature, and a track record of success at the foundation, Ngn2 induction of the NPCs/neurons and expression of the target genes.

	• The main rationale is that candidate mutations are TGOF and cause abnormal gene expression in iPSC-derived NPCs. Therefore, the application will screen multiple candidate ASOs that restore normal gene expression profiles in NPCs. • Yes, the rationale behind ASO therapy for these conditions is clear. • The ASO rationale is sound. The choice to focus on neural progenitor cells (NPCs) is amenable to rapid analysis, but NPCs are not the in vivo target in patients, rather it will be their non-cycling and active neurons. • Since selected patients' mutations are associated with pediatric neurodevelopmental and neurodegenerative disorders, candidate ASOs must be also tested with mature neurons. • It is also likely that simplistic determination of global gene expression in NPCs is not sufficient to determine specific pathogenic effects of each mutation and evaluate therapeutic value of candidate ASOs. • Moreover, it is highly unlikely that in vitro screens of ASOs on NPC gene expression will be sufficient to determine the safety and efficacy of candidate ASOs and allow transition directly to the clinical trials as the applicant suggests. • It will be imperative to test safety and efficacy in vivo in animal models. In vivo data even from a simple mouse model could provide far more important safety and efficacy assurance than vitro observations of patient NPCs. • Lastly, the selected patients have neurodevelopmental and neurodegenerative disorders, meaning that these mutations may have already induced permanent pathological changes in patient brains, including irreversible loss of neurons in specific regions. Loss of neurons and the subsequent loss of function are generally responsible for disease symptoms common for children with NNDs including impairments in memory, language, behavior, motor skills, learning, speech, social skills, and emotions. Therefore, it is reasonable to expect that administration of ASOs to patients with advanced stages of disease could simply slow the disease progression but not cure or improve the condition. • Preliminary data is focused on one disorder, not addressed in the proposal, that seems to show efficacy of ASO in altering gene expression in neural progenitor cells. • The choice of cells is not well rationalized.
GWG Votes	**Is the project well planned and designed?**
Yes: 11 **No:** 2	• The project is well constructed and high quality, and uses a proven pipeline already developed by the applicants in a prior CIRM funded study. • The project design is straightforward and patient iPSCs and candidate ASOs are, or will be, available for proposed gene expression evaluations. • The system described in Fig 5 is most conventionally used to generate excitatory neurons, not NPCs. The expression analysis in Fig 7 indicates the NPC marker nestin is only highly expressed at week 1 and is replaced by neuronal markers like MAP2 by week 2. It is confusing why the cells are described as NPCs when they do not appear to self-renew. • Later experiments suggest that immature and mature neurons will be made in the absence of basic fibroblast growth factor (bFGF). To obtain active neurons, it is essential to co-culture them with astrocytes. While rescue of the gene profile assays is the direct impact of the ASOs in vitro, this cannot be assessed in the eventual, treated patients. No experiments are proposed to investigate in vitro neuronal activity by electrophysiology, and this is a phenotype that may be useful as a biomarker in treated patients. • Being an abundant histone protein, the high transcript levels of H3F3A may require very efficient delivery and effectiveness of their ASOs in the brain. The lower levels of the EZH1 and NARS1 transcripts may make their ASOs more likely to succeed, and these could be prioritized. • The rationale for generating “knock in lines for each gene to use as isogenic control” in Aim 1 is not clear. Applicants state that “Several patient-derived lines for each gene will

	be compared with control IPSC lines in which the identical mutant allele is introduced via CRISPR. We will compare DGE signatures in controls vs patient cell line vs control line in which the mutation is knocked in". It is understandable that having an additional unrelated control iPSCs with similar mutation knocked-in could be useful, but since patient iPSCs with mutations already exists, the isogenic control is usually "rescued" patient iPSCs where the mutation is corrected by gene editing approaches. However, the proposal is not considering this critical control sample. • Sequencing of patient iPSCs planned for Aim 2 critical for designing of allele-specific ASOs should have been provided as a preliminary study. These data are important to support feasibility and for properly planning the experimental design. Such targeted sequencing, even with a long-range platform, is affordable, relatively simple and does not require dedicated Aim. • The project should identify ASO therapeutics for further development within timeframe of the proposal.
GWG Votes	**Is the project feasible?**
Yes: 13 **No:** 0	• Milestones are logical and feasible. An early task is to differentiate cells to NPCs and generate aliquots for later experiments. It appears from the description on p.21 that this means puromyin selected iPSC lines express the lentivirus. NPCs will only be made transiently in later passages by doxycycline induction of Ngn2. • The proposed milestones and expected project outcomes sound logical. • Yes, the project should yield candidates for handoff to the foundation. The prior review raised questions about further development of the ASO. The proposal does not address these aspects, but it outlines a strategy for going forward. It would have been better to have more detail on future preclinical development plans.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13 **No:** 0	• DEI is discussed at length for underserved communities. The free for life pledge by the foundation is a strength. Letters of support are included from support groups indicating that their perspectives will be incorporated. • DEI is extensively described, but it is still unclear if the selected patients and iPSCs represent race, ethnicity, sex and gender diversity • The DEI statement is weak.

Application #	DISC2-16801
Title (as written by the applicant)	A small molecule therapeutic to differentiate cancer stem cells
Research Objective (as written by the applicant)	We will develop a small molecule that blocks the growth of human triple negative breast cancer stem cells in vitro and in vivo.
Impact (as written by the applicant)	This work will lead to a new treatment for cancer stem cell driven triple negative breast cancer, and it will improve patient prognosis.
Major Proposed Activities (as written by the applicant)	• Identify the target of the candidate therapeutic • Iterate on the structure of the candidate therapeutic to maximize cancer stem cell differentiating activity as well as absorption, distribution, metabolism, excretion, and toxicology. • Conduct in vivo pharmacokinetics studies to identify the best performing versions of the candidate therapeutic. • Conduct in vivo pharmacodynamic studies in patient derived xenograft models to identify the best dosing strategy for the best performing versions of the candidate therapeutic. • Conduct efficacy studies of the lead candidate therapeutic in-patient derived xenograft models to demonstrate treatment performance Identify the mechanism of action of the lead candidate therapeutic.
Statement of Benefit to California (as written by the applicant)	Triple negative breast cancer is prevalent in the State of California. Because this research will lead to development of new treatments for these diseases, the citizens of California will directly benefit. Triple negative breast cancer affects people of all ethnicities and socio-economic status. Thus, if successful, the new therapeutic will improve outcomes for patients throughout the State of California.
Funds Requested	$2,772,002
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	75
Median	80
Standard Deviation	11
Highest	85
Lowest	50
Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	2
(1-84): Not recommended for funding	11

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 9 **No:** 4	• This project would develop a small molecule drug candidate that selectively induces differentiation of cancer stem cells (CSC) for treatment of triple-negative breast cancer (TNBC) and possibly other malignancies. • TNBC is a leading cause of mortality in women, and new therapies are urgently needed. The proposed candidate has a novel mechanism of action via inducing CSC differentiation and the loss of tumor-initiating potential that may lead to development of a new class of cancer drugs. • The proposed technology is a small molecule that can differentiate CSCs, and there is already a candidate that has worked in preliminary studies. • The candidate, if successful, would significantly improve patient response to current therapeutics and decrease resistance. • This is a resubmission of a previously reviewed application. The revised application is responsive to the reviewers' critiques. However, new preliminary data shows that the lead molecule has only a modest inhibition of tumor-initiating activity in vivo. • Improved therapy for cancers such as pancreatic ductal adenocarcinoma and triple negative breast cancer is an urgent unmet medical need. However, there is a low probability that the proposed technology will impact that need. • The applicant proposes that the work will lead to definition of a candidate for translation - namely a small molecule that selectively induces differentiation of cancer stem cells. The data and proposed work presented do not give much confidence that this will be the case. • The data presented do not strongly support the original premise of the mechanism of action or encourage the belief that the program is likely to generate a potent therapeutic compound. • Preliminary data suggest that the approach did not render the expected results.
GWG Votes	**Is the rationale sound?**
Yes: 9 **No:** 4	• Selective targeting of CSCs while sparing normal stem cells could offer a potentially effective and non-toxic cancer therapy. • Preliminary data suggests that target molecule acts via activation of the proposed signaling pathway in CSCs that could inform development of new analogues, however, there is no evidence that this activation alone is sufficient for inducing CSC differentiation. • There is a basis to argue that driving cancer stem cells into a non self-renewing state would be an effective tool for cancer therapy. This has been an active goal in cancer research at least since publication of a key review paper by the Clark and Weissman groups in Nature (2001). However, there is little precedent to show that this actually can be achieved, for example, by agonists of the signaling pathways as proposed. • The critical test called for in reviews of the previous application was in vivo demonstration that the lead compound prevents cancer stem cells (CSCs) from initiating tumors. New data presented in current application show only approximately a 2-fold

	decrease in tumor initiating cells. That seems too weak to have a significant therapeutic impact, and calls into question the fundamental premise of the project. • The rationale that targeting the proposed pathway would be sufficient are not compelling. • Evidence that the direct target of the compound is the proposed signaling pathway seems indirect. There are other examples of compounds that have been hyped to target CSC through a specific signaling pathway, but now appear more likely to work through different mechanisms. For example, consider a STAT inhibitor, which was widely touted to target CSC by blocking STAT3 signaling. However, its primary action against cancer now appears to be elevated production of reactive oxygen species.
GWG Votes	**Is the project well planned and designed?**
Yes: 10 **No:** 3	• The experimental design is logical. There are well designed in vivo studies, including the use of patient-derived xenograft models. • The project is well-constructed, and the response to the resubmission presented a sufficient advance in the technology that increases the compelling nature of the proposal. • The project takes advantage of the state-of-the-art methods for identifying molecular targets of lead molecule followed by the corresponding knockout library testing. • The proposed aims will identify the protein targets of the lead molecule. • The applicant proposes that additional variants of lead molecule will be characterized. • The applicant needs much more rigorous approach to testing that the activity actually is as advertised - inducing differentiation of CSC to a state in which they no longer can function as tumor-initiating cells. • It seems unlikely that the project will yield a product candidate that is ready to advance to translational studies. The notion that the action of lead molecule is through activating the proposed pathway is supported weakly by the data presented. The target-finding effort based on interaction of test compounds with cellular proteins might be informative but has many pitfalls. The connection between these activities and the specific claims about interaction with key element(s) of the pathway studied is not presented clearly. • Efforts to generate a translation-ready therapeutic candidate through screening seem likely to fail because the basis for the activity of lead molecule is poorly defined. The rest of the proposed chemistry becomes irrelevant if the activity is not primarily through differentiation of CSC as originally envisioned. • The plan proposes going down multiple tracks that don't enhance the likelihood of obtaining a useful therapeutic compound. This includes: the proposed knockout study of proteomics-identified targets; the characterization of models' systems to promote DEI, the molecular docking studies, etc. None of these is of great value if the basic concept of the biological impact of the lead molecule on CSC is incorrect. • The PI's publication record shows evidence of creative productivity in a variety of areas, mainly reflecting a background in chemical and biomolecular engineering. A strong collaboration with an expert cancer biologist with particular expertise in cancer stem cells might fill a significant gap.
GWG Votes	**Is the project feasible?**
Yes: 11 **No:** 2	• The proposed milestones and expected project outcome are logical and likely to be achieved within the proposed timeline. • PI and co-investigators have complementary expertise, forming a strong team for this project. • The team is qualified to develop small molecules. There is new expertise in mass spectrometry added. • Milestones as laid out are not likely to lead to a translation-ready candidate within the timeline.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**

Yes: 12 **No:** 1	• The project plan adequately addresses and accounts for the influence of race, ethnicity, sex and gender diversity. • The applicant reviews factors that may contribute to higher prevalence and worse outcomes of certain cancers among people of low socioeconomic status, and people of African or Latin descent. It is laudable to have models available that comprise a range of genotypes representative of a diverse population. However, the relevance of this to reaching the primary target of the project is not clear. In particular, it is not obvious that accessing many human tumor lines to test compounds for potential differences in efficacy as a function of ethnic/racial background will increase the probability of producing a viable therapeutic candidate, let alone one that will be of equal value across the full population. Having already locked into a specific starting compound, it seems a stretch to imagine that the success of further development will be impacted by the diversity of test lines. • It is unclear why the applicant chose the primary cancer type on which to focus (e.g., triple negative breast cancer) based specifically on its disproportionately high impact on minority populations. In the end, what would seem most critical is to find out against what types of cancer the candidate molecule is most effective against. It does not seem safe to assume that CSCs of all cancers would be equally susceptible to the compound.

Application #	DISC2-16538
Title (as written by the applicant)	A Gene Therapy Approach to Cardiac Troponin I Cardiomyopathy
Research Objective (as written by the applicant)	Developing an AAV gene therapy to deliver functional Troponin I3, targeting genetic roots of cardiomyopathy for effective treatment.
Impact (as written by the applicant)	The research aims to treat TNNI3 cardiomyopathy by delivering a healthy gene to heart cells, potentially improving heart function and patient outcomes.
Major Proposed Activities (as written by the applicant)	• Capsid and Promoter Tweaks: Improving AAV vectors to deliver TNNI3 gene effectively in heart cells, enhancing gene therapy precision. • Animal Tests for Dosage: Assessing therapy in mice with heart issues to gauge effectiveness and find the right dose for best results with fewest risks. • Cell Studies with Heart Cells: Testing therapy on human heart cells grown from stem cells to confirm it fixes genetic defects and improves heart function on a cellular level. • Big Heart Experiments: Trying therapy on pigs with human-like heart problems to see if it works well and is safe, refining dosing for future human trials.
Statement of Benefit to California (as written by the applicant)	The proposed research focusing on TNNI3 cardiomyopathy offers a beacon of hope for Californians affected by this specific heart condition. This proposal has the potential to change the treatment landscape, providing targeted solutions to the needs of individuals with TNNI3-related heart issues. Through these efforts, the research aims to alleviate the burden of TNNI3 cardiomyopathy within California, enhancing the quality of life for those impacted by the condition and their families.
Funds Requested	$2,772,000
GWG Recommendation	***(1-84): Not recommended for funding***
Process Vote	*All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."* *Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."*

SCORING DATA

Final Score: 80

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

Mean	73
Median	80
Standard Deviation	15
Highest	85
Lowest	45
Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	3
(1-84): Not recommended for funding	10

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 11 **No:** 2	• Troponin I3 (TNNI3) mutation causes thin filament cardiomyopathy, a severe form of heart disease. Traditional treatments focus largely on symptom management rather than correcting the genetic cause. The applicant proposes an adeno-associated virus (AAV) based gene therapy, to directly express wild-type TNNI3 within heart muscle cells via a one-time administration. • The candidate product is an AAV vector gene therapy encoding human TNNI3 gene expression under the control of a cardiac specific promoter. However, the promoter and sequence optimization are not adequately detailed. • The proposal aims to create and evaluate a cardiotropic capsid to deliver a gene therapy to treat cardiac troponin I cardiomyopathy. As a gene therapy, this would represent a cure for this mutation. • As the proposal mentions, TNNI3 expression is considered a late step in maturation of iPSC-derived cardiomyocytes (iPSC-CMs). The iPSC-CM model can be used for TNN13 protein overexpression measurement but might not enable evaluation of the associated functional phenotype. • The proposed technology is a reasonable approach to address hypertrophic cardiomyopathy caused by TNNI3 mutations. There are some concerns about the milestones proposed, suggesting that the project is not likely to significantly improve a gene therapy product. • The proposal needs more evidence showing high quality of iPSC-CM generated and used for AAV delivery.
GWG Votes	**Is the rationale sound?**
Yes: 12 **No:** 1	• TNNI3 cardiomyopathies encompass a spectrum of clinical presentations, with no strict association between TNNI3 mutation and clinical phenotype. The diversity of phenotypes belies their common cause: defective or absent cardiac troponin I in the sarcomere, encoded by a mutated TNNI3 gene. • The rationale is that directly delivering wild-type TNNI3 within the cardiac sarcomere structure holds the potential to treat all the manifestations of TNNI3 cardiomyopathy. • The general rationale is sound to deliver TNNI3 transgenes using AAV into iPSC derived cardiomyocytes and animal models. • The preliminary data demonstrates functional TNNI3 protein integrates correctly into the thin filament structures of the sarcomere in wildtype mice. It also appears to extend lifespan in mutant mice. • Preliminary data for iPSC differentiation into cardiomyocytes and phenotyping are not shown. The only data are from a TNNI3 western blot, in Fig 3a, of the different constructs after transduction. There is no description of the final construct design, just that they differ in codon optimization and promoter sequence.

	• Proper localization of the human TNNI3 is shown in mouse hearts. Mouse models include the TNNI3 KI homozygous model. Treatment with the AAV vector showed gene transfer, and protein expression, but only modest improvement in survival. • Further transgene optimization is done in cell lines (Fig 6) and shows it is expressed in HEK293 and HELA cells suggesting it is not cardiac specific. The prioritized construct is higher expressed in cardiac AC16 cells, but this line was made by cell fusion, and it is not clear if their karyotype is normal. • The method to make cardiomyocytes is claimed to be a recent and improved protocol, but it refers to a standard method derived from a publication in 2017, and no effort is proposed to engineer 3D cardiac tissues.
GWG Votes	**Is the project well planned and designed?**
Yes: 9 **No:** 4	• Overall, the project is appropriately planned and designed. The preliminary data are mostly supportive. The mouse model and large pig model for product evaluation in vivo are its strengths. • In Pilot Study #1, they tested two AAV vector capsids in iPSCs derived CM in vitro and in wildtype mice. Data showed that only the highest dose of AAV was sufficient to drive detectable expression of human TNNI3. Therefore, they seek to test a new engineered AAV capsid, for which they do not yet have any data. • In Pilot Study #3, they optimized the TNNI3 expression cassette to increase TNNI3 expression and found an optimized construct as the candidate. However, the proposal does not include detailed optimization information for this candidate. • They have developed a small and large animal model of TNNI3 mutants, which is a clear strength of the proposal. This will provide information about cardiac function, TNI incorporation, biodistribution, some safety characteristics, and a clinically relevant dose. • The path forward is well delineated by the team. • The applicant plans to measure cardiac TNNI3 protein expression, which is a critical primary endpoint and will help lead to approval of the AAV-TNNI3 gene therapy. They will look at dosing, biodistribution and gene expression. • Some proof of concept data may be obtained, but the planning and design of project is not optimal. The animal models are homozygous, whereas human HCM is heterozygous. This may complicate interpretation. • The project is not well constructed. The iPSC aim is underdeveloped. Apart from examining protein levels, the only phenotyping that will be done is on isolated myofibrils. HCM in iPSC derived CMs is more commonly evaluated by intact cell contraction assays using Ca2+ imaging, an Xcelligence device, or 3D contractile engineered tissues. Hypertrophy of CM size can also be assessed. • Myosin inhibitors have been tested in iPSC HCM models, and the applicants could compare the effectiveness of their vector versus these compounds. It is not clear if the three patient iPSC lines are available and have, they been previously analyzed by other groups. Exploring effects on diverse iPSC lines is not discussed. • Pitfalls include the possibility that iPSC-CMs express primarily troponin I1 (TNNI1) and that the TNNI3 mutation might not reveal a phenotype. The isoform ratio and a hypertrophic phenotype really needs to be validated as a preliminary result.
GWG Votes	**Is the project feasible?**
Yes: 10 **No:** 3	• The design follows logic and is achievable with the previously established animal models and available resources. • The PI is an Assistant Project Scientist and has no other funding support. PI has a secure Letter of Support from his current supervisor, attesting to his capabilities and readiness to serve as PI on this project. • This proposal has an achievable level of work and will prepare the applicant for the next steps to an IND meeting.

