

DISC4 AWARDS

3/26/26

\$108,531,482 GWG RECOMMENDED

\$80,531,482 CIRM TEAM RECOMMENDED

\$84,000,000 AMOUNT AVAILABLE

\$0 BOARD APPROVED

APP #	TITLE	BUDGET REQ	GWG Recmd	CIRM Recmd	SCORE (MEDIAN)	Score Range				Number of GWG Votes		Previous CIRM Funding	Disease Indication
						Mean	SD	Low	High	Y	N		
DISC4-19200	Defining the Developmental Origins and Neuronal Regulation of Glioblastoma Stem Cells	\$13,578,858	Y	Y	90	89	4	80	95	12	3	N	glioblastoma
DISC4-19271	Tracing the metabolic basis of neurological disorders	\$12,995,613	Y	Y	90	87	5	75	90	11	2	Y	Parkinson's disease, Alzheimer's disease
DISC4-19334	Advancing Therapeutic Discovery Through Multimodal Analyses of Genetic and Cellular Mechanisms in Neuropsychiatric Disorders	\$13,957,175	Y	Y	90	86	6	75	90	9	5	Y	autism, schizophrenia
DISC4-19371	Transplantation of human forebrain assembloids as a platform for therapeutic screening in neurodevelopmental disorders	\$13,999,999	Y	Y	89	89	2	85	92	14	0	Y	epileptic encephalopathy, autism
DISC4-19291	Reversing age-dependent neurodegeneration by elimination of RNA pollution	\$13,000,000	Y	Y	87	87	2	85	90	12	0	N	Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis
DISC4-19391	Dissecting cell-specific genetic and molecular drivers of amyotrophic lateral sclerosis for therapeutic insights	\$12,999,837	Y	Y	85	84	2	80	87	11	3	Y	amyotrophic lateral sclerosis
DISC4-19196	Reprogramming the Spatial Transcriptome for Precision Neurodegenerative Therapies	\$14,000,000	Y	N	85	82	4	70	86	8	6	N	amyotrophic lateral sclerosis
DISC4-19226	The immune system of the human brain: A platform for neuroimmunotherapies	\$14,000,000	Y	N	85	80	10	50	88	8	6	N	Alzheimer's disease
DISC4-19444	Utilizing advanced iPSC organ-chip models of ALS and AI technology to discover subtypes, biomarkers and novel therapeutic targets	\$13,000,000	N	N	80	81	5	70	92	3	10	Y	amyotrophic lateral sclerosis
DISC4-19319	How does Parkinson's Disease impact brainstem and limbic system neurons outside the motor system?	\$12,999,478	N	N	80	80	1	75	81	0	14	N	Parkinson's disease
DISC4-19227	Multi-omic Mapping of the Human Autonomic Nervous System to Accelerate Therapeutic Discovery for Dysautonomia	\$14,000,000	N	N	80	79	5	70	90	2	11	N	dysautonomia
DISC4-19452	Developing novel organoid-derived cellular therapies to restore hippocampal circuit function in mesial temporal lobe epilepsy	\$12,965,718	N	N	80	79	4	70	85	2	13	N	mesial temporal lobe epilepsy
DISC4-19278	Cellular drivers and cell therapies elucidated from stem cell models for brain cancer	\$14,000,000	N	N	79	78	3	70	80	0	14	N	glioblastoma
DISC4-19214	Personalized Paradigms for Discovery and Therapeutics in Glioblastoma	\$12,999,997	N	N	75	80	6	72	88	5	10	N	glioblastoma
DISC4-19364	Engineering Stem Cells to Treat Demyelinating Diseases	\$13,000,000	N	N	70	68	7	50	75	0	15	N	multiple sclerosis
DISC4-19350	Deciphering Neuro-Immune-Vascular Mechanisms in NeuroHIV employing Stem Cell Models and Circuit Analysis: DyNAMIC-NeuroHIV	\$12,999,521	N	N	68	67	4	60	70	0	13	N	HIV neuropathology
DISC4-19207	Restoring network function to treat classes of genetic neurodevelopmental diseases	\$12,356,743	N	N	65	64	6	50	75	0	15	N	epileptic encephalopathy



Application#	DISC4-19200
Title (as written by the applicant)	Defining the Developmental Origins and Neuronal Regulation of Glioblastoma Stem Cells
Project Objective & Impact (as written by the applicant)	Glioblastoma (GBM) is the most common and aggressive primary brain tumor in adults and displays striking cellular heterogeneity, largely driven by multipotent glioma stem cells (GSCs) that sustain tumor growth and resist therapy. The precise cellular origin of GBM and GSCs remains unresolved. We aim to identify and test drug targets to therapeutically halt GBM growth and treat GBM patients.
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> Defining the origin and transformation pathways of GSCs and functionally identifying molecular drivers of transformation. Investigate the mechanisms of neuron-GSC crosstalk by identifying neuron-to-GBM connectivity and synaptic composition, and identify the role of voltage-sensitive signaling in GSC proliferation. Targeting GSC stemness and synaptic pathways for therapy, and development of an AI-driven framework to predict clinical outcomes.
Statement of Benefit to California (as written by the applicant)	This research will benefit California by developing new glioblastoma treatments, improving outcomes for affected citizens and potentially reducing healthcare burdens. While specific California incidence isn't detailed, the US sees over 12,000 new glioblastoma cases annually. California's strong cancer research infrastructure, supported by initiatives like its Cancer Registry, will help translate these advancements into better health equity and survival rates for its residents.
Funds Requested	\$13,578,858
GWG Recommendation	(85-100): Exceptional merit and warrants funding, if funds are available
Process Vote	<p>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."</p> <p>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."</p>