	• This is a small but committed team with strong Letters of Support. They will be able to leverage these resources to help to move the experiments along. • They appear to have all the facilities in place to conduct the experiments. • The team has relevant expertise for the project described in a half page. The lead is an Assistant Project Scientist and has one Letter of Support from their supervisor attesting to their ability and stating that the project is the lead's initiative. The Key Personnel for the iPSC work lists relevant skill sets, but team publications refer to large animal models only. • The iPSC milestone involves characterizing 3 different mutations in a total of 4 months. This is not feasible. The other milestones are more likely to succeed, but there are concerns about the relevance of the homozygous animal models. • The stated resources are a half-page list of a number of core facilities. There is no description of iPSC culture or phenotyping resources, or wet lab/office equipment.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13 **No:** 0	• DEI is well-discussed across underserved communities that are well represented in the local hospitals. A Letter of Support is provided by a hypertrophic cardiomyopathy association, documenting that patient perspectives will be taken into account. • The purpose of this work is to create a AAV gene therapy for patients with TNNI3-associated HCM. Because the target is any individual with a pathogenic TNNI3 genetic variant resulting in cardiomyopathy, there will be no sex, ethnicity, or race selection criteria for the proposed studies. • The applicant will test different cell lines and both male and female animal models. This product would provide a universal aid to all populations.

Application #	DISC2-16569
Title (as written by the applicant)	Pioneering the next generation of MSH3 mRNA-silencing strategies to halt somatic expansion in Huntington's Disease
Research Objective (as written by the applicant)	The therapeutic candidate aims to reduce MSH3 mRNA levels via RNA splicing modulation, preventing CAG repeat expansion in Huntington's Disease and slowing neurodegeneration.
Impact (as written by the applicant)	Huntington's Disease, Myotonic Dystrophy, Friedreich's Ataxia, ALS, Spinal Cerebellar Ataxia, Fuchs Corneal dystrophy
Major Proposed Activities (as written by the applicant)	• Develop tools for assessing MSH3 lowering and its impact on human HTT CAG repeat expansion • Identify a MSH3 lowering lead compound with ADME-tox profile suitable for proof-of-concept in vivo studies • Determine the pharmacological impact of MSH3 lowering using a small molecule splicing modulator in vivo in human HD models
Statement of Benefit to California (as written by the applicant)	The proposed research, a collaboration with companies across California, will develop a novel treatment for Huntington's Disease, benefiting thousands of Californians affected by this debilitating condition. By pioneering a new therapy that prevents disease progression, the project will enhance patient outcomes, reduce healthcare costs, and bolster California's biotechnology industry through local job creation, advancing the state's leadership in innovative healthcare solutions.
Funds Requested	$1,806,418
GWG Recommendation	***(1-84): Not recommended for funding***
Process Vote	*All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."* *Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."*

SCORING DATA

Final Score: 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	77
Median	79
Standard Deviation	2
Highest	80
Lowest	74
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	14

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 13 **No:** 1	• Huntington's Disease (HD) is an incurable autosomal dominant neurodegenerative disease with a cytosine-adenine-guanine (CAG) expansion hallmark. The disease lacks effective treatments. • This proposal aims to develop a small molecule to lower MSH3 mRNA levels as a potential therapy for HD. There are no available therapies so this could meet an unmet need. There are however several similar strategies much further on in clinical development and in clinical trials. • There is no treatment for HD. A small molecule to reverse disease process would be a significant breakthrough. • The oral delivery is appealing and would improve cost and availability of therapy. The next steps are defined as preclinical optimization of safety and efficiency, dose-ranging and long-term safety, PK and PD modeling, toxicology, and then an IND application. The therapeutic may potentially benefit other conditions. • The proposed therapy is intended to reduce MSH3 mRNA levels by modulating MSH3 mRNA splicing, which could reduce CAG repeat expansion, a major disease progression driver in some individuals. The project proposes lead candidate discovery via innovative methods. • Based on the mechanism of action, it might be necessary to deliver the proposed drug indefinitely. Long-term durability of effects is not studied in this proposal. The applicant speculates that reduced volume in key brain regions might be a biomarker, and that early detection and delivery could be disease modifying. • The proposed project's endpoint is efficacy in a patient striatal organoid (i.e., rather than in an HD animal model). Very little detail is provided about the expected small molecule(s) therapy, making it difficult to judge its capability for oral delivery and minimal off-target effects. • The small molecule therapeutic identified through this project could prove to be orally bioavailable and blood-brain barrier permeant. These are potential advantages over intrathecal delivery of oligonucleotide therapeutics. However, this is speculative. • This project requires novel compound discovery and may not lead to an effective therapeutic candidate.
GWG Votes	**Is the rationale sound?**
Yes: 12 **No:** 2	• The proposed therapeutic candidate will promote degradation of MSH3 mRNA by harnessing cellular machinery for nucleic acid cleavage that in turn promotes nonsense-mediated mRNA decay and blocks protein production. The small molecule candidate would target human striatal neurons (the relevant target cells for HD) and modulate splicing of MSH3. • Human genetic studies have identified MSH3 variation as a significant modifier of HD onset, with reduced activity linked to less somatic expansion of the toxic huntingtin (HTT) allele. Preclinical models demonstrate that genetic knockout or reduction of MSH3 in HD models delays CAG repeat expansion and neuronal degeneration. • MSH3 is a modifier of Huntington's Disease (HD), and there are a number of knock-down approaches in development. • The proposed screening systems are in place, and some preliminary hits have been identified.

	• The applicant indicates that the effective small molecule therapeutic could lead to clinical readouts such as changes in regional volumes in the brain and reduced HD symptoms. • Yes. The MSH3 approach is effective in HD models, and the knockout mouse survives. • It's not clear how HD neuron selectivity occurs? • This is a very complex, multiply outsourced program. It is difficult to understand the status of the many pieces. • The scientific rationale for the project is justified by data in Figures 1-4. Fig 1b. Figures 1-3 are convincing. Figure 4 is labeled as a human iPSC organoid expressing TH and DAT DA transporter, but this result is very hard to see in the images.
GWG Votes	**Is the project well planned and designed?**
Yes: 11 **No:** 3	• It is a well-planned project with a clear step-wise approach. Pitfalls, especially related to therapeutic potential and suitability is well presented but only partially mediated via contingency plans. • There are several Letters of Support including from the consultant CMO, who is a psychiatrist and directed a program targeting MSH3 with antisense oligo (ASO). • Yes. Studies include lead optimization, efficacy work in human cells, PK in animal models, and efficacy studies in humanized mouse. • The organoid model is not sufficiently tested. • It is unclear what constitutes proof-of-concept data for the product candidate. • There is a mention of off-target effects, but methods to reduce them are not very clear. MSH3 polymorphisms are implicated in rectal cancer. • The ambitious timeline carries the risk of not delivering on time.
GWG Votes	**Is the project feasible?**
Yes: 11 **No:** 3	• Preliminary data are included that support the approach. Yet, the project is early stage and would benefit from more preliminary data to fully assess feasibility. • Establishment and validation of the proposed models is complex and may prove difficult. Availability of striatal organoids does not guarantee an organoid disease model with reproducible and quantitative readouts. Not enough consideration is given to the stage of disease progression at which the compound should be administered, or to potential chronic toxicity. • Potential difficulties with the proposed human models are not given sufficient consideration. It would be better if there were preliminary data on repeat expansion in these models. • It is not clear that the compound to be discovered will be sufficiently advanced to be prepared for an INTERACT meeting (in which proof of concept should already be established). • The team appears to lack input from an experienced neurologist. • There are several critical product development steps that are outsourced but are identified. • The project is proposed to be conducted in 2 years and additional funds are requested. Support for a large number of people is requested. The compressed timeline puts more pressure on outsourced deliverables. • The justification for the abbreviated timeline is not very compelling. • The two-year timeframe is very ambitious. • The applicant does not address off target effects, durability and timing of therapy.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14 **No:**	• DEI is well incorporated in the plan and samples will be sourced from diverse individuals.

0	• The applicant proposes to test samples from males and females of differing genetic backgrounds. The diagnostic frequency by race is provided. Males have a slightly higher frequency, but females are more symptomatic. • The product is expected to act independently of demographic factors and may be more cost effective than alternatives. • Diverse iPSC cell lines will be included for testing. Six of seven cell lines are currently from women. The applicant proposes to stratify samples-based polymorphisms (SNPs) associated with disease progression rate. • The applicant may stratify based on SNPs in MSH3. • Letters of Support from HD organizations indicate recognition of the promise of the new therapeutic and visibility through relevant meetings.

Application #	DISC2-16763
Title (as written by the applicant)	Development of a safe and effective transplantation strategy for engineered stem cell-derived islets for the treatment of diabetes
Research Objective (as written by the applicant)	We propose engineered vascular niche-containing human stem cell-derived islets to restore blood sugar levels in diabetes, without the need for insulin injections
Impact (as written by the applicant)	Our work would overcome three major bottlenecks for cell replacement for diabetes: dearth of supply, poor engraftment and function of beta cells, and safety risks associated with hepatic site.
Major Proposed Activities (as written by the applicant)	• Identify, obtain, scale-up, and perform QC for commercially consented hPSC lines • Identify eligible hPSC line that yields functional SC-beta cells w/ disease-modifying activity • Introduce universal safety switch to permit clearance of all hPSC-derived cells • Define conditions that support efficient SC-beta engraftment, function at non-invasive site • Demonstrate disease-modifying activity of engineered SC-islets at non-invasive site • Draft target product profile
Statement of Benefit to California (as written by the applicant)	Type I Diabetes (T1D) is a significant burden in California, especially for children; according to estimates provided by the California Diabetes Program, ~2.3 out of every 1,000 children between the ages of 5-19 in California had diagnosed diabetes in 2008, with 83% having T1D. Research proposed here would represent a significant step towards the holy grail of T1D treatment: a therapy for patients without the need for the administration of insulin or frequent blood testing.
Funds Requested	$2,839,239
GWG Recommendation	***(1-84): Not recommended for funding***
Process Vote	*All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."* *Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."*

SCORING DATA

Final Score: 78

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

Mean	78
Median	78
Standard Deviation	2
Highest	80
Lowest	75
Count	14

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

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 11 **No:** 2	• This is a high significance project, which proposes to derive an effective stem cell-based treatment for type 1 diabetes, T1D. The candidate has the potential to impact an unmet medical need. The technology will consist of transplantable allogeneic pancreatic islet beta-like cells (SC-BLCs) differentiated from human pluripotent stem cells (hPSC). The SC-BLCs will be derived from the donor-eligible and commercially consented hPSC line(s). • The transplantation will be done at an alternative site (liver is normally used for islet transplantation). Instead, the SC-BLCs will be transplanted at alternative site(s) for easy monitoring with non-invasive procedures and a simplified retrieval, if needed. The cells will be genetically engineered with an inducible, universal safety switch to permit clearance of all grafted hPSC-derived cells in the case of an adverse event. • The proposed technology is designed to identify cGMP lines that can efficiently differentiate to islet-like cells, to introduce a safety switch and to improve their engraftment in non-invasive sites by accessorizing the SC-islets with vascular cell types. If successful, this approach can improve the life of people living with T1D and eligible for transplantation. • This product will accelerate the likelihood of developing a stem cell-based therapy for T1D. • The applicant presents a clear plan to produce cGMP cells with a safety switch for clinical use and mentions the possibility of collaborating with other groups producing vascular cells for the deployment of the SC-islets in alternative sites.
GWG Votes	**Is the rationale sound?**
Yes: 13 **No:** 2	• The proposed project is based on sound scientific rationale as there is a clear need to: 1) identify cGMP lines that can efficiently generate islets, 2) introduce safety switches and 3) improve engraftment in non-invasive sites. • The scientific rationale of the project is sound. The PI notes that one of key barriers to translation of hPSC-based technologies to treatment of T1D is a poor survival of stem cell-derived beta like cells (SC-BLCs) upon transplantation. The PI hypothesizes that combining the SC-BLCs with vascular cells to form a protective niche for SC-BLCs would increase *in vivo* survival of the insulin producing cells. • The applicant provides strong preliminary data demonstrating that engineered SC-islets with vascular cells perform better *in vitro* (improved glucose-stimulated insulin secretion) and *in vivo* (improved survival and insulin release). • The second hypothesis to be tested in the project is that the safety of the system can be assured by incorporating an inducible safety switch into hPSCs that would make possible the elimination of the transplanted cells in case of adverse events. Both are reasonable hypotheses.

	• The applicant has published data demonstrating that safety switches can be successfully introduced into the cells and perform as needed. This strongly supports the feasibility of this proposal. • However, while the applicant shows that SC-islets plus vascular cells lower glycemia in diabetic mice, the team does not show normalization of glycemia when these cells are transplanted under the kidney capsule, which is concerning. • Additionally, the differentiation efficiency to SC-islets seems highly variable with no indication of what the other cells in these clusters are. Analysis of other cell types should be included in their assessment.
GWG Votes	**Is the project well planned and designed?**
Yes: 9 **No:** 4	• The project is appropriately planned and designed to achieve the expected outcome. As discussed above, the most challenging part would be the protocol optimization for different lines, the need to prove that the sc-islets can normalize glycemia and the requirement to better characterize the final product by including additional on-target and off-target marker analysis. • The project is well-planned and is likely to achieve the expected outcome, including proof-of-concept data for further translation of the technology. The preliminary data are supportive of the project. However, several Figures could be improved by including additional controls. • This project is planned well and includes preliminary data to support the experiments. • Yes, this project contains state of art methods and quality controls for their experiments. • This is a well-constructed project. • Potential pitfalls have been identified and contingency plans are in place. • The team has outlined potential pitfalls in their proposal. • The project timeline demonstrates an urgency that is aligned with CIRM's mission. • Donor-consented cells have already been generated under another CIRM-funded project. It's not clear why this needs to be done again. • The population is not well defined.
GWG Votes	**Is the project feasible?**
Yes: 11 **No:** 2	• The proposed milestones are likely to be achievable within the proposed timeline. • The milestones are feasible. • The team is qualified. • The team has access to state-of-the-art facilities. • The budget is appropriate. • Expertise to generate the data required is not well demonstrated.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13 **No:** 0	• The proposed project, wherever possible, will balance the selection of donor-eligible, commercially consented hPSC lines to account for the racial and ethnic and gender diversity of the US population. • The team will try to factor in sex and race when choosing the cGMP lines. • The product would be available to the all people living with T1D, with no racial/ethnic restrictions. • The applicant does not have plans to incorporate perspectives and experience from the T1D population during the course of the award.