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	4
Highest	95
Lowest	80
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	12
(1-84): Not recommended for funding	3



FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

Key Strengths and Weaknesses

- The proposal is viewed as high impact with an incredible team of investigators with complementary expertise. Weaknesses include lack of cohesion between Projects 1-2 and Project 3.
- There are power concerns given the limited number of cell lines.
- Leading technical expertise.
- Outstanding team with potential for transformative impact.
- Key strengths: The investigator team, the strong publication record and expertise for the technology.
- Key weaknesses: Low statistical power, and some concerns about the feasibility.

Significance: Evaluate the project's significance and potential for impact

- Glioblastoma (GBM) is the most common and aggressive primary brain tumor with no effective treatment options.
- The cellular origins of GBM and glioma remain incompletely understood which limits our ability to target these cells therapeutically.
- The aims outlined in this proposal will provide an unprecedented database of the GBM landscape, provide novel insight into the mechanisms by which neuronal connectivity drives tumor growth and determine new targets and prognostic markers based on these data.
- If successful, the proposed project will lead to a better understanding of tumor origin and tumor growth drivers of glioma.
- The proposed project will generate a large amount of multi-omics datasets from diverse glioma samples.
- The project may lead to the identification of potential targets for therapeutic development for glioma.
- Project 1 is extremely impactful and alone warrants funding support for this proposal.

Innovation: Evaluate the project for innovation relative to the current state of research

- The PI's lab recently described several novel neural and glial progenitor stages unique to human brain development. The tumor-initiating potential of these populations has not been investigated.
- One of the key strengths of this proposal is the breadth of techniques that will be employed. Not only that, the complementary approaches proposed will be able to address key questions regarding fundamental biology in GBM.



- This project is considered to be both technically and conceptually novel.
- The project will also apply system neuroscience approaches to understand the tumor associated synapses and regulatory signaling pathways that may drive tumor growth.
- The proposed project will apply state-of-the-art multiomic approaches at single cell resolution to define the tumor cell lineage progression.
- Discovery science is innovative in Project 1-2.
- Conceptually, the hypothesis in itself is not particularly novel.

Rationale: Evaluate the scientific rationale in the proposal

- The scientific rationale for studying the progenitor cells as possible glioma-initiating cells is sound and supported by published data.
- The use of somatic single-nucleotide variations to perform clonal analysis and lineage tracing is a clear strength, and the team has experience with this approach. Many of the other methods proposed are best in class, with appropriate endpoints defined for each.
- The proposed studies are extremely ambitious. However, the assembled team has the necessary expertise and access to sufficient tissue samples to carry out the proposed omics studies.
- Glioma is the most lethal human brain cancer and lacks effective treatment due to its high heterogeneity. A deep understanding of tumor origin and the drivers of tumor growth is pivotal for targeted therapeutic development.
- The proposed project aims to identify the tumor stem cell origin and to define the molecular and synaptic drivers of its transformation and growth.
- Development of an AI framework is potentially risky. Another potential risk is that multiomic data may not be routinely available, making it critical to distill a minimal set of markers sufficient for a usable prognostic.
- The rationale for Projects 1–2 is very strong, but Project 3 has unclear connectivity. It is unclear which targets will be prioritized and whether long-term treatment will be possible without off-target effects.

Plan & Design: Evaluate the project plan and design

- The proposal is well designed and well planned.
- The proposed experiments will generate several complementary datasets that will define aspects of cellular origin and mechanisms underlying transformation.
- Pitfalls associated with analysis of surgically resected samples, apoptosis, and RNA quality are considered and appropriately addressed in Aim 1. Similarly, in Aims 2 and 3, alternative approaches are discussed should technical problems arise with the various techniques outlined.
- Team members are all well-established experts in their respective fields, with demonstrated expertise in each of the single-cell–based techniques proposed. One of the Co-Investigators is a leader in neuronal–glial/glioma communication and is ideally suited to perform the experiments proposed in Aims 2 and 3.