Application #	DISC2-16784
Title (as written by the applicant)	Mechanism for the Curative Potential of Fucosylated Autologous Hematopoietic Stem Cell Transplantation in a Mouse EAE Model of Multiple Sclerosis
Research Objective (as written by the applicant)	Improving the curative potential of autologous hematopoietic stem cell transplantation as a safe and cost-effective treatment option for patients with Relapsing Remitting Multiple Sclerosis
Impact (as written by the applicant)	Treatment Resistant Relapsing Remitting Multiple Sclerosis
Major Proposed Activities (as written by the applicant)	• Research grade enzyme optimization and downstream processing activities • In vitro optimization for the fucosylation of peripheral blood (PB) mobilized hematopoietic stem cells (HSCs) • Safety and efficacy of fucosylated aHSCT (Fuco-aHSCT) on clinical indices and behavior in the in vivo experimental autoimmune encephalitis (EAE) mouse model • Increased homing and persistence of regulatory T cells (Tregs) in the CNS with fucosylated aHSCT • Reduction in the formation of lesions using imaging assessments in EAE mice receiving aHSCT • Measurement of biomarker end points in the CNS of EAE mice post Fuco-aHSCT
Statement of Benefit to California (as written by the applicant)	Multiple sclerosis (MS) afflicts about 1 million people in the US and studies from California have indicated that the burden of MS amongst the Black and Hispanic communities has long been under-recognized. aHSCT is a promising curative treatment option but has significant shortcomings related to safety and mortality. Positive outcomes from the present study can rectify many of the issues associated with aHSCT thereby transforming it into a safe, effective, and cost-efficient treatment option for MS
Funds Requested	$1,749,999
GWG Recommendation	***(1-84): Not recommended for funding***
Process Vote	*All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."* *Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."*

SCORING DATA

Final Score: 75

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

Mean	77
Median	75
Standard Deviation	3
Highest	85
Lowest	72

Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	1
(1-84): Not recommended for funding	14

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 13 **No:** 1	• Although there are some treatments for multiple sclerosis (MS) available, there is still an unmet need for more effective therapies and cures. The project seeks to improve safety and efficacy of an already existing therapy with potential for better treatment. • New treatments for MS are needed. While current high efficacy disease modifying therapies (DMTs) are able to prevent inflammatory disease in MS, they require repetitive administration, are not curative and are largely unable to prevent disease progression. In contrast, successful hematopoietic stem cell transplantation (HSCT) can improve progression-free survival rate. An ongoing trial is directly comparing autologous HSCT (Auto-HSCT) with current DMTs. • Auto-HSCT is used clinically for the treatment of MS but has several drawbacks which limit its utility. This project will address one limitation by modifying HSCs using fucosylation to improve homing and engraftment. • The proposed product provides a clinically relevant approach to engineering autologous stem cells to improve their ability to home into the bone marrow and increase engraftment efficiency. • improving HSC engraftment would be a significant achievement to avoid risks associated with post-transplant cytopenias. The applicant has shown, in preliminary data and published work, that their fucosyltransferase can significantly increase fucose on the surface of HSC after a brief incubation, and this enhances engraftment in vivo. The simplicity of the approach is a strength of this overall program. • The applicant team has already advanced this technology for autologous transplant for patients with hematopoietic malignancy. They are conducting a trial currently. The current proposal seeks to test the same process in the context of Auto-HSCT for treatment of MS. • Auto-HSCT is a therapy used to treat some patients with MS. The applicant hopes to improve upon the Auto-HSCT engraftment in this setting and will model it using a murine model of MS. • The proposed product has an Open IND and is in late stage clinical development. • The project plan comprises manufacture of clinical grade product, DMF submission and design of an initial phase 1/2a trail (to commence approximately 12 months after this project). • There is a discussion on the next steps following the completion of this proof-of-concept study. • Overall, the premise and simplicity of this technology are strengths, and the applicant addresses an important challenge in the field of HSCT. This approach could be useful for

	HSCT across a variety of disease indications where HSCT is appropriate (autologous and perhaps allogeneic as well). • Concern exists that this will not lead to a candidate product, as extensive animal work is needed to see if this is ready for translation.
GWG Votes	**Is the rationale sound?**
Yes: 13 **No:** 1	• The use of immunoablative or myeloablative therapy followed by autologous hematopoietic stem cell transplantation (Auto-HSCT) has been used for the treatment of MS for more than two decades with varying results. • This study seeks to find mechanistic insight to be used for better therapeutic effect and provide proof of concept for this in a mouse model. This is important, but it's unclear this effort will be successful. • The rationale for Auto-HSCT in MS is sound and is currently under investigation in clinical trials. The rationale for fucosylation as a means to improve homing and engraftment of HSCs is very strong, having been established in GVHD in preclinical studies and initial clinical trials. • It is unclear whether homing is a limitation in clinical practice. Can increasing the dose of unmodified HSCs by a factor of 5-fold result in a similar level of engraftment? This is a relatively minor weakness as it appears that immunomodulatory Treg cells in particular have low fucosylation and would benefit greatly from this approach. • Preliminary data establish that the proposed enzyme product can increase the fucosylation of HSCs. • Data show that fucosylated human CD34+ cells engrafted more efficiently in NSG mice via FACS and can be visualized using in vivo bioluminescence. • Preliminary data were published in a 2015 publication in the journal *Blood*. No new preliminary data are provided. • EAE data in Figure 16 require clarification. The model used is unclear, and there is no mention of sample size. Is this is published data by this group or from another group? Additionally, there are no preliminary data of HSCT in mouse EAE. • The rationale for specifically testing fucosylation in EAE/MS is not clear. The current understanding of the role of HSCT in MS is that it provides an opportunity to use high-dose non-specific chemotherapy to kill autoimmune T and B cells with the hope that deep elimination is achieved and the regenerating immune compartment is purged of autoreactive clones allowing an immune “reset”. Thus, the “therapy” is really the chemotherapy that kills autoimmune cells, and the stem cell transplant is a rescue due to killing of HSCs. Thus, if their technology is really focused on improvement of HSC engraftment, as they clearly state, it is unclear why this needs to be tested in EAE/MS if the regeneration is being shown in advanced stage clinical trials (for malignancy). It would make sense if there is reason to believe that fucosylation of the cells would impact their engraftment differently in EAE/MS compared to the malignancy context, but the investigators do not provide such rationale or discuss this.
GWG Votes	**Is the project well planned and designed?**
Yes: 13 **No:** 1	• The project plan is well described, but it is unclear if and how it will lead to better therapy. • The successful completion of this project will achieve the stated goal, which is to provide proof-of-concept in mouse EAE that fucosylation improves cell homing and provides meaningful therapeutic benefit (both via clinical score and using biomarkers of tissue integrity such as histological and MRI imaging end-points). • Sample size comes across as somewhat arbitrary. There are no power analysis or relevant preliminary data utilizing aHSCT in EAE to justify the experimental design. • Only female animals are used in the project. • Long term follow up appears to be only 4 days after the initial MRI (at 45 days). • The proposed histological analysis of spinal cords is somewhat rudimentary. Electron microscopy is mentioned, but it is not clear which animals will be utilized with this

	approach. More modern approaches, such as lineage tracing, to label new oligodendrocytes would provide a better measure of remyelination vs. axonal preservation. • Single cell transcriptomics will likely not provide much in the way of meaningful data that could be utilized to improve the efficacy of this therapeutic approach. The use of bulk techniques such a real-time PCR to validate single cell approaches is confusing and unlikely to succeed. • MRI imaging of EAE brain adds relatively little to the proposed experiments. Demyelination does not occur in the brain in MOG EAE. The specificity of any observed effects would need to be examined separately; any observed changes may be due to the overall inflammatory nature of the model, rather than as a consequence of inflammatory demyelination. • Surprisingly, it appears that clinical score measurements will only be performed at predetermined time points rather than daily analysis. This is a weakness as the timing of disease can vary somewhat between experiments. • There is some discussion of problems and alternative approaches. • Chemotherapeutics used to induce myeloablation will cause progenitor dysfunction in the brain and clinically are often associated with chemotherapy-induced cognitive impairment. As such, it would be important to determine whether Auto-HSCT causes off-target effects on animal cognition and progenitor dysfunction. • The time line describes research grade manufacturing of the enzyme product will be completed in 8 months, yet the budget asks for substantial support to the PI's team in years when most or all work will be performed in a collaborator's lab. • The applicants will pursue the possibility that HSC fucosylation may enhance Treg levels, and that this could be a mechanism to mediate immune suppression in the CNS and mitigate EAE/MS. However, they miss the opportunity to delve into this mechanistic hypothesis. • Firstly, when the team state that their enzyme enhances Tregs and reference Figure 8, it is clear that engraftment of all lineages is enhanced (as they also recognize). So this is just consistent with more rapid hematopoietic engraftment in general, not something specific to Tregs. Interestingly, they don't actually show the engraftment of Tregs in Figure 8, which is surprising given the emphasis on pursuing Tregs as a potential mechanism. • Additionally, in Figure 8 is hard to know what they are showing (% of engraftment). Absolute cell numbers would have been a clear way to present the data. So, this reported increase in Tregs is not compelling rationale supporting their active role in mitigating disease. • If they believe that Tregs are important, they miss the opportunity to implicate Tregs more conclusively. Do they believe that Tregs in the transplant product are important, or is it Tregs emanating from engrafted HSCs (only the former would be fucosylated)? This basic question is not addressed. For example, they do not propose to measure/characterize Tregs in the graft. They do not propose Treg depletion experiments that could determine their importance. • In addition to comments above related to consideration of Tregs, it's unclear why there is so much attention to production of the enzyme when they are already using it in clinical trials. • The dose of HSC used is not justified. A single dose is proposed. Why not do a dosing study to see at what level the treatment improves engraftment over control? • Activity 2 includes analyses of biomarkers and scRNA-seq of CNS tissues after HSCT in EAE. Applicants provide more than ample technical detail of experimental procedure but no focused scientific rationale, hypothesis, or questions.
GWG Votes	**Is the project feasible?**
Yes: 14	• The given timeframe is realistic.

No: 0	• Yes. The proposal is logically designed starting with the development of a reagent grade enzyme for use in mice, validation of the approach in mobilized HSCs, in vitro confirmation, and assessment in animal models of MS. The approach utilizes both chronic and relapsing EAE models. • The PI has demonstrated expertise in the preparation of the proposed enzyme product and its ability to generate fucosylated HSCs for HSCT. The collaborator is an expert in MS and associated animal models. • The team has the required expertise. The critical reagent(s), fucosyltransferases, are already in advanced stage development. The team has the requisite expertise including EAE modeling. • Based on the timeline, the principal role of the PI's organization (manufacturing research grade enzyme) will be completed in the first year. However, a large portion of funding is requested for the PI's organization in years two and three. • Furthermore, there is insufficient justification for the variations in salary support for staff at the PI's organization. Salary support increases dramatically mid award and is then reduced in the last year. The PI's milestones end after year 1, yet the budget is increased for personnel and still very substantial after this time. • Scientific advisor, secretarial and accounting staff must be removed from the budget. This is not an appropriate use of CIRM funds. • The budget request lacked sufficient detail. • Yes, there are no feasibility concerns to note
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14 **No:** 0	• The applicant states that DEI considerations are not applicable for a preclinical study. While the issues are certainly not the same as for a clinical trial, they are not entirely irrelevant. • MS affects racial/ethnic communities differently, and this is described in proposal. Other considerations of DEI are not described. • This was not addressed in the project outline.

Application #	DISC2-16808
Title (as written by the applicant)	Gene corrected patient derived iPSC-based autologous platform for delivery of BDNF to treat Huntington's Disease
Research Objective (as written by the applicant)	Human induced pluripotent stem cells (iPSC)-based platform for the delivery of the neuroprotective factor BDNF to prevent neurodegeneration in Huntington's Disease.
Impact (as written by the applicant)	Huntington's disease
Major Proposed Activities (as written by the applicant)	• Gene correction of HD patient-derived iPSCs and engineering to overexpress BDNF and kill switch proteins • Differentiation of engineered iPSC into striatal progenitor cells for transplantation studies • Determine the lowest cell and BDNF doses of gc-STRpcs able to prevent the HD phenotype and assess their short- and long-term efficacy in HD mouse models • Validate that activating the safety switch with rapamycin can eliminate the engrafted gc-STRpcs without causing neuroinflammation in the striatum of Q175 mice • Determine a pilot process for engineering iPSC at multiple genomic loci without genotoxicity
Statement of Benefit to California (as written by the applicant)	Almost all cases of HD manifest in the 35-to-64-year age group, with debilitating symptoms presenting by early 30's, meaning most patients are unable to participate in paid-employment. Our goal is to develop a gene-editing therapy that is effective at correcting at providing neuroprotection and is accessible to all patients. Our study will support California's lead in driving innovative stem cell research for studying disease mechanisms and developing innovative stem cell-based gene therapies.
Funds Requested	$2,407,578
GWG Recommendation	***(1-84): Not recommended for funding***
Process Vote	*All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."* *Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."*

SCORING DATA

Final Score: 75

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

Mean	77
Median	75
Standard Deviation	2
Highest	80
Lowest	75
Count	15

(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 15 **No:** 0	• Huntington's Disease is a progressive neurodegenerative disease leading to striatal atrophy without effective therapy and thus presents a clear unmet medical need. Recent therapeutic failures with oligonucleotides have placed new emphasis on the potential importance of transplanted cells. • Thus far in the field, success of tissue and cell grafting for HD has been limited. • Fetal cell transplant therapy in HD has had mixed success, and the largest study was not successful, possibly due to immune rejection. • The candidate is a novel cell therapy designed to deliver a therapeutic agent BDNF to the affected region of the brain in HD. There is no treatment for this neurodegenerative disease. • The product seeks to both protect and replace neurons lost in Huntington's Disease (HD). Each strategy alone, or combined, would meet an unmet clinical need since there are no available cures of restorative therapies in for HD patients. • The generation of patient-specific gene-corrected cells could advance the field of therapy for neurodegenerative disease and potentially mitigate immune rejection problems. • The proposed therapy of genetically corrected HD patient-specific iPSCs is very complex. Its primary MOA is neuroprotection to slow disease progression, with a potential for cell replacement. A safety kill switch to induce apoptosis is incorporated. • Yes, a clear milestone plan is provided for the DISC award. Further translation through IND-enabling studies and then into clinical trials is not well defined. • It's not clear how long it would take to prepare a patient-specific gene-corrected cell product.
GWG Votes	**Is the rationale sound?**
Yes: 13 **No:** 2	• The primary rationale is BDNF delivery for neuroprotection. Efforts to enhance neurotrophin delivery have been tested for many years already. • Both neuroprotection using BDNF and cell replacement are based on good scientific rationale. The combination of these would also be good, but maybe not in the same product? Also, the rationale for moving onto an autologous, gene corrected product is not entirely clear. • There is precedent for a role of BDNF depletion in HD pathology in mice and patients, for reversing HD through administration of BDNF, or by administration of neural progenitor cells. Evidence exists in patients that BDNF levels are reduced in the striatum. Prior studies of BDNF therapy have not reversed disease.

	• Yes, the applicants have provided a compelling POC in a well-established mouse HD model that demonstrated key HD features. • One mouse model reproduced aspects of HD, such as intranuclear mHtt aggregates and progressive cognitive and motor deficits. They were suppressed using several antibodies. BDNF levels were somewhat elevated but inconsistent, and post mitotic neurons were few. • Figure 4 shows reduced motor and cognitive deficits. The BDNF increase is not that striking. • Preliminary data in figure 4 showing improvement in a mouse HD model are not overwhelming. • Safety switch approaches have not been shown to be highly effective - thus safety remains an issue. • Safety is likely to be a concern with this iPSC multi-edited therapy, and when to activate the kill switch is an important question. Whole genome and SNP testing is proposed. • Milestone 3 includes testing of the minimum effective cell dose. Short-term and longer-term mice will be used for kill switch testing. This lowest dose testing will require quite high levels of reproducibility to be valid. • Difficulties with immune suppression are one reason to emphasize autologous cells. • The benefit of autologous grafting is not clear.
GWG Votes	**Is the project well planned and designed?**
Yes: 14 **No:** 1	• The project is well planned and described in the proposal and follows a long trajectory of work by the group. It is ambitious but feasible. • The milestones are well defined. • In the TPP critical Milestone 5, one clone from 3 different patients is created with the desired edits and genome stability that maintains pluripotency, BDNF expression and kill switch efficacy as well as no off-target mutations after propagation. It is ambitious but appears to be possible, with certain unknowns. • After correction and addition of BDNF genes, differentiation to forebrain and striaital progenitor cells will be assessed using different protocols. To confirm cells for transplantation studies, the minimum expression of key markers is defined. • This project will build cells expressing different levels of BDNF. Extensive gene editing is required. Genetic stability will be assessed by SNP array; this may fail to pick up significant alterations, e.g., p53 mutations. The possible presence of off target cell populations is not examined. Edited clones may not differentiate well; ideally cell lines should be cloned before genetic manipulation. • Potential issues may arise with the requirement for multiple rounds of edits and the accompanying clonal selection. • It is uncertain whether the efficiency of the safety switch is sufficient to guarantee protection of patient. • Alternatives to low target clone frequency and low striatal progenitor differentiation efficiency are presented. • It's a very complex plan, and unanticipated delays may occur.
GWG Votes	**Is the project feasible?**
Yes: 14 **No:** 1	• The team is well composed and covers the expertise necessary. All resources and infrastructures are in place. • Clones producing different levels of BDNF will be obtained. • It's an ambitious project, but milestone success is defined. • Yes, this is an excellent team. the PI is a well-trained MD, PHD clinician scientist, while another key personnel is an eminent neurologist. The PI has extensive hematological transplant and stem cell experience. Together they provide substantial clinical translation experience.

	• The team comprises experts in gene editing, neurology, and hPSC neural differentiation. • It's not clear whether this team could advance the project into clinical trials, but there should be adequate collaborators. • There is good preliminary data for the neuroprotection and some also for replacement based upon the use of wild type cells in same animal model supporting its feasibility. The preliminary data in Fig 3 and 4 are incomplete.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 15 **No:** 0	• DEI is well described and accounted for in experimental plan for example by using iPSCs from diverse patient groups. • Applicants propose to develop the cellular product from different ethnic backgrounds, emphasize that HD impacts diverse populations, and note that the HTT gene was discovered from villagers in Venezuela. Milestone 5 proposes to develop robust methods to derive cellular product from different genders and ethnicities. • Yes, it upholds DEI principles if the cells can be developed from a range of donors. It is, however, likely to be an expensive therapy. • This therapy, like many others of the kind, will be expensive and likely delivered via specialized clinics. This is acknowledged, and strategies to mediated this are in the proposal. • That is a bit premature at this stage of development, but they are involved with CIRM alpha clinic efforts. • Extensive efforts to engage in DEI training and foster a DEI culture are reported by the applicants. • The patient perspective is included. • Team members participate in various institutional DEI education and awareness groups.

Application #	DISC2-16581
Title (as written by the applicant)	Targeting protein-RNA interaction in cancer stem cells
Research Objective (as written by the applicant)	Our goal is to develop and optimize novel drugs that can attack cancer stem cells. These drugs interfere with a target protein and will prevent relapse of disease.
Impact (as written by the applicant)	By targeting blood cancer stem cells, these compounds can be used to treat and prevent recurrence of cancer in patients. During this research, we will extend this use to other types of cancer.
Major Proposed Activities (as written by the applicant)	• Generate new chemical compounds that are similar to drugs, based on our prior discoveries and test whether they directly bind to the target protein. • Test whether the new compounds interfere with cancer cells' ability to survive and replicate. • Test the best of the compounds in mouse models and analyze what happens to cancer stem cells when we treat the cells. Test whether the compounds act by stopping the target protein from carrying out its function in cancer stem cells.
Statement of Benefit to California (as written by the applicant)	This project will move forward a single candidate drug that targets a unique pathway that is important for cancer stem cells. The completion of this project will lay the groundwork for future clinical development and testing of drugs, which will benefit several thousands of Californians, including many who are from historically underserved populations, who are diagnosed with cancer each year, and for whom current treatments do not work.
Funds Requested	$2,727,716
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	15
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 12 **No:** 2	• The proposed small molecule inhibitor targets the RNA-binding protein that is pathologically overexpressed in 15-20% of cancers, including leukemia and other aggressive cancer types. The inhibitor has shown significant potential in preclinical studies to decrease cancer stem cell populations and improve patient outcomes, addressing a critical unmet need in cancer therapy. • The project aims to develop a highly potent and specific inhibitor, which could transform the treatment landscape for cancers with high target protein expression. • The development of this drug could pave the way for new treatments targeting cancer stem cells, overcoming the limitations of current therapies that often fail to eliminate these resistant cells. The small molecule approach offers a scalable and cost-effective alternative to more complex therapies, potentially accelerating the availability of effective treatments. • The project addresses a bottleneck in targeting RNA-binding proteins, traditionally considered undruggable, which could open new avenues for therapeutic development. • The proposal includes a comprehensive plan for advancing from candidate discovery to translational studies, with clear milestones and timelines. Detailed strategies for optimizing the chemical structure and improving pharmacological properties of the inhibitor are presented, ensuring a smooth transition to clinical development. • The inclusion of safety and efficacy assessments in preclinical models and engagement with regulatory agencies demonstrate a thoughtful approach to translation. • The project seeks to identify a potent inhibitor of an RNA binding protein that is expressed in cancer cells, including cancer stem cells. A therapeutic agent that specifically targets cancer stem cells may make a major impact on cancer treatment. • Acute myeloid leukemia, pancreatic cancer, and glioblastoma are diseases in need of novel therapies. • The agent is designed to target cancer stem cells. • The proposal logically presents plans to identify a more specific inhibitor and carry out preclinical testing to demonstrate efficacy. • The project hold the necessary significance and potential for impact with a promising target. • Research is somewhat incremental. This is not really a stem cell or gene therapy product.
GWG Votes	**Is the rationale sound?**
Yes: 12 **No:** 2	• The scientific rationale is based on robust preliminary data showing the critical role of the target in cancer stem cell survival and its dispensability in normal adult tissues. The proposal leverages well-established techniques in RNA biology and cancer research to develop a novel therapeutic approach. The use of genetic and biochemical assays to validate the target and mechanism of action supports the scientific rationale. • Preliminary data indicate that target inhibitors can significantly reduce cancer stem cell populations and inhibit tumor growth in preclinical models. The identification of a promising pharmacological core and initial structure-activity relationship (SAR) studies

	provide a strong foundation for further development. The data from in vivo studies demonstrate the feasibility and potential efficacy of the proposed therapy. • The project leverages the unique properties of human cancer stem cells to develop a targeted therapy, addressing a key challenge in cancer treatment. The use of human-derived models and patient-derived xenografts ensures the relevance and applicability of the findings to human disease. The innovative approach to targeting RNA-binding proteins could significantly advance the field of stem cell-based and genetic therapies. • The investigators have identified the target as a therapeutic target within cancers, and lead compounds have been identified. • It is not clear why the currently available compounds are not suitable for further development. • There is some concern regarding the rationale. Why not move forward currently developed inhibitors? Development of new inhibitors seems superfluous. • The problem with existing drug is not stated. It is unclear why is a new product needed.
GWG Votes	**Is the project well planned and designed?**
Yes: 12 **No:** 2	• The applicant will test the efficacy and safety of the candidate product. The plan for iterative optimization of the small molecule based on SAR and pharmacokinetic data is well-conceived. • The inclusion of interdisciplinary expertise in chemistry, biology, and clinical research supports the overall quality of the project. • The project is well designed and planned with appropriate milestones. • This project from the team described alternative approaches, such as modifying chemical structures or using different assay systems. • The proposal identifies several potential risks, including off-target effects and issues with compound stability, and presents strategies to mitigate these risks. • The project is well planned with regard to the development and identification of compounds and in vitro and in vivo testing against leukemia cell lines and clinical samples. • More details are needed with the current compounds that demonstrate significant efficacy. • The deficiencies that the new compounds will abrogate compared to the one identified in the first screen need to be explicitly stated. It's unclear why the applicant wants to screen additional drugs.
GWG Votes	**Is the project feasible?**
Yes: 13 **No:** 1	• Each milestone is methodically planned to follow a logical sequence, making them attainable within the projected timeline. • The team has experts with the necessary qualifications and experience in cancer research, medicinal chemistry, and translational medicine. • Yes, this team has necessary resources to perform the experiments. • This team has necessary budget to perform the experiments. • The project is feasible based upon the preliminary data. • It's unclear if the inhibitor elicits any effect on efficacy. More data on lead compound development and activity in preclinical models would de-risk the proposal. Survival data using the inhibitor would be preferred.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14 **No:** 0	• The project is designed with a commitment to inclusivity, specifically targeting a wide demographic spectrum. • Special attention is given to addressing the needs of racial and ethnic minorities disproportionately affected by leukemia and other cancers. • Collaboration with community organizations and patient advocacy groups is planned.

	• Yes, the project upholds the principles of diversity, equity and inclusion (DEI).

Application #	DISC2-16706
Title (as written by the applicant)	Cell-based, personalized gene therapy to precisely treat pulmonary hypertension
Research Objective (as written by the applicant)	The project will deploy endothelial cells modified specifically to produce high quantities of nitric oxide precisely, and exclusively, to the pulmonary circulation.
Impact (as written by the applicant)	High blood pressure in the lung, pulmonary hypertension, will be directly addressed by the proposed studies.
Major Proposed Activities (as written by the applicant)	• Demonstrate that endothelial cells can be isolated from blood and expanded in vitro • Demonstrate that expression of a nitric oxide can be selectively and durably increased in endothelial cells in culture • Demonstrate that endothelial cells can be grown on implantable, intravascular stents • Provide proof of principle that this strategy can both prevent and treat pulmonary hypertension in large animal model.
Statement of Benefit to California (as written by the applicant)	This research will provide a novel strategy to deliver autologous cell-based therapies that will be rapidly tested in clinical trials. Contingent upon positive results, the overall strategy will benefit Californians with pulmonary hypertension as well as their families. To move the strategy into the clinical arena, systems will be created for endothelial cell isolation, expansion, gene modification, and delivery; thereby expanding the economy and increasing employment.
Funds Requested	$1,745,879
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	4
Highest	80
Lowest	70
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 14 **No:** 0	• The proposed technology is highly significant and if the project is successful the candidate will strongly impact an unmet medical need. The therapeutic target of the technology is pulmonary arterial hypertension (PAH) - a severe, life-threatening condition, where decreased nitric oxide (NO) production is one of the key factors contributing to pathophysiology of the disease. • Definitely an area of unmet need. Being able to increase NO locally in pulmonary vasculature would be a step change.
GWG Votes	**Is the rationale sound?**
Yes: 13 **No:** 1	• The proposal is based on a strong scientific rationale. It is well recognized that NO released from pulmonary endothelium is a major determinant of pulmonary vascular tone and that pulmonary hypertension (PH) leads to ventricular hypertrophy, low cardiac output and heart failure. • The ability of NO to increase vasodilation and reduce remodeling is known and an important approach to pulmonary hypertension. The concept of gene therapy via the proposed method for pulmonary hypertension is novel. • The preliminary data support the ability to use gene editing technology to achieve high expression of nitric oxide in endothelial cells. This will be translated to primary endothelial cells grown from circulating blood outgrowth endothelial cells. • Will there be down-regulation of receptors over time?
GWG Votes	**Is the project well planned and designed?**
Yes: 11 **No:** 3	• The project is generally well-designed and is likely to generate proof-of-concept data for translational studies, especially as it relates to Aims 1-3. • All this work is being done (Milestones 1-3) in human cells but then switches to swine cells for the large animal model. Does the validation work need to be repeated? How does the gene edited swine cell correspond to the human edited cell? There is minimal detail about this experiment and no info regarding the duration of model nor about how many time points the assessment will be at (other than mentioning the final 6 months in the healthy swine). • Unfortunately, for Aim 4, no discussion is presented if the team expects that the technical protocols optimized for human endothelial cells would be directly transferable to swine endothelial cells. Also, there is no discussion if engineered swine endothelial cells grown on stents will be functionally tested prior to placements of the stents in vivo. • Overall, Aim 4 is underdeveloped. For example, the applicant states that "there is a significant risk that the [endothelial cells] on the stent may produce either too much or too little nitric oxide. This stoichiometric challenge can be overcome but might require several experimental steps to ultimately solve". This limitation may result in a significant impediment to the success of this project; more discussion of potential options would be useful here. • What does "too much" NO mean? What is too little? What is target allele incorporation? The in vitro experiments have power calculations that were confusing - effect sizes were not justified, nor data presented for their standard deviations. • The rationale for the source for endothelial cells is unclear. No data about the donor are provided. Will this include smokers/will it reflect diversity?

	• There is no information about the stent material nor size. What are the targets for degree of coverage/rate of proliferation? Do you expect the cells to reach senescent phase? Will this affect gene expression? • Pitfalls are insufficient. There are some uncorrected informal notes e.g., "please provide reference" etc... in Aim 2.
GWG Votes	**Is the project feasible?**
Yes: 10 **No:** 4	• The milestones and proposed project outcomes are logical. However, the project is high risk and not all milestones might be achievable within the proposed timeline. • The feasibility of the timeline is difficult to evaluate as it is unclear how long the swine model will run. • Stent design is a concern. The stent itself could cause issues. The budget seems reasonable.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14 **No:** 0	• It is proposed to use autologous cells obtained from the peripheral blood to over-express NO. Therefore, if this project is successful, it will address the needs of the patients of all races, ethnicities and genders affected by PAH. • Plans for taking into account diversity have not been well addressed. • It is unclear whether the applicant has plans to incorporate perspectives and experience from the intended population during the course of the award.

Application #	DISC2-16674
Title (as written by the applicant)	Developing small molecule therapeutics for Parkinson's disease
Research Objective (as written by the applicant)	We will use iPSC-derived neurons and mice as preclinical models to validate small molecules to treat Parkinson's disease (PD)
Impact (as written by the applicant)	There are many challenges for finding a cure for PD, due to the lack of effective therapeutic targets. The success of proposal will help develop an effective therapy for PD
Major Proposed Activities (as written by the applicant)	• To characterize disease relevant phenotypes in human neurons. • To evaluate the disease-modifying activity of the compound series in human neurons. • To determine target engagement. • To reveal the mechanisms underlying the benefits. • To test the lead compound in vivo. • To determine the pharmaco-physiological profiles of Miro1 Reducer
Statement of Benefit to California (as written by the applicant)	PD is an age-dependent disorder and a leading cause of disability that afflicts about 1% of the population over 60 years of age, and 4% over 80. About 500,000 PD patients are currently living in the U.S, and approximate 1/10 of them live in California. An effective treatment for PD is desperately needed. We will develop small molecule therapeutics with the hope to treat PD. This study is closely relevant to public health of the state of California and will greatly benefit its citizens.
Funds Requested	$2,661,248
GWG Recommendation	***(1-84): Not recommended for funding***
Process Vote	*All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."* *Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."*

SCORING DATA

Final Score: 75

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

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

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 15 **No:** 0	• The proposal will identify small molecules that target a key player in mitophagy pathway in Parkinson's Disease (PD) that would deal with underlying pathogenesis rather than provide symptomatic relief. Preliminary findings indicate this is a key mechanism in sporadic and familial disease. • Despite drugs and deep brain stimulation, PD remains a relentless neurodegenerative disease. The proposed therapeutic is a small molecule mitochondrial rho GTPase 1 (Miro1) reducer that directly binds to and destabilizes a mitochondrial protein, Miro1. • Development of small molecules as disease modifying therapies in Parkinson's Disease would have a large impact. • Miro1 reducers accelerate Miro removal. This project will undertake lead optimization in human and patient induced pluripotent stem cell (iPSC) lines and a PD mouse model. • Applicant plans to determine the disease-modifying activity, target engagement, and pharmaco-physiological properties though the use of stem cell models. • The candidate being developed is planned to be delivered orally with a weekly dosing regimen. Non-invasive delivery is advantageous and the likely hood of resulting in available therapies once developed is increased. • An oral small molecule has advantages over other delivery methods. • The bioassay concept using PD patient fibroblasts will require validation across a larger range of patients. • Progression to clinical use is described but not in detail.
GWG Votes	**Is the rationale sound?**
Yes: 14 **No:** 1	• Small molecules are good candidates to pursue to target Miro1. They bind directly to the target and destabilize Miro1 protein - with the idea to remove Miro1 from damaged mitochondria, promote mitophagy, and by doing so protect the neurons from the disease process. • In prior work using several PD patient cells, applicants removed Miro1 from damaged mitochondria and validated a lead compound in an in-silico screen to reduce Miro1. They discovered a Miro1 Reducer compound series and provided initial validation selective human neuron and fly PD models. • Depolarized mitochondria can be toxic within cells. Defective mitophagy is a significant source of cellular dysfunction in PD. Applicants published that PD is associated with defective clearance of damaged mitochondria. In mutant cells, Miro1 remains longer than normal. • Applicants provide a well-structured nomination plan for a lead compound to proceed to further testing. • Applicants use iPSC models in screening. • The applicant has extensive data from basic research studies conducted over many years to support the overall concept. • The project seeks to develop a small molecule that destabilize Miro1 protein which may lead to better survival of the dopamine neurons. This part is well established and supported by previous publications and preliminary data. However, that this will have an effect on PD symptoms is not yet established. Proof of concept studies to address this is planned in the proposal but conducted only in last year. Before this is established, large efforts on drug development seems premature. • The preliminary data, as well as previous publications from the team, supports the use of Miro1 as a target to increase cell survival. But it has not yet been established that this

	will have a therapeutic effect. Not sufficient preliminary data that screening method based on iPSCs will be successful or in vivo efficacy. • Some concerns about insufficient preliminary data.
GWG Votes	**Is the project well planned and designed?**
Yes: 7 **No:** 8	• Applicant has addressed most issues raised in prior reviews. Plans for further screening in human cell models are good. • Well planned and designed, however, there are several unknowns that may affect the proposal, such as the successful identification of an effective candidate. • The project is well planned but premature. The first task is to establish phenotypic readouts in human neurons and there is a plausible plan for this but not data yet. Also, there is no actual data that supports that their screening method works and if it fails, the whole project fails. • Not clear whether relevant pathology can be analyzed in the model and will appear in a time frame that is manageable. • Yes, if all assessed compounds have unfavorable half-maximal inhibitory concentration (IC50), the applicant proposes to synthesize analogs, but this could significantly delay the project. • The responsiveness of patient-generated cell lines may vary. • No verified animal's model. • It is not clear if a product will be ready at the end of the proposal to move to the next steps.
GWG Votes	**Is the project feasible?**
Yes: 11 **No:** 4	• The PI (20%) and two other postdocs (100%) will conduct the research. The PI is an established, well-funded researcher who has done other NIH-funded work that appears to be related to this project. • The project has a logic design but contain two major risks. The first related to the screening system that will be established at start of project. If they fail to establish robust assays to detect relevant phenotypes in a reasonable time frame the whole project will fall short. The second relates to lack of efficacy data. • Milestone 1 seems feasible. Other milestones are for in vivo studies for which no data is presented, adding risk. • Significant details for the screening studies are lacking. • What if compounds show unexpected toxicity or if animal model does not replicate mitochondrial pathology? • Use of Contract Research Organization (CRO) for safety and efficacy is reasonable but compound failure in safety or efficacy testing will arrest the program. Applicants would benefit from collaboration
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13 **No:** 2	• Yes, a small molecule may have a lower cost and be available more broadly to PD patients. Applicant will study several cell lines including males and females. • Applicants refer to foundation literature regarding PD demographics and unequal distribution of current therapies such as deep brain stimulation (DBS). • With only 5 cell lines this is hard to assess. But both male and female lines are planned to be used. • DEI statement is weak.

Application #	DISC2-16660
Title (as written by the applicant)	HSC-inspired Metabolic Optical Biomarkers for Leukemia Stem Cells and Therapeutic Prediction
Research Objective (as written by the applicant)	The proposed biomarker is a set of metabolic optical biomarkers (MOBs) and their subcellular features in acute myeloid leukemia (AML) and leukemic stem cells (LSCs).
Impact (as written by the applicant)	If successful, the biomarkers will significantly impact the treatment of AML in older or vulnerable patients by guiding low intensity chemo, targeted, and combination therapy options.
Major Proposed Activities (as written by the applicant)	• Establish a comprehensive MOB feature library for resolving AML metabolic heterogeneity. • Correlate MOB features with underlying molecular features in AML. • Identify key MOB features resolving metabolic heterogeneity in primary AML samples. • Identify MOB features that distinguish normal HSPCs, LSCs, and progenitors and validate LSC function of MOB feature enriched LSCs in vivo • Establish a MOB-based metabolic response assay for AML drug responses. • Establish a MOB-based single cell division assay for assessing LSC-targeted therapies.
Statement of Benefit to California (as written by the applicant)	AML disproportionately affects specific demographic groups in the context of California's diverse and multiracial population. Increasing accessibility and accuracy of biomarkers for prognostic evaluation and therapeutic prediction are crucial for the disadvantaged demographic groups. Our developed biomarkers and associated assays will be rapid, accurate, and low-cost. It will benefit a diverse group of AML patients and those with AML and other leukemic diseases in the State of California.
Funds Requested	$2,438,261
GWG Recommendation	***(1-84): Not recommended for funding***
Process Vote	*All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."* *Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."*

SCORING DATA

Final Score: 75

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

Mean	74
Median	75
Standard Deviation	2
Highest	75
Lowest	70
Count	15

(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 13 **No:** 1	• The proposed product is a set of metabolic optical biomarkers (MOBs) and their subcellular features to identify leukemic stem cells (LSCs). These MOB may be able to inform the metabolic and biological states of AML, identify the disease-driving LSCs, and predict their responses to different drug types and doses. If this is true, it would be a clear benefit to AML patients. • The proposed technology is a metabolic optical biomarker (MOB) useful to identify heterogeneity in samples of AML, predictive of resistance to treatment response, and useful as response biomarkers for effective treatments. There is high significance recognized for the differential metabolic features of stem cells compared to differentiated cells, and a biomarker that can distinguish leukemic stem cells would have a high impact. • The studies will be carried out in AML, in which high relapse rates are driven by drug-resistant LSCs. Therapies based on the biology of LSCs are limited, and the studies may open up new methods to identify these rare cells. • The proposed studies will develop novel assays based on imaging of metabolic changes as a means of predicting outcomes, including responses to therapies, and better identifying and studying LSCs. • This technology and methods developed here could also be useful for other indications in the stem cell field. • The technology is primarily designed to discriminate different cell types based on their metabolic profile, size, etc. This may provide prognostic information but is unlikely to be therapeutic. • It is not exactly clear how this technology would progress into the clinical arena.
GWG Votes	**Is the rationale sound?**
Yes: 12 **No:** 2	• It is thought that LSCs differ from bulk AML blasts, and potentially normal HSCs, by their metabolic features. Therefore, it is possible that this technology provides a novel method by which to identify and study various cell types. • It is likely that metabolic differences are based on a variety of genetic, epigenetic, transcriptional, etc. changes, and it is possible that the studies proposed will better link these known changes with metabolic features. • Data are presented that the MOBs appear to be distinguish normal HSCs from progenitors, different leukemia types, etc. • The techniques will distinguish normal from leukemic stem cells. • The rationale that metabolic features of cells can distinguish states of differentiation is well accepted. The preliminary data demonstrates that MOB can distinguish stem cells from differentiated cells. The data suggests that MOB may relate to molecular features of

	the cell, such as gene expression. The preliminary data is suggestive but not convincing. Figure 5, for instance, is not convincing to the naked eye. • LSCs are rare and difficult to distinguish from normal hematopoietic stem cells (HSCs) by conventional surface markers, but they are thought to have a distinct metabolism from HSCs. However, metabolic states can vary over time and disease state, so the heterogeneity, even in the same patient, may prove to cause too much variation to make a useful biomarker. • There is some concern that metabolism is too noisy to serve as a useful biomarker. • It is not clear whether the isolation of cells is feasible, especially in the numbers for the proposed in vivo studies.
GWG Votes	**Is the project well planned and designed?**
Yes: 8 **No:** 6	• Many of the methodological steps are feasible based on the data provided. • Yes, the proposed studies are appropriate for each use of MOBx proposed in three distinct aims. In Objective 3, there is no proof-of-concept with homogeneous, genetically defined cell lines that MOB features can be predictive of drug sensitivity or resistance. • The proposed studies are extensive, and it is possible that at any step there may be technical issues. • It is not clear whether the studies will lead to a viable clinical strategy based on the findings. • It is not clear whether the relatively small numbers of cases to be studied will provide robust and reliable results. • This application is using cutting edge tools and methods. It is not clear if all the methods needed to phenotype patient samples are in common use in the lab. • The discussion on pitfalls and alternatives is modest. • The timeline is aggressive. • It is not clear how this would work as a product to move to the next phase. A service to process samples? A tool that is sold to institutions? • The future outlook of the project is not clear.
GWG Votes	**Is the project feasible?**
Yes: 8 **No:** 6	• The project milestones are logical and linear. This a very involved project, and early setbacks could limit later milestones. • The project is overambitious. • The team is adequate based on the data provided and previous publications. • It's not clear where all the work will be done. Letters of support from core labs or other facilities would add strength to the proposal. No letters of support are provided.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13 **No:** 1	• Yes, the applicant addresses principles of DEI. • While they do not state they will try to test from a wide variety of AML samples, they will try to pull patient demographic when possible. It would strengthen the application if they tried to pull a diverse sample set at the start.

Application #	DISC2-16557
Title (as written by the applicant)	Providing a cure for pulmonary fibrosis using adeno-associated viral mediated SGPL1 gene therapy
Research Objective (as written by the applicant)	AAV-SPL is a viral gene therapy that delivers the SGPL1 gene to lung cells. By reducing the levels of a pro-fibrotic molecule, AAV-SPL prevents or reverses lung fibrosis and its lethal consequences.
Impact (as written by the applicant)	Our treatment will be used to prevent disease progression in idiopathic pulmonary fibrosis (IPF), a debilitating lung disease associated with a 2–5-year survival.
Major Proposed Activities (as written by the applicant)	• Test the ability of AAV-SPL to prevent or even reverse disease progression in a genetic mouse model of IPF • Test the effectiveness of AAV-SPL if given late in the disease course • Test whether AAV-SPL is able to prevent fibrosis in cultures of human lung tissues • Test whether circulating blood levels of the pro-fibrotic molecule sphingosine-1-phosphate can predict responsiveness to AAV-SPL • Test the ability of delivering AAV-SPL by aerosol spray to the lungs is more effective than delivering it intravenously • Test the safety and body distribution of AAV-SPL
Statement of Benefit to California (as written by the applicant)	Pulmonary fibrosis (PF) affects over 5,000 Californians. Associated morbidity, mortality and reduced quality of life are incalculable. In addition, PF impacts work productivity due to absenteeism and work limitations. Notably, the burdens may have greatest impact on patients of low socioeconomic status and in rural locations. PF also levies a heavy economic burden, translating to millions of dollars per year that California and its citizens pay in health care, loss of productivity, and benefits.
Funds Requested	$2,806,731
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	4
Highest	75
Lowest	65
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	0

(1-84): Not recommended for funding	15

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 12 **No:** 2	• This is a gene therapy grant proposal designed to treat pulmonary fibrosis (PF). If successful, this would address an unmet need. PF is idiosyncratic and usually fatal within 5 years or so. • In addition to development of a gene therapy for PF, this work might help to establish serum sphingosine-1-phosphate (S1P) as a biomarker for PF, which would be useful in the clinic. • The proposal contains a detailed and thoughtful progression plan for translation to the clinic. • The use of the adeno-associated virus (AAV) vector is not novel for isolated cells and has been demonstrated to be effective in infecting target cells. There is a concern that there are no data on how much increase is necessary to prevent fibrosis. Also, there are no data on the efficacy of this vector on fibrotic tissue. If the proposal is successful, because there are no approved effective anti-fibrotic therapies that can reverse or control the progression of IPF, the potential is high. • There is a significant unmet need for idiopathic pulmonary fibrosis (IPF). The data showing S1P drives IPF in humans are not compelling. If it does, it is likely to be in a small subset as raised S1P levels are not seen in all IPF patients • AAV based gene therapy has not been successful in any lung disease to date in patients. There is very little in the application that addresses this prior bottleneck.
GWG Votes	**Is the rationale sound?**
Yes: 11 **No:** 3	• Sphingosine phosphate lyase (SPL) enzyme degrades sphingosine-1-phosphate, which can reduce lung inflammation and scarring. Delivering SPL enzyme to the lung by AAV transgenesis seems like a sound approach. • In the mouse model, SPL is increased, but this is insufficient to reduce S1P levels (these also increase). The central research hypothesis is that the body lacks sufficient ability to overproduce protective levels of SPL in response to fibrosis, but this might be overcome by overexpression of SPL to high levels by AAV delivery. A risk is that even with very high SPL (by AAV) this still might not suffice. However, the project shows promise on this point. • The data that aberrant sphingosine signaling occurs in mouse model and modifiable by SPL overexpression in mice are generally supportive. However, in the mouse model in the proposal the S1P level is not actually lower in the gene treated group than the empty vector. There is a reduction in hydroxyproline and sirius red staining, but the latter is open to bias and there are inconsistencies in the numbers in the hydroxyproline (n=8) and sirius red (n=4) groups. • It is not entirely clear that S1P excess is a major cause of pulmonary fibrosis. • A major concern is that raised S1P is found in almost all lung diseases and are unlikely to be specific to fibrosis. Persons with SPLIS do not develop pulmonary fibrosis despite higher S1P – there is confusion regarding this. • There are minimal data relating to the actual proposed pathophysiological mechanism in human cells and tissues driving fibrosis and a lack of evidence about S1P in lung tissue

	from patients with IPF and how S1P and its signaling mediates fibrosis in primary human cells. • Part of the preliminary data and proposed experiments involve cell lines that are not adequate for evaluating the interventions. IPF primary cell lines are more appropriate. It has been demonstrated smooth muscle actin (SMA) and fibroblasts express lower collagen levels than adventitial, inflammatory, and collagen triple helix repeat containing 1 (CTHCR1)+. In this application, all the rationale is based on SMA+ cells. There is not a single mention of the subtypes and the effect of the therapy on those.
GWG Votes	**Is the project well planned and designed?**
Yes: 9 **No:** 5	• In the mouse model, SPL increases, but this is not enough to prevent increases in S1P. The hypothesis from this preliminary data is to see if increasing SPL levels even more (by AAV) will cause S1P to decrease. • There is some concern that delivering virus to the lung might exacerbate already compromised lung function. • The murine studies seem fair and use an engineered mouse that develops short telomeres in the alveolar epithelial type II (ATII) cells. This has some merits but still limitations. Only 25% patients with non-familial IPF have short telomeres and the diseased cells in this model are the ATII cells, not addressing the role of fibroblasts or endothelial abnormalities. A power calculation based on the mouse model is given and justified. Some risks and mitigation steps are discussed in the proposal. • The human cellular studies are inadequate. This center has access to and experience in isolating primary ATII cells and fibroblasts from both healthy and diseased lungs. Not using primary adult cells is unlikely to be helpful (A549s really undergo EMT changes) and fatal lung cells resist senescence (a feature of this disease) but also take up virus more readily. Primary cells here could be used to test path-mechanisms much more extensively. • The applicant refers to precision-cut lung slices (PCLS) from biobank – it is unclear if this means using frozen tissue and resuscitating for PCLS culture. There are no data presented to support this and the referenced paper (not the applicant's) is on fresh tissue. There is no data supporting the statement that biobanked tissues can be kept in culture for 5 days. • Cells used seem not appropriate, details regarding quantification of human tissue are limited. • Arguably the human cell and PCLS experiments are the most important in this proposal since they should define whether S1P really is profibrotic in human tissue (and therefore whether developing lyase therapy for IPF is likely to be helpful) but the experiments are not well designed with no preliminary data showing they can do PCLS or drive fibrosis in PCLS. • Use of the biobank to test plasma S1P in IPF is helpful and to test whether the S1P correlates with the degree of murine studies are generally well designed, human tissue ones not. • Yes, generally for the mice, but there's no demonstration that the applicant understands the problems with PCLS (which will be even greater in those with established fibrosis). • Use of frozen tissue has not been demonstrated. • Mouse model is not appropriate.
GWG Votes	**Is the project feasible?**
Yes: 12 **No:** 2	• Adenoviral delivery of transgenes (in this case SGPL1 encoding sphingosine phosphate lyase) is feasible. Delivery to the lung should be possible in high efficiency by inhalation, but there are concerns about making the condition worse with a viral infection. • Murine studies - yes. PCLS unlikely to be deliverable. • Yes, but need more consideration of human studies Reviewer suggests that PCLS has to be used first. This is the best human pre-clinical model available. Efficacy must first be demonstrated in PCLS before moving to animal models for pharmacological studies. The team is outstanding. The concern is the genuine involvement of key personnel related to pulmonary, critical care in the project.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**

Yes: 13 **No:** 1	• Yes, even though this is mostly at the stage of cell and mouse research. However, lung samples from a diverse and appropriate racial spectrum will be utilized in the proposed work which adds to the quality of informative studies for the race variable. • Yes, this treatment would be especially useful for the Hispanic population, which is stricken with high levels of pulmonary fibrosis. Black individuals are diagnosed at unusually young age with PF and would also benefit. • According to the applicants, mortality by IPF is higher for Hispanics. and because 40% of the population in the state of California is of Hispanic heritage, the results can have a significant impact on underrepresented populations. This could be addressed better in the biobank section where the investigators could ensure the samples being tested reflect diversity of population. • DEI plan not clear.

Application #	DISC2-16664
Title (as written by the applicant)	Pluripotent stem cell-derived liver organoids for treatment of liver disease
Research Objective (as written by the applicant)	The proposed technology is the development of allogeneic pluripotent stem cell (PSC)-derived liver organoids for implantation into patients with liver disease as functional tissue replacement therapy.
Impact (as written by the applicant)	PSC-derived liver organoids will provide a stable and scalable source of hepatocytes that can be used as adjuncts or alternatives to transplantation in the treatment of severe liver dysfunction.
Major Proposed Activities (as written by the applicant)	• Aim I: Identify clinically compatible pluripotent stem cell (PSC) lines that are optimal for development of therapeutic liver organoids to treat a diverse population. • Aim II: Determine the functional robustness and tumorigenic risk of liver organoids developed from the most promising clinically compatible PSC lines. • Aim III: Determine the disease modifying activity of the leading PSC-derived liver organoid therapeutic candidate in mouse models of non-fibrotic and fibrotic liver disease.
Statement of Benefit to California (as written by the applicant)	Pluripotent cell stem-derived liver organoids will provide a stable and scalable source of functional hepatocytes that can be used as adjuncts or alternatives to liver transplantation in the treatment of severe liver dysfunction. This novel therapeutic will alleviate the suffering of those with severe liver disease and promote equitable access to life-saving functional tissue replacement therapy.
Funds Requested	$2,845,500
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	3
Highest	75
Lowest	70
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 13 **No:** 1	• Liver diseases represent a major health care challenge worldwide and the only treatment for end stage disease is organ transplantation. Thus, developing alternative therapies will have a major impact and help a large number of patients. Livers diseases can be divided into four categories: Chronic, acute, inherited, and cancer. The proposal aims to develop cell-based therapy against chronic and inherited diseases, which affect by far the higher number of patients. • Liver injury and failure is a medical problem because the need for liver transplants exceeds the availability of donor liver lobes. The use of stem cell derived organoids, if they can be effectively engrafted, could alleviate this need. • The proposal focuses on cell-based therapy against liver diseases and has a clear translation strategy including the utilization of GMP grade hiPSC, defined culture conditions transferrable to GMP, and QC including tumor formation • There is a significant need for alternative treatments for individuals who require liver transplantation because of liver failure or genetic disease, as the demand for cadaveric livers greatly exceeds the supply, and living donors are rarely available. In principle, large amounts of functioning liver tissue generated from pluripotent stem cells might help to address that need. However, growing liver organoids from iPSC does not seem likely to offer a practical solution to the unmet need. • Cell based therapy against liver disease is a promising approach. However, primary hepatocytes are difficult to obtain or to grow in vitro. Thus, using hiPSC derived cells could provide an advantageous supply. However, several challenges need to be addressed including large scale production. The liver is a very big organ which contains around 300 billion hepatocytes. • There are still major gaps to progress to translation that are inadequately addressed by the applicant. These include: 1) achieving allogeneic transplantation with an "off-the-shelf" product; 2) demonstration that the organoids indeed generate a product consisting mainly of highly functional, mature adult (not fetal) hepatocytes; 3) producing the material to the required scale; 4) safely transplanting the organoids into recipients. • There is some concern about novelty. Many research efforts are underway (some funded by CIRM) with similar aims and strong approaches.
GWG Votes	**Is the rationale sound?**
Yes: 10 **No:** 4	• Cell based therapy against liver disease has already been achieved using primary hepatocytes. However, good quality primary hepatocytes are extremely rare since most available organs are used for transplantation. Thus, the development of cell-based therapy for liver disease has not progressed for almost two decades. hiPSC derived cells provide an advantageous alternative to primary cells. • It is clear that it is possible to generate iPSC-derived hepatic lineage-specific cells expressing liver-specific genes (at the mRNA level), and that these generally function better in organoids than two-dimensional cultures. It is also clear that matrigel is a poor material to use for clinical translation so that an alternate approach to organoid formation is desirable. • The choice to work on inherited diseases is a very good new approach in this revision, as their treatment would meet an unmet medical need. Further, compete engraftment efficiency is not required for effective treatment of these disorders, and what is learned

	from such efforts might lead to eventual treatment of patients with non-genetic generalized liver failure. • The use of bioreactor in suspension is probably the only approach available to produce the quantity of cells necessary for therapeutic dose (around 2-3 billion). However, there is no information about the scale-up capacity regarding the method proposed by the applicant. • Advancing from seeing activity in a murine model of a genetic disease to initiating a serious translational effort to yield a safe, effective product requires a much larger jump than the applicants indicate. • Cell biological and functional characterization is minimal. It is unclear what is the distribution of cell types in the organoids. No preliminary data are presented on functionality after transplantation in vivo. Generating the first data of that type is not sufficient to reach "translation ready" status by the end of the project. • There is some concern about the use of allogenic organoids. Though existing liver transplants are allogenic, the use of PSCs (especially iPSCs) could provide an autologous source of organoids. It is not clear why this route was not chosen, at least in part. However, this effort will identify some relatively useful allogenic PSC line(s) that bear HLA genetics suitable for engraftment into matched patient groups. • There are also some data that show that electroporation of the host (patient) liver creates beneficial damage (probably creating niches) for improved engraftment efficiencies. These seems to work in fibrotic liver as well. However, there is no quantitation of these approaches, only histology is presented. • Overall, the preliminary characterization of the PSC seems only adequate, not extensive. It is unclear why more CYPs not assessed, why were more secreted factors not studied. Also, the cellular architecture and composition of liver organoids are not shown. • Better evidence of a mature organoid or hepatocytes is needed.
GWG Votes	**Is the project well planned and designed?**
Yes: 10 **No:** 4	• This is a resubmission of a grant that received a strong score previously. The revised application is responsive to concerns about the mouse model, which has now been replaced to avoid a selective advantage for engrafted cells. In addition, the new models and possible human patients to be treated allay concerns about portal vein degradation. • In this revision, the mouse models to be used have been changed in response to reviewer feedback. This is a good improvement, and this group now proposes to use two mutant mouse lines that model PKU and CN1 disease. These result in non-fibrotic liver dysfunction, thus portal vein injection in patients would not be an issue upon translation. • The aims are logical. • The project is divided in 3 aims. The first aim will aim to identify GMP grade hiPSC lines which can generate hepatocytes like cells using the protocols developed by the applicants. The use of primary hepatocytes as control is essential. • Proof-of-concept for correction of a genetic deficiency in liver function in mice by transplanted human iPSC derived liver lineage organoids would be a useful step. However, this would not suffice to advance to translational studies. • The project is designed reasonably to show biological activity expected of liver cells matured from iPSC. However, this is not particularly novel. As a step leading directly to translation, this is not a well-constructed project. • Aim 3 will test the hepatocyte like cells in different animal model for inherited disorders and chronic diseases. This part is extremely important and interesting. The use of immune suppression is a major drawback which will be covered by the back crossing planned in the grant. This is time consuming and unlikely to be achieved. • The applicants could explain in more detail the rationale behind the dose of cells they plan to inject. It would also be useful to understand how many cells can be produced

	with their bioreactor. Their system might not be compatible with production capacity necessary to reach the 2-3 billion cells necessary for therapeutic dose. • Aim 2 will control the cell preparation for contamination by undifferentiated hiPSC. This is a necessary step since their protocol generate an heterogeneous population of cells. The rationale to use LIN28 to detect hiPSC could be better explained. Oct-4 seems more appropriate. The soft agar assay is unlikely to work since these conditions are extremely selective. Teratoma formation (proposed in Aim 3) will be more informative if performed in the right conditions. • The iPSC model is not well developed; there are no images of organoids.
GWG Votes	**Is the project feasible?**
Yes: 10 **No:** 4	• The timeline seems to be achievable. This group is talented and appropriate for the proposed work. • The plan is rational and well organized. This is a well-constructed project which will achieve essential milestone, but the clinical relevance is limited. • Demonstration of some functional correction of the genetic deficiencies, at least, seems feasible. Generating a strong product candidate seems unlikely. • The Principal Investigator (PI) has published several papers on the electroporation method proposed to prepare the liver to receive transplanted cells by creating a cellular vacuum. No evidence is presented for experience of team members in the development of a cellular product for medical use. • The only team member discussed in the application is the PI. The team's qualifications are summarized in a single general sentence. The PI's publication record includes one paper on the liver organoid method on which the proposal is based (Stem Cells, 2023). • The preliminary data show that the applicant can produce hepatic-like cells in vitro which express specific markers and display important function. However, these cells still express AFP and represent an heterogeneous population. Furthermore, the absence of direct comparison with primary hepatocytes renders difficult the functional evaluation of these cells. These cells are clearly not fully functional. • Scaling up does not seem feasible.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14 **No:** 0	• The DEI component of this grant is quite strong (e.g., the table on page 10). Using such data, this grant aims to identify one or more PSC lines that would potentially be HLA matched for individuals of non-European origin. • The application includes a theoretical discussion of frequency of different HLA haplotypes across racial and ethnic groups. This seems irrelevant, as the basic proposal is developing an off-the-shelf product for transplantation from a single donor line chosen primarily on the basis of efficient differentiation to hepatocytes, utilizing standard immunosuppression. • The proposal plans to use female and male hiPSC line. It could have been interesting to also include cell lines from different ethnicities.

Application #	DISC2-16680
Title (as written by the applicant)	Dual plasma protein and imaging biomarker of discogenic low back pain for stem cell therapy application
Research Objective (as written by the applicant)	We aim to discover and develop dual imaging and protein biomarker that in combination will be a specific surrogate to disc health and will help diagnosis of disc disease and monitor stem cell therapy.
Impact (as written by the applicant)	The developed biomarker will enable minimally invasive stem cell therapy for discogenic low back pain.
Major Proposed Activities (as written by the applicant)	• Optimize the imaging biomarker using integration of multiple parameters using Multitasking MRI for better prediction of discogenic pain. • Identify blood borne biomarker (or biomarkers) in a large animal model. • Test the sensitivity of the blood borne biomarkers. • Proof-of-concept pilot study in human patients.
Statement of Benefit to California (as written by the applicant)	Intervertebral disc (IVD) degeneration associated low back pain is a leading cause of disability. While it affects all adults, many people belong to underserved communities that more often carry government-sponsored health insurances. Despite decades of research, there are no robust therapies targeting the underlying causes of IVD degeneration. Spinal disc injections with the proposed treatment candidate may provide an IVD rejuvenating, inexhaustible off-the-shelf treatment accessible to all.
Funds Requested	$2,292,638
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	4
Highest	80
Lowest	60
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 12 **No:** 3	• The development for a biomarker and imaging technology would have relevance for understanding and possibly treating degenerative low back pain. • Low back pain is a prevalent problem for which treatments are of variable efficacy, leaving many individuals with chronic pain. • If the biomarker had high sensitivity and specificity, it might guide earlier intervention. • Degenerative low back pain is a major orthopedic issue. Treatment strategies are extremely limited. So, while a diagnosis would be impactful, the next step would be "what to do with that information". It is unclear whether there is a "gold-standard" for painful discs. This is a major concern. • Currently, diagnosis is established clinically together with MRI and x-rays to assess segmental motion. Applicants propose to combine MRI and biomarkers to create a per-level isogenic pain score. A problem with the idea is that many abnormal discs on MRI are not reported as painful. Also, clinical exams can be quite accurate for discogenic pain. • Finding a biomarker from disc damage does not predict or mean it would be a relevant marker to monitor and evaluate the outcome of a possible cell-based solution. • There have been good suggestions of how this project would contribute; however, there is still much missing for us to be certain this will be a useful investment of time and effort from a stem cell programs-based perspective. • It seems that the biomarker study is actually to support the applicant's research in stem cell treatments of intervertebral disc degeneration. • The prospect for successful stem cell therapy for intervertebral disc (IVD) degeneration remains uncertain and likely linked to the timing of the intervention. • Stem cell therapies have been studied because there is strong evidence that the nucleus pulposus is progressively damaged at affected levels, typically those with the greatest biomechanical stress. However, despite several anecdotal reports, definitive evidence of the efficacy of such therapies has not been established. • Applicants propose to attempt a correlation between the relevant preclinical model and human datasets. • It seems odd that applicants would favor developing a biomarker assay in a model when they already have patient samples, and the actual study of humans is likely to be of greater relevance. • There has been a move away from invasive discography, and the proposed disc imaging biomarker may be valuable when more extensively validated.
GWG Votes	**Is the rationale sound?**
Yes: 12 **No:** 3	• This is a diagnostic biomarker and imaging focused solution that might eventually support the development of cell-based solutions. • The background and idea are solid and highly relevant, if solved. However, it remains unclear if and how this project, even if fully successful, would allow the next step towards successful clinical application.

	• The preliminary data are solid and provide the necessary information. There is confidence in the group and its proposal. • Prior correlations to MRI imaging and molecular markers were with extracted disc material; some of the markers peaked at one time point and went down at a later time point, indicating that the temporal window of disc injury may be important to detection. • It is difficult to understand the correlation of human and price variables presented in Figure 2. It seems to indicate a correlation between MRI and pain-positive discograms. • There is no established, published, validated spinal pain model in the proposed species. This is a major problem with the study. • Milestone 4 is the most clinically relevant, but the concordance of model and human biomarkers may be difficult to study. • Figure 6 seems to be the most important and ideal for further validation between people's disc samples and serum from cases and controls. • In other studies, the applicants are testing intradiscal stem cell implants. The source of stressed progenitor cells for these experiments is not clear. • It would be better to focus this line of research on people, as the ethics of a pain model in the proposed animal species is questionable. • While the lumbar disc is a contributor to lumbar back pain, focusing on the disc exclusively fails to account for the other pain generating structures in the spine. • The injection of stressed IVD cells, as well as creating an annular injury, may bias the findings. • Not clear why CSF samples will not be obtained and studied. • There's no gold standard for "back pain." The proposal is not using a validated model, and there's no ability to "quantify" back pain.
GWG Votes	**Is the project well planned and designed?**
Yes: 9 **No:** 6	• The proposal is clear and well-structured with an efficient and original and comprehensive approach to the work that needs to be done. • The work makes sense, is well organized and focuses upon progress and comprehensive improvement. • The project is timely and realistic, if milestones and goals are met. • Aim 1 is to validate MRI and protein biomarkers in a large animal stress-induced discogenic pain model. One concern is that imaging biomarker does not seem to be associated with pain in the model. There are 4 groups proposed. The role of the stressed cell group is not clear. That the human cells will be rejected seems to be anticipated. • In Aim 1a the MRI biomarker is to be optimized. It is proposed to compare human lower back pain outcomes, but it's not clear that these parameters have been tested in people. • Aim 2 assesses whether the number of injured discs correlates to protein biomarkers. The source of stressed human cells is not clear, and the relevant preclinical model's rejection of human cells may confound the biomarker and pain outcome findings. • The proposal does not discuss covariates such as diabetes and cardiovascular disease, which may confound biomarker profiling and cannot be meaningfully tested in the proposed model. • Aim 3 is a human study in discogenic lower back pain subjects from whom disc material will be obtained as well as serum with correlation to two different pain assessment outcomes. • For Aim 3, applicants propose to combine two biomarkers, apparently protein and imaging, to increase sensitivity and specificity. However, how this biomarker model is to be derived is unclear. • There is some mention of pitfalls and possible challenges, and the approach is comprehensive and flexible enough to adapt. It is unclear how the imaging information and blood biomarker studies will be combined to provide a relevant human biomarker. That is the main weakness and challenge remaining.

	• The biphasic design is unique. There are concerns about the animal model being consistent with humans. • It is a very ambitious project for which attempts at animal to human comparison as detailed is not convincing. • It is unclear whether disc degeneration should be a priority for CIRM. Changes in discs are a manifestation of aging and no more a disease than other aging senescent processes in other tissues.
GWG Votes	**Is the project feasible?**
Yes: 11 **No:** 4	• Yes. The team is qualified, and the study is budgeted appropriately. • There do not seem to be many feasibility concerns if the models work, and the patient info can be acquired. • Yes, it's very feasible, although video analysis numbers and minutes do not add up, and the PhD students' hours seem undervalued. • The level of support for the bioinformatician is low. There is no clinical pain expert on the project. This is important, as you cannot actually image pain at the spacial level. • The budget seems considerable and should cover the needs of the project. • An enormous amount of work is proposed which will generate extensive data for analysis. The inclusion of the human pilot proof of concept study seems excessive. • Budget is acceptable.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14 **No:** 1	• That aspect is well described and seems appropriate if the found markers are applicable in wider population • No concerns. Spine degenerative joint disease is universal. • Yes, the MRI biomarkers may be useful. • Lower back pain may be more prevalent in Black and Latino men. The argument to use only female animals is not compelling, and there may be pain differences between males and females. Acute disc injury is much more prevalent in men.

Application #	DISC2-16624
Title (as written by the applicant)	Engineering tunable biomimetic adhesive hydrogel to deliver and enhance MSCs function for corneal regeneration
Research Objective (as written by the applicant)	We propose a minimally invasive adhesive MSC-laden hydrogel with tunable properties that can sustainably release MSCs in the cornea while mimicking its biomechanics to repair ocular injuries.
Impact (as written by the applicant)	Engineered MSC-laden adhesive hydrogels can enhance MSC survival and retention, control release and dosage, support cell ingrowth, facilitate tissue regeneration, and seal and repair stromal injuries.
Major Proposed Activities (as written by the applicant)	• Aim 1: Engineer MSC-laden adhesive hydrogels based on modified gelatin and hyaluronic acid with tunable physical properties • 1.1. Synthesis of a soft adhesive hydrogel for promoting epithelial growth, 1.2. Synthesis of a strong adhesive hydrogel for the repair of stromal injuries • Aim 2: In vitro characterization of the engineered MSC-laden adhesive hydrogels • 2.1. Assess MSCs survival and secretion of factors within the soft hydrogel and its effects on epithelial growth under the hydrogel in vitro • 2.2. Study in vitro migration of MSCs from the strong hydrogel as well as epithelial growth on top of the hydrogel • Aim 3. In vivo characterization of the engineered MSC-laden adhesive hydrogels in a corneal epithelial wound model and stromal injury model
Statement of Benefit to California (as written by the applicant)	The proposed research addresses the high rate of ocular injuries in California (~52,000 emergency department visits) by developing an alternative treatment using MSC therapy. By addressing issues of tissue donor shortages and invasive surgical methods, this new therapy will broaden treatment options for patients and promote health equity in California. The incorporation of DEI concepts further ensures that the therapy benefits all patients, regardless of their background or socioeconomic status.
Funds Requested	$2,393,522
GWG Recommendation	***(1-84): Not recommended for funding***
Process Vote	*All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."* *Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."*

SCORING DATA

Final Score: 70

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

Mean	69
Median	70
Standard Deviation	4
Highest	79

Lowest	60
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 10 **No:** 4	• Millions of eye injuries are reported yearly. This product (a mixture of an optimized hydrogel and MSCs derived from bone marrow) might speed healing by releasing advantageous cytokines and promoting corneal epithelial repair. Thus, repair of corneal injury is a partially unmet need, though corneal transplant is a largely effective existing therapy. • The candidate product is a biomimetic hydrogel that is mixed with live mesenchymal stem cells (MSCs) prior to application and photopolymerization onto the cornea. This product is expected to be applied to the cornea in the setting of severe injuries and other non-healing corneal defects to promote corneal tissue repair while inhibiting pathologic scarring. Thus, the product is expected to help preserve and potentially restore vision. • This is a very well written biomaterials application by excellent PIs with a long history of excellent corneal wound repair research. • Hydrogels are a great polymer to develop as a stem cell delivery vehicle. • This grant, though strong on a biomaterial's development front, does not contain strong preliminary data to show that MSCs are highly efficacious for corneal repair. Plans to investigate this are presented. However, there is considerable risk, as there are little proof-of-concept data available on MSC efficacy per se. There is some support in the literature, but if such previous data are strong, this is not showcased in this grant. • MSCs have recently been shown to be efficacious in a open label phase 1 trial, but progress has been limited by delivery challenges and the limited ability of MSCs to stay on the cornea. There are limited means of delivering MSCs and this project does fulfill an unmet need. • A bottleneck for MSC based corneal therapy is delivery of MSCs to the corneal environment. • Hydrogels and biomaterials can aid the survival and delivery of the cells, but also make the product more complex. • There is a need to promote repair and regeneration in retinal disease. This proposal meets this partly. • A major concern is that many feel the underlying mechanism of action (MOA) for MSCs is yet to be well documented and confirmed. • This product may not be sufficiently stem cell - oriented to be a priority for CIRM. • Corneal transplants are already available - the advancement here is not clear.
GWG Votes	**Is the rationale sound?**

Yes: 8 **No:** 6	• Hydrogels with HA are well-studied biocompatible polymers. The applicant is working with clinical and research grade MSCs which already have a completed phase 1 study with no safety concerns. So, the hydrogel is novel. These MSCs are Good Manufacturing Practice (GMP) with a certificate of analysis (COA). • Using hydrogels and other biomaterials with tunable properties is a good strategy to deliver cells while promoting survival and function. However, the direct mechanism of action of the MSCs is not clear and, thus, also the support provided by polymer. • The efficacy of MSCs not well described. • The rationale is based on the notion that corneal repair can be achieved by delivering MSCs to the cornea. There is some evidence that this is helpful, but this aspect is not well-presented in this grant. • The notion that MSCs are efficacious for corneal repair is not terribly well-presented in this grant. This claim is supported by references 18 and 19, two review articles in relatively obscure journals. Figure 5 does contain some data to show that MSCs prevent vascularization of cornea after injury (which promotes clarity). Proof of potential efficacy largely rests on Fig. 5. • The preliminary data on hydrogel formulation and delivery of hydrogel and adhesives to damaged rat cornea are strong enough, but in general, this is not much data on corneal repair (at the level of restored biological function) having to do with therapeutic effects of MSCs. • Overall, the biomaterials aspect of this grant are strong, but the preliminary data on efficacy of MSCs is relatively weak. • Lacking efficacy data. Better delivery methods are clearly needed, unclear if MSC's are the way to go. Not sure how the corona repair works. There are several ECM's delivery type devices that might aid repair in addition to the MSC or cell therapy.
GWG Votes	**Is the project well planned and designed?**
Yes: 8 **No:** 6	• This proposal includes MSC allogeneic cell components, and the cell source meets donor eligibility requirements and has been appropriately consented for intended use. Applicant is working with a GMP grade facility manufacturing clinical grade MSCs. • Yes. The applicant has 3 aims to assess essentially the characteristics of the a biomimetic polymer gel with MSCs that can stay on the cornea from 5 days to 14 days. They are incorporating assays to assess in vitro and in vivo feasibility. • The applicant did a good and complete job addressing the prior reviews and comments. • The project is well planned but lacks insight into therapeutic mode of action of MSCs and how the hydrogel could promote this. • The research plan is logical, but mostly focused on preparing hydrogels that are optimized and supporting the survival of embedded MSCs. • The development of the hydrogel and its properties is well planned and takes a step wise approach in terms of survival times and criteria. Not enough characterization of MSCs and their mechanism. • Plans to study and optimize hydrogel degradation (and hence MSC release) are better developed in this revision. There are also reasonable plans to study secreted factors from MSCs that might be beneficial. • Pitfalls and alternatives for biomaterials (hydrogels and adhesives) are good. Pitfalls and alternatives for difficulties with efficacy are less developed.
GWG Votes	**Is the project feasible?**
Yes: 13 **No:** 1	• This is a good team with biomaterials and ocular expertise. • The project is feasible but there is limited preliminary data on efficacy at the moment and no mechanism of action described or efficacy proven. This should be in place before improvements are developed.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**

Yes: 14 **No:** 0	• Corneal cells from a diverse cohort will be used in testing and validation experiments. This is good however donor MSCs are from a company and presumably the ethnicities of these MSCs are unknown. If race or sex of donor cells are important, this will not be ascertained. • Diversity in the patient population and how this can be modeled is partially taken into account. • DEI considerations are addressed to some extent - many people who are underserved due to social determinants of health (SDOH) are at risk for significant corneal trauma (agriculture workers/farmers, military, construction workers, etc.). There is a higher risk of infections in people with diabetes. • The nature of the research focus on eye injury probably makes DEI considerations somewhat indirect. The PI is sensitive to DEI practices within the research environment at their institution and collaborating institutions.

Application #	DISC2-16549
Title (as written by the applicant)	Pluripotent Stem Cells for Osteochondral Repair and Regeneration
Research Objective (as written by the applicant)	The therapeutic candidate is a tissue engineered graft that is composed of bone and cartilage. This composite graft will be used to repair tissue defects in the knee joint.
Impact (as written by the applicant)	The treatment of joint injuries will be significantly impacted. The incidence of developing osteoarthritis after joint trauma will be reduced. The need for joint replacement will be reduced
Major Proposed Activities (as written by the applicant)	• We will show proof of concept of manufacturing bone and cartilage tissues using clusters of cells called microspheroids assembled from stem cells. • We will show proof of concept that microspheroids can repair lost bone and cartilage tissues, in laboratory experiments • We will show proof of concept that microspheroids can repair lost bone and cartilage tissues, in animal experiments • We will develop a manufacturing process to fabricate tissues large enough to be implanted in humans • We will develop a manufacturing process to freeze the manufactured tissues for long term storage so that these grafts will be readily available when needed by patients • We will prepare a package of documents to inform the FDA of our plans for preclinical testing and for clinical trials.
Statement of Benefit to California (as written by the applicant)	Annually, a significant number of Californians sustain joint injuries that result in loss of cartilage and bone in the knee, are challenging to repair, and often lead to early osteoarthritis. There is no FDA-approved treatment that can change the progress of osteoarthritis. Nearly 50,000 joints are replaced every year in CA alone. Our therapeutic candidate, if successful in repairing lost tissue due to joint injuries, is likely to significantly reduce the need for joint replacement.
Funds Requested	$2,996,600
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: 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	66
Median	70
Standard Deviation	6
Highest	70
Lowest	50

Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 11 **No:** 3	• The proposed project is significant because it will develop a tissue engineering approach for generation of osteochondral constructs from an abundant allogeneic cell source for treatment of knee cartilage defects resulting from trauma or osteoarthritis. Given that current therapeutic approaches for diseased knee cartilage restoration are often ineffective, the project holds a strong potential to impact an unmet medical need. • There is a need for the solution that would help treat osteochondral defects and even more so for an arthritis limiting or postponing solution as stated. Unfortunately, it is very unlikely that the proposed solution would bring that. It also very unlikely that this solution would be realistically feasible and clinically applicable. • The proposed solution reads as ticking all the boxes of what one would like to see but even though there has been previous funding there is insufficient evidence or data to accept the claims and to expect that the claims would be realized. • The focus with this product is on osteochondral defect (OCD) but that is a limited cohort of patients (especially given their exclusion criteria). This would not be a product focused on arthritis which the area of unmet need. However, once a patient has an osteochondral defect, there are limited solutions currently clinically available. The track record for these products, in the past, has not been terribly good and so the bar is low for "success". • There is a lot of language and many great suggestions, however there is not a well described path. There are many missing areas that would allow us to be confident of the true translational path of the project. This could be, as previous funding outcomes suggest, an extended lab process that keeps going. • An off the shelf treatment would be great but with a cellular product that is not realistic. A frozen allogeneic product has no basis in current evidence nor do the proposed studies suggest they will provide that. • Lots of competition in this space and many products are much further along. • The group and the track record are strong, and the personnel clearly have ample experience.
GWG Votes	**Is the rationale sound?**
Yes: 7 **No:** 7	• The scientific rationale is generally sound, but additional preliminary data would further strengthen the rationale. • This is a very stem cell specific project that fits the goals of CIRM very well. • There are concerns about the ability to execute this in humans but in a small animal model, the rationale is sound. There are also concerns about the relevance of a small animal model for cartilage injury. • To the best of my knowledge there is no previous evidence of the success and feasibility of allogeneic stem cells grafting cartilage or bone in patients. These are contrary

	suggestions that go against current understanding. Also the suggestion of frozen off the shelf cartilage is not supported by current understanding of the field. • The proposed approach for lab studies is sound and the methods are well explained and feasible by this group. However, it remains very unlikely that given the proposed methods and goals that these would be feasible. • The presented data and preliminary findings support the groups claims and allow the contemplation of subsequent steps. The missing info is in the upscaling and the treatment step to large defects in humans. This is the missing info that will not be solved by the proposed studies even if completed successfully. • The source of cells is not described or rationalized.
GWG Votes	**Is the project well planned and designed?**
Yes: 6 **No:** 8	• This grant is well written with clear and aspirational goals. It's a good quality proposal and research that can be done and will provide some level of interesting novel information. • The project's design is appropriate for obtaining the initial proof-of-concept data. The concern, however, is that the analyses of the candidate constructs are insufficiently detailed to determine how well the neo-tissues mimic native cartilage and bone, and thus how suitable they are for functional cartilage restoration. • No plans are presented for assessing possible functional heterogeneity of the neo-tissues. To these ends, RNAseq analysis and analysis of mechanical properties of the engineered tissues conducted side-by-side with a native cartilage/bone would be valuable. These data would serve as a useful guide for optimization of the neo tissue manufacturing. • Large animal studies would be better and more translatable. • For a small animal model, the project is well planned. But will this correlate to a large animal model and then a human? There would be much more excitement about this project if they were looking at a large animal model. • Project pitfalls and alternatives are limited and insufficient to be convincing. However, the group and leaders have ample experience and would be expected to solve challenges as they occur. • The urgency of the project plan and timeline this is not described in detail, but this would be a project that is more than 10 years away from implementation, if not more. • From a time and capabilities perspective yes but from a scientific realism perspective the project plan and design are uncertain.
GWG Votes	**Is the project feasible?**
Yes: 11 **No:** 3	• The project is realistic and well described. • The milestones and outcomes are logically presented, and it should be possible to achieve them within the duration of the project. • Very much so. Given the time and location, there is likely a large team with adequate funding and time for this project. • The technicians and labs are well established and have shown they are able to complete such work. • The team is excellent and experienced. However, the project may not be budgeted in a manner that is commensurate with the work necessary.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 13 **No:** 1	• Where applicable, they have addressed DEI. In this phase, DEI is not the most obvious part of the proposal. However, the proposed technology and the disease to be treated is very inclusive and widely applicable to many. • The project proposes to develop a universal off-the-shelf allogeneic cell product, which will benefit diverse populations of all races, ethnicities and genders. • If successful, yes.

	• The clinical problem of OCD (that is, the patients that seek out treatment for this condition) may not impact a diverse group of people. Alternatively, this study could have examined osteoarthritis rather than OCD. • The impact for the global population may be small.

Application #	DISC2-16730
Title (as written by the applicant)	Self-delivery of a liver-targeting CRISPR-Cas9 fusion protein for the treatment of acute intermittent porphyria
Research Objective (as written by the applicant)	A liver-targeting CRISPR-Cas9 protein-based therapeutic to selectively inactivate ALAS1 in the liver for the treatment of acute intermittent porphyria
Impact (as written by the applicant)	Acute Intermittent Porphyria
Major Proposed Activities (as written by the applicant)	• Confirm optimized Cas9 fusion proteins have enriched liver biodistribution and high uptake in hepatocytes. • Select an endosomal escape agent and cleavable linker that results in highly efficient, receptor-mediated editing via self-delivery. • Confirm selected candidate possesses on-target ALAS1 editing and minimal off-target editing by self-delivery in human hepatocytes. • Confirm candidate reduces ALAS1 protein levels and enzymatic activity in human hepatocytes, which is the cell type that generates the pathogenic metabolites that drive attacks in AIP patients. • Demonstrate systemic administration of the candidate results in liver biodistribution, hepatic ALAS1 editing and no obvious toxicity. • Confirm candidate is efficacious in a clinically relevant AIP disease mouse model. The therapeutic candidate will be benchmarked to the current standard-of-care givosiran.
Statement of Benefit to California (as written by the applicant)	AIP disproportionately affects women, with ~80% of cases seen in women. AIP causes debilitating pain for women but can go undiagnosed for years, as pain experienced by women is often discounted by care providers. AIP can manifest as depression, anxiety, and other mental health disorders. Our proposed research will result in a broadly accessible and highly efficacious single-administration therapeutic for AIP, which will benefit both female and male California citizens, across all ethnicities.
Funds Requested	$2,023,515
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	68
Median	65
Standard Deviation	4
Highest	75
Lowest	65

Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 11 **No:** 3	• The overall objective of this proposal is to develop gene therapy against genetic disorders affecting the liver. The application focuses on acute intermittent porphyria which is a disease affecting mainly women and which can strongly decrease quality of life. There is currently a treatment based on SiRNA which has a lot of side effect, and which can have variable efficacy. So, gene therapy could bring important improvement. • Acute intermittent porphyria (AIP) is a genetic disorder caused by increased levels of ALAS1, which encodes a liver enzyme. A treatment for AIP already exists (based on siRNAs that target mutant ALAS1) but this required repeated administration and has some toxicity problems. Thus, the proposed project (CRISPR/Cas9 targeting of ALAS1) would improve upon a medical need that is at present partially satisfied. • Impact will be based on whether or not the gene editing approach is significantly better than than the approved siRNA therapeutic agent. • In AIP, ALAS1 is over-expressed due to a causative mutation in PBGD. Since PBGD metabolizes ALAS1 protein (enzyme) genetic partial loss of function of PBGD leads to over accumulation of ALAS1, leading to AIP. Since this approach will target ALAS1 (a normal gene in patients) it should result in reduction of ALAS1. However, there is unknown risk; what are the consequences of CRISPR-induced ALAS1 mutation in such patients? • If successful, this approach strongly improves quality of life of patient with AIP. In addition, such an approach could be applied to the broad number of genetic diseases affecting the liver. • The application is well structured but mainly focused on R&D; translation remains limited to proof of principle.
GWG Votes	**Is the rationale sound?**
Yes: 9 **No:** 5	• Yes - the etiology of AIP is well understood and reducing ALAS1 levels should help. However, risk of inducing ALAS1 mutations is not well addressed. • An ALAS1 knockout mouse exists, and it is surprising this was not discussed in the grant. Homozygous deletion of ALAS 1 is embryonic lethal, hets exhibit heme abnormalities. Thus, the targeting efficiency of ALAS1 will be crucial, and it is unknown if there is a window of reduction of ALAS1 that will be therapeutic for AIP treatment, but not so severe that it will cause toxicity. • The grant contains a description of strategies that the applicant organization has used to improve RNP stability and delivery. In addition, they have worked on how to increase uptake and endosomal release of endocytosed RNPs. The company employs nucleic acid modifications known to promoter targeting [redacted] and backbone structures [redacted] that stabilize the nucleic acid components of RNPs. The grant describes endosomal promoting molecules [redacted] which are not described well.

	• It is unclear if editing *in vivo* will be as much, or greater than the 10-15% observed *in vitro* in a cell line and if this will be sufficient to impact AIP attacks. • In Figure 5, a strategy to increase hepatocyte delivery of editing RNPs is presented, based on binding to a receptor. However, molecular details are not provided (proprietary?). This makes this work difficult to evaluate in terms of the detailed molecular biology, but the editing efficiency in a cell line seems greatly increased. • In Figure 4, there are some data that are interpreted that gene editing occurs in vivo. However, the assay in Fig. 4C is based on immunofluorescence for TdTomato. There is not sufficient explanation as to how this experiment was performed to be able to evaluate whether or not gene editing has occurred or what the efficiency might be. • Using CRISPR/Cas9 to edit the genome of hepatocytes in the context of AIP does not makes sense. A full knock out might not correct the disease. CRIPSR/Cas9 has already been used successfully to correct liver diseases in the context of Transthyretin Amyloidosis. LNP are already used successfully in the clinic to deliver CRISPR/Cas9 in the liver. Why develop something so complicated? • The proposal, if successful, will provide a proof of principle that CRISPR/Cas9 can be used to treat inherited metabolic disorders which affect a wide diversity of patients. However, the applicant should be focusing on proof of principle and then the safety/toxicology profile to enable INDs. Overall, this program is very early stage and not mature enough for funding under this program.
GWG Votes	**Is the project well planned and designed?**
Yes: 8 **No:** 6	• The project is well planned. It includes 6 milestones which first aim to address key technical development associated with their approach (fusing Cas9/CRISPR protein) and then proof of principle *in vivo* and *in vitro*. • The experiments are well explained, rational and will deliver important results. • The budget seems appropriate based on the different milestones included. However, this project strongly focuses on R&D that could benefit multiple projects. • The project design quality in this grant is difficult to assess because molecular details are largely absent, probably for proprietary reasons. For instance, in Milestone 1, it is stated that 30 editing fusion proteins will be screened, but the identity of the "mini proteins" to generate these are not presented. Also, these have been evaluated in a cell line (kidney origin), but they hope to deliver to liver hepatocytes. There is concern findings will not translate to hepatocytes. • In Milestone 3, off-target effects in hepatocytes will be evaluated *in vitro*. Risk of off target effects *in vivo* are not addressed. Milestone 5 (*in vivo* targeting) will look at bio-distribution in other organs but will not address off-target events in other organs. • Milestone 1's pitfalls section is inadequate; it just states that they anticipate success. Other milestones have pitfalls and alternatives presented very briefly, but adequately. • There is a strong possibility of significant side effects with the knockdown.
GWG Votes	**Is the project feasible?**
Yes: 11 **No:** 3	• Yes, the milestones can probably be achieved if there is high commitment to adhere to the schedule. • The preliminary data show that the technology could work, but there is a lack of information regarding all the different steps necessary to improve efficacy. There is a lot of work to be done. • The proposal includes pitfalls. However, Milestones 1 and 2 are critical for the success of the program, and if they don't work the project will end. • The team includes complementary skills and has a lot of experience in gene therapy / CRISPR/Cs9. There is no doubt that they can deliver a successful program.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14 **No:**	• 80% of AIP cases are in women, so this work will assist women predominantly.

0	• AIP is prevalent in women but shows no particular correlations with race. To the extent that underrepresented communities are under-diagnosed for AIP, this may serve to help them. • This proposal focuses on AIP which mainly affects women and thus will account for the influence of gender. • If successful, this approach could be applied to additional genetic diseases affecting the liver and which can have an increased incidence in underserved communities.

Application #	DISC2-16640
Title (as written by the applicant)	Novel gene therapy vector using endogenous promoter to treat SLC6A1-related disorders
Research Objective (as written by the applicant)	Novel gene therapy vector incorporating endogenous promoter to treat SLC6A1-related neurodevelopmental disorders.
Impact (as written by the applicant)	SLC6A1-related neurodevelopmental disorders associated with autism, epilepsy, and encephalopathy.
Major Proposed Activities (as written by the applicant)	• Assess the efficacy of a new gene therapy in patient-derived GABAergic cells as proof of concept (Months 1-24). • Test the efficacy of a new gene therapy in two neonatal mouse models of the Slc6a1 disorder (Months 1–24). • Test the efficacy of a new gene therapy at the adolescent age in two mouse models of Slc6a1 disorder. (Months 13–36).
Statement of Benefit to California (as written by the applicant)	SLC6A1 is a risk gene for autism and epilepsy. The disorder resulting from SLC6A1 dysfunction is likely underdiagnosed evidenced by the fact that in our registry of 111 subjects, only 3% identify as African American and 5% as Asian, compared to 5.8% and 14.8%, respectively, in the general California population. It is likely that all populations are equally susceptible to SLC6A1 disorder, for which there is no cure. Our program aims to test a therapy that is applicable to all SLC6A1 patients.
Funds Requested	$2,167,744
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	70
Lowest	50
Count	12
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	12

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 9 **No:** 3	• Incidence of solute carrier family 6 member 1 (SLC6A1) related disorders is estimated to be 2.65 per 100,000 births. There is no cure, and the application addresses a large unmet need. • Like many rare disorders, there are no current therapies for SLC6A1-related disorders. SLC6A1 is also a likely/potential cause of seizure-related disorders and autism. • There is no disease-specific therapy and what does exist is only moderately effective. It is proposed that a gene replacement strategy would provide the most likely strategy for a clinically meaningful outcome. • This project is designed to develop a novel gene therapy that could be used to address a variety of neurological disorders, including SLC6A1-related disorders. • Proposed therapy will address SLC6A1-related disorders that are defined by failed γ-aminobutyric acid (GABA) clearance due to impaired GABA transporter 1 (GAT-1). Pathologic accumulation of GABA at the synaptic cleft and extrasynaptic space results in developmental delay, autism, and childhood-onset epilepsy. • If the vector design is successful, this will be a candidate for treating epilepsy patients and has relevance for autism spectrum disorder. • The objective of this project is to bring a gene therapy towards the clinic by performing preclinical rodent studies. • There is essentially a single candidate being examined in which gene replacement is sought in a tissue-specific manner by using the endogenous promoter for the SLC6A1 promoter. • Experienced consortium to advance a successful preclinical outcome.
GWG Votes	**Is the rationale sound?**
Yes: 3 **No:** 9	• Applicant proposed proof of concept approach by constructing and testing a single novel gene therapy candidate in both in vivo mouse models and in vitro with patient induced pluripotent stem cells (iPSC) derived neurons. Existing mouse models reproduce human pathology and provide a suitable preclinical model. • The justification for gene replacement is well justified. The tissue-restricted approach is appropriate. • The preliminary data are largely indirectly supportive of this project such as: the mice exist for these studies; the vectors have been designed (but not tested); a clinically relevant rodent phenotype exists; human GAT-1 functions in mice. • This is a project that is essentially exclusively focused upon testing a singular vector for gene therapy/replacement. • The proposal entirely rests on the assumption that the endogenous human promoter will drive physiological levels in the correct cell types at the correct times in the mouse brain. The applicant is not testing the levels and benchmarking it to wild type animals. No preliminary data are provided that would support this claim. • The rationale for the proposed promoter is weak. If all one needed for appropriate expression was an endogenous promoter element, then gene therapy expression issues would have been solved decades ago.

	• The proposed promoter has not been tested by the applicants and is based on an unnamed paper that describes an enhancer of a different length, but this paper uses a construct that is not cell specific. In other words, there are no preliminary results for the promoter construct and it may not be brain specific. • No specific preliminary data are provided as to cell type specific restoration of GAT-1 function. Some preliminary data need to provided showing that the promoter is suitable. Without this, the proposal remains premature. • Unreasonable assumptions for the use of the promoter. • The approach rests on driving expression in neurons only. Driving expression in neurons as well as astrocytes should be considered a necessary preclinical step to show efficacy. • Interneurons from one line of patient iPSC and isogenic controls will be tested. As with any product, it is essential to validate findings in more than one patient to ensure reproducibility. Milestones in mice are strong and will be done in parallel, but they are all interdependent on a successful cell specific vector design.
GWG Votes	**Is the project well planned and designed?**
Yes: 4 **No:** 8	• This is a well-conceived project that leverages existing animal models that represent a large proportion of the target population. • The experimental design is very strong and well controlled. Excellent off-site collaborators. • Milestone 1 will use iPSC-derived GABAergic cells from an affected patient to measure extracellular GABA level (as a proxy for GABA reuptake) upon transgene delivery. While the goal is to restore normal level of GAT-1 function, it does not clear how "normal" levels will be defined. It is also not clear how long-term monitoring of GAT-1 function is achieved. • The mice are commercially available, and the vector is being produced commercially/collaboratively. The inclusion of the iPSC experiments does not seem necessary as similar results could be (and should be) obtained during the in vivo experiments. • The iPSC experiments seem somewhat superfluous as the same outcome (neuronal expression) could easily be measured from in in vivo context. • There is preliminary data, but they are not directly supporting the milestones (vector expression not known; initial hints into efficacy are not known; over-expression concerns are not known). These will be addressed within the proposal within the milestones but there is no preliminary data directly supporting these important points. • Overall, it is premature to propose detailed mouse and iPSC phenotyping on a single construct without first having made a concerted effort to optimize expression of the transgene. • The planning of the vector construct needs improvement raising doubts it will achieve the expected outcome. • Over-expression is stated by the applicants to be a problem in transgenic mice, and no effort has been made to incorporate a microRNA feedback control into the vector to reduce this possibility. Recent approved trials for methyl CpG binding protein 2 (MECP2) gene therapy take this strategy, and it should be considered. • A specified promoter is proposed as a control, but this will also express in excitatory neurons. No experiments are planned for astrocytes. • For the iPSC interneurons, the assays are limited to GABA content and DNA damage, and no electrophysiology or network studies using multielectrode arrays will be performed to look for seizures or activity changes. • Interdependent aims. • Regarding the “Pitfalls and alternatives” for Milestone #1, it is stated that if the vector does not express in a neuronal culture, then alternative vectors will be explored. It is not

	clear if this is specifically to test the activity of the promoter/SLC6A1 expression in neuronal cells or if changes to the vector would be contemplated at this time. • While stated, the proposed plan only includes a single mutation at the iPSC stage, although others appear available. An antisense oligonucleotide (ASO) is mentioned as a potential means to reduce endogenous expression, although this is not a trivial endeavor to develop an effective and specific ASO.
GWG Votes	**Is the project feasible?**
Yes: 6 **No:** 6	• The milestones are logical and can be accomplished in the timeline. Success depends on the one transgene construct. • Technically feasible. • The team is well qualified and utilizes a number of external collaborators for technical expertise. • The project draws upon the expertise of the PI's lab but also leverages a number of external collaborators to essentially outsource some of the time consuming and laborious activities.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 11 **No:** 1	• The applicant will only use one patient iPSC line due to apparent budget limitations, although potential opportunities to test additional patients are discussed. • Male and female mice will be used; Milestone 1 will initially use one line but an extension to include more lines is planned. • The co-investigators work intimately with the patient-led organization and a letter of support is provided. • The proposal discusses how there will be outreach efforts to further expand the patient pool through an existing rare disease network. • The team works directly with a patient-led organization to ensure a broad-based representation (genetically, socio-economically, etc). • DEI-related training occurs at each site involved in the project. • According to the applicant, diagnosis of this disease is highly biased against underserved communities. A patient organization is working towards extending the reach of diagnoses into underserved communities and areas. • There is discussion on the frequency of this disease in underrepresented populations and states that SLC6A1-associated disease is likely underdiagnosed due to expense and the general lower quality health care of many individuals. • SLC6A1-associated disease is rare and it is possible that underserved communities are poorly represented in the current mutation database. • The project includes a caveat section that states that additional iPSC lines could be included but the current proposal does not intend on using additional mutations or lines.

Application #	DISC2-16752
Title (as written by the applicant)	Hyaluronidase-equipped NK-92 engineered with a chimeric antigen receptor as effective therapeutic candidate for pancreatic ductal adenocarcinoma
Research Objective (as written by the applicant)	NK-92 cells genetically engineered with a bispecific chimeric antigen receptor specific for the B7 homolog 3 (B7-H3) and TROP-2 target tumor antigens and expressing hyaluronidase.
Impact (as written by the applicant)	If succesful, the proposed study has the potential to improve the treatment of pancreatic ductal adenocarcinoma patients, for whom no effective therapy is currently available.
Major Proposed Activities (as written by the applicant)	• Design optimization of the chimeric antigen receptor (CAR) constructs and generation of CAR NK-92 cells. • Functional assessment of CAR-engineered NK-92 cells. • Generation and characterization of hyaluronidase-expressing CAR NK-92 cells. • In vitro functional assessment of hyaluronidase-expressing CAR NK-92 cells. • In vivo functional assessment of hyaluronidase-expressing CAR NK-92 cells. • Regulatory preparation and phase I/II clinical trial design.
Statement of Benefit to California (as written by the applicant)	B7-H3 and TROP-2 are two promising targetable tumor antigens expressed by pancreatic ductal adenocaricnoma (PDAC). California currently has no access to cellular therapies targeting those tumor antigens for patients with PDAC. Cedars-Sinai Medical Center is a large center which provides tertiary and quaternary care to assist patients from the whole state and country and is equipped with a good manufacturing practice (GMP) facility which warrants feasibility and prompt patient recruitment.
Funds Requested	$2,581,729
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	--
Highest	--
Lowest	--
Count	15

(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 10 **No:** 4	• The proposal to engineer the NK-92 natural killer cell line to express a dually targeted chimeric antigen receptor plus express the enzyme hyaluronidase conceivably could result in a cell-based therapy candidate that could be useful to treat pancreatic ductal adenocarcinoma (PDAC) and other cancers. The unmet need is clearly substantial. However, the likelihood of success rests on several untested assumptions, and therefore does not appear high. • The project holds the necessary significance and potential for impact; PDAC necessitates new therapies. • The project would engineer an NK-92 cell line to express a dual specific split chimeric antigen receptor (CAR) and hyaluronidase for immunotherapy of pancreatic ductal adenocarcinoma (PDAC). • PDAC is one of the mostly deadly types of cancer and represents an unmet medical need. • NK-92 cell line is readily available for a large-scale manufacturing of the "off-the-shelf" cell therapy products that can be used for multiple infusions. • However, NK-92-derived therapeutic products, including CAR-NK-92 cells have been tested in numerous clinical trials over the last 30 years. Although safe, these cells have very short persistence post infusion to patients and do not produce durable responses even in hematologic malignancies.
GWG Votes	**Is the rationale sound?**
Yes: 5 **No:** 9	• The rationale is sound, but there are concerns the cell line will not persist. • The accumulated results of clinical testing of NK-92-derived products are not supportive for the use of this cell line in the proposed project. • There is no rationale for the use of "and" gating in the proposed CAR construct, specific for B7H3 and TROP-2. Tumor cells must express both antigens in order to be killed, and they can escape by down-regulating one of them. Such design cannot be justified by safety concerns: irradiated NK-92 cannot expand/persist and there is an inducible caspase-9 safety switch in the construct. • There is a clear rationale to test the use of NK-92 modified with carefully chosen chimeric antigen receptors (CARs) to treat cancer. However, problems of using an allogeneic NK line are not considered as carefully as one would hope. The rationale for modifying the cells to express hyaluronidase is not well supported. • It has already been shown that NK-92 cell line does not persist *in vivo*.
GWG Votes	**Is the project well planned and designed?**
Yes: 5 **No:** 9	• There are a number of in vitro and in vivo model to test NK-92 cells expressing various CAR constructs alone and then with hyaluronidase. In particular, 3D organoid cultures

	and in vivo patient-derived xenograft (PDX) models are innovative and more informative than 2D cultures or transplantable cell lines. • The use of the "and" gating, split CAR design is not justified. • Some experiments have non-informative endpoints. For example, assessing CAR NK-92 cell proliferation ability following co-culture with PDAC cells (section 1.4.2 and other places) is not relevant to the clinical application, since these cells must be lethally irradiated before infusion to patients and won't be able to proliferate. • No. The elaborate cell line construction is based on some concepts that have gained currency and may have value - i.e., the targeting of B7-H3 and TROP-2 (with the novel notion of doing so with a dual-targeted CAR, for which feasibility remains to be established). The combination of this with hyaluronidase expression by the NK line seems poorly justified. Some of the pieces make sense. Overall, the construction seems based on assembled guesses rather than systematic testing of each component. • Pitfalls of the approach are inadequately considered. • It's unclear how this project will overcome challenges such as T cell persistence and overcome issues such as heterogeneity and immunosuppression.
GWG Votes	**Is the project feasible?**
Yes: 9 **No:** 5	• The proposed experiments are technically feasible. • The construction seems feasible. There are weaknesses in the design and testing that suggest a low probability of generating a novel line that will be the best of cell-based therapies for PDAC or other cancers that utilize NK-92, target B7-H3 and/or TROP, or overcome the resistance of PDAC to immunotherapy. • The project is feasible.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 14 **No:** 0	• The project plan adequately addresses and accounts for the influence of race, ethnicity, sex and gender diversity. • The applicant notes that PDAC has higher incidence and worse outcomes on average in black patients than white patients. The use of patient-derived models from individuals reflecting the diverse population of CA and the US is worthwhile. • The project upholds the principles of diversity, equity and inclusion (DEI).

Application #	DISC2-16732
Title (as written by the applicant)	Dopaminergic regeneration of a novel nuclear Nurr1-positive neuronal progenitor derived from human embryonic stem cells by small molecule induction
Research Objective (as written by the applicant)	The therapeutic candidate is a hESC-derived Nurr1+/Nestin- dopaminergic (DA) neuronal progenitor (hDANP) suitable for safe and effective DA neuron regeneration therapy for Parkinson's Disease (PD).
Impact (as written by the applicant)	This project enables clinical translation of hESC technology and IP as a much-needed therapeutic solution for PD, overcoming a major bottleneck and having a groundbreaking impact on advancing medicine.
Major Proposed Activities (as written by the applicant)	• To demonstrate hDANP is a homogeneous population of DA neuronal progenitors. Milestone: >90% positive for Nurr1 and differentiate into DA neurons but negative (<1%) for pluripotence/nonneural markers. • To affirm its homogeneity and neuronal identity with no residual pluripotent cells of safety concern by highly sensitive miR profiling. Milestone: >100-fold-down of miR-302, >100-fold-up of miR-10b. • To demonstrate the hESC-derived hDANP is highly neurogenic and safely engraftable following transplantation into the brain. Milestone: > 50% yields DA neurons and a lack of tumor formation (<1%). • To establish preclinical safety and efficacy of the hDANP for DA neuron regeneration in an animal model of PD. Milestone: >50% of the graft yields DA neurons and <1% forms inappropriate cell types. • To develop the target product profile (TPP) for the hDANP and conduct INTERACT meeting. Milestone: The TPP with preclinical safety and efficacy data is established for entry into TRAN1.
Statement of Benefit to California (as written by the applicant)	In regenerative medicine, hESC research holds huge promise for treating major human diseases that have been challenging for traditional medicine. Millions of people are pinning their hopes on hESC research. This project enables clinical translation of hESC technology/IP as a much-needed solution for PD, presenting hESC as a novel, advanced strategy for a wide range of incurable or hitherto untreatable neurological diseases, bringing tremendous benefits to California economy and healthcare.
Funds Requested	$2,804,000
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: --

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Mean	--
Median	--
Standard Deviation	--

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

KEY QUESTIONS AND COMMENTS

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

GWG Votes	Does the project hold the necessary significance and potential for impact?
Yes: 1 **No:** 14	• PD is an incurable disease that causes symptoms and signs through loss of dopaminergic neurons. Current treatments such as drugs and deep brain stimulation address symptoms but the disease remains progressive. Cell therapy is theoretically a realistic approach for PD to reduce dopamine deficiency. • There is an unmet clinical need for regenerative and reparative therapies for PD but this proposal is not likely to contribute to this for several reasons. There is no clear advantage of this cell product over once that are years ahead in development and have already shown safety and efficacy in clinical trials. • The program is unlikely to produce anything novel that will address the need for cell therapy for PD. • There is a lack of critical distinction of this cell product from competitors aside from various critical statements about the value of other programs. • Responses to the prior GWG review are tangential. The applicant indicates that nuclear localised Nurr1 sets the product apart from that of other competitors who "will certainly fail in their clinical trials." The applicant criticizes iPSC cells as likely to cause cancer. • Regarding animal model testing, the applicant launches into criticisms about other researchers without addressing the question. • The ability to effectively treat PD with cell therapy remains unclear, although some promising reports exist, and the learning in the field has been extensive. As the understanding of PD has deepened, the approach of only providing dopamine replacement is currently regarded as an over-simplification. • The candidate will provide neural progenitors with dopaminergic identity cells at scale. • The methodology to generate the neuronal progenitors seems to have been developed around 2011 and patented in 2014, and it is not clear why it hasn't progressed more quickly. • There is no clear plan for translation in humans and many missing details.
GWG Votes	**Is the rationale sound?**
Yes: 0 **No:** 15	• There is a very long and extensive history of tissue and cell transplantation for PD. Significant challenges included graft rejection and survival and circuit integration with modest evidence of benefits. Even with significant survival of cells and increased PET signal there may be little clinical benefit. Disease progression may also involve the transplanted cells.

	• Given the clinical effects of PD including dementia and autonomic dysfunction there is now a more realistic view of the scope for DA neural cells to have clinical effects, which are basically to provide a source of dopamine within the relevant circuit. • In this proposal there are several major flaws in rationale. The first one being that pure progenitors generates pure dopaminergic (DA) grafts and that this is better than grafts with a mix of mature cells. There is no evidence for this, and in fact several studies shows that non-DA neurons, such as astrocytes, can have an important role in supporting survival and maturation of DA neurons. • The idea that a pure population of neural progenitors will maintain in vitro features after implantation is flawed as there are many signals that may affect phenotype. • It is incorrect that the previous therapies are not based on cell replacement. • Additionally, the strategy based on grafting into the proposed location may be successful in rodents but the distance in the human brain is much much larger and not likely to have a therapeutic effect. • Many groups well ahead in this field and applicants repeated criticism of these approaches are unfounded. Other investigators have produced very well characterized and well validated dopaminergic cells for use in PD. • Applicants state this candidate is superior to others through graft dependent neural regeneration as compared to neuroprotection. However, no engraftment data are provided. • Preliminary data do not demonstrate any unique features of the new product. Immunostaining of poor quality and in particular does not show convincing nuclear localization of Nurr1. • Applicant's claims for their differentiation protocol is unsubstantiated. Methods for assessment of off target cell types inadequate and poorly validated. • There is extensive background work that supports the cell derivation project using hESC and the proposed platform. However, there is very little data about how the cells behave after being implanted into the brain. • The lack of testing of the cell product in PD animal models makes it difficult to anticipate the possible efficacy. • No data are provided to support the statements made. • Scientifically this is a largely incorrect proposal.
GWG Votes	**Is the project well planned and designed?**
Yes: 0 **No:** 15	• The applicant institute has hESC platform therapeutics, apparently at scale, for the cell derivation. The small molecule that induces differentiation appears to be a molecule which also leads to Nurr 1 nuclear translocation. Superiority over hiPSC sources is claimed. • Preliminary data in cell differentiation in vitro are not sufficient to convincingly support claims on superior differentiation into DAs. The in vivo data are sparse and do not allow for assessment of key parameters such as graft survival, maturation, function and innervation. • Without functional characterization of cells in vitro, or adequate characterization, this project will not get to a defined endpoint. • Methods for assessment of off target cell types inadequate and poorly validated. • No, no functional assessment of DA cells. Inadequate assessment of off-target cells. No mechanism of action data from in vitro or in vivo work; any improvements seen in the animal model could be due to unspecified trophic effects of grafted cells. Functional integration is not assessed in the animal model. • There is a substantial amount of work proposed to be done by a TBD scientist. The facilities for the conduct of PD-animal experiments are not evident. • The ability to conduct animal studies is unclear. • No experimental details.

	• No immune suppression in the xenograft. • Program de-risking forward from TPP is not discussed. Two patents are awarded, but the potential to partner around the expensive downstream requirements is not discussed.
GWG Votes	**Is the project feasible?**
Yes: 0 **No:** 15	• The project is unlikely to produce any product ready for further development. • Based on prior publications and provided preliminary data, it seems that some milestones (1-2) may already have been achieved, and animal brain engraftment is the main result to be accomplished to provide proof of concept to define a Target Product Profile. • The animal models used are standard in the field but where the graft will be placed, when and how it will be assessed and outcome evaluated is not clear. Also, the plan is to use wildtype rats and only use immune suppression as a contingency plan even though the need to protect the grafts from rejection in a xenograft setting is well documented. • Access to animal PD models and relevant testing is unclear. • The ability to carry out Milestone 4 is unclear. Animal numbers and power analysis are not provided. Milestone 5 cannot occur without the success of Milestone 4. • The project is controlled fully by applicant PI with some infrastructure support and advisors. Based in the application, the PI lacks sufficient knowledge of the field and models used. • The PI is a stem cell scientist; no other staff members are identified. It's not clear if the PI has experience in rodent PD models. • No expertise in PD is demonstrated. • The project is not realistic, with PI and one postdoctoral fellow. • The annual travel budget is high.
GWG Votes	**Does the project uphold the principles of diversity, equity and inclusion (DEI)?**
Yes: 6 **No:** 9	• This is discussed to some extent in proposal. • PI advocates for DEI. • Application states: "PD affects persons of all races, ethnicities, and genders, including 39% of Latino/Hispanics, 36% white, 15% Asian, 6% black, < 1% Natives in California". It's not clear where these figures come from, and they are not accurate. • The PI indicates that they are a stem cell advocate. • Applicant claims her advocacy has influenced federal policies to increase stem cell access, but no evidence is provided. • Statement contains some assertions that lack support.