- The primary leadership team will coordinate the activities described in this proposal. Monthly progress meetings will be held, and a plan is described for data sharing, conflict resolution, and publications.
- Given the high heterogeneity of glioma, the sample size is too small to draw meaningful conclusions regarding the identification of non-coding SNV variants as molecular drivers of tumor progression or to determine whether such findings can be generalized. In addition, it is unclear how many recurrent samples can be obtained from these patients.
- Two GBM lines will be used for Project 2, but no information is provided about these lines. As GBM cell lines do not capture the full heterogeneity of glioma, there is concern regarding whether findings from these experiments will be generalizable and translatable.
- There is a lack of explanation for why different cell lines and different in vitro models are chosen for the proposed experiments. Although combining analyses at the stem cell and synaptic levels is stated as a strength, the experiments for these aims remain largely separated into two parallel arms.
- The teams have the necessary expertise for the proposed project, and the infrastructure and facilities are appropriate.

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations

- The experimental design accounts for genetic, environmental, and other external factors that may influence research findings.
- Genetic factors are carefully considered in all experiments. Environmental and other external factors could be analyzed through meta-analysis.
- Samples are taken from both male and female patients. Although the majority of participants are White, smaller proportions identifying as Asian, Black, or other groups are included, and more than 5% identify as Hispanic. Given the sample size (>1,000), the dataset is expected to capture much of the diversity of GBM patients.
- The PI and co-investigators are involved in relevant glioma- and tumor-related patient advocacy organizations and have established educational partnerships with non-R1 institutions.
- The tumor samples used in the proposed project will be obtained from patients of varying ages, sexes, and ethnic backgrounds, with the majority from non-Hispanic origins, reflecting the patient population in California.
- Some team members are actively engaged in patient advocacy foundations and organizations and will continue these efforts.
- All participating PIs are committed to the training and education of the next generation of scientists and physicians.



Application#	DISC4-19271
Title (as written by the applicant)	Tracing the metabolic basis of neurological disorders
Project Objective & Impact (as written by the applicant)	Define and therapeutically exploit glial neurometabolic flux alterations in Alzheimer's and Parkinson's disease as a common disease mechanism using human stem cell-derived models, single-cell flux profiling, clinical flux measurements, and tunable gene therapy tools to produce biomarkers, targets, and therapeutic platforms that advance CIRM's basic science and translational goals.
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> • Define core metabolic flux alterations in glia associated with sporadic AD and PD • Interrogate neurodegeneration risk genes and core metabolic pathways using Perturb-seq and functional testing • Identify metabolic flux alterations and biomarkers in patients with AD or PD • Develop a metabolic engineering platform for therapeutic modulation of systemic serine
Statement of Benefit to California (as written by the applicant)	California will benefit from new stem cell-based disease models, neurometabolic flux biomarkers, technology for measuring metabolism, and gene therapy platforms that catalyze therapies for Alzheimer's and Parkinson's disease as well as other disorders. The project will strengthen CIRM-linked academic-clinical networks, attract industry collaborations, and reduce the health and economic burden of neurodegeneration in the state's aging population.
Funds Requested	\$12,995,613
GWG Recommendation	(85-100): Exceptional merit and warrants funding, if funds are available
Process Vote	<p>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."</p> <p>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."</p>

SCORING DATA

Final Score: 90

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

Mean	87
Median	90
Standard Deviation	5
Highest	90
Lowest	75
Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	11



(1-84): Not recommended for funding

2

FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

Key Strengths and Weaknesses

- Strengths: Focus on metabolic flux, not steady state; focus on glia, especially microglia in AD; development of new metabolic analysis methodologies; the clinical in vivo study is very exciting.
- The combination of approaches between in vitro, (in vivo), and patient data is overall excellent and very impressive. The feasibility of carrying out the proposed experiments is high based on prior and preliminary data.
- The selection and sizes of studies are a bit unclear. It would have been meaningful to include a clearer discussion of power analysis, including discrepancies between the various lines and patient groups

Significance: Evaluate the project's significance and potential for impact

- The proposal highlights and focuses on the role of metabolic pathways in neurodegeneration. This is a hot topic at the moment, and the findings of the project will make a major contribution to the field.
- The project will use and develop new and important technologies for studying metabolic pathways, such as spatial MS imaging, spatial metabolic flux analysis, and single-cell metabolomics.
- If successful, the work will identify new targets for therapeutic development in metabolic pathways.
- The CRISPR and Perturb-seq approaches in Aim 2 will use single-cell RNA-seq to build on the -omics observations to dissect mechanistic pathways, followed by functional analysis.
- This proposal offers a very novel mechanistic framework that addresses two major neurodegenerative diseases—AD and PD. It proposes that compromised sphingolipid and sterol homeostasis disrupts membrane dynamics, endosomal transport, and Ca²⁺ reuptake to drive neurodegeneration.
- Targeting serine modulation is very intriguing, and the clinical fluxomics aim has the potential to identify mechanistically grounded biomarkers in blood, CSF, and skin. Non-invasive epidermal sampling via tape stripping is a clever and attractive way to monitor sphingolipid fluxes.
- The proposal seems much more exploratory compared to some of the other proposals. This is not necessarily a criticism, but risk management with resource allocation may need to be carefully considered.
- A successful project would lead to detailed mapping of metabolic flux alterations and metabolic pathways in AD and PD patients and in iPSC models derived from patients with these diseases. It would also lead to unique and shareable data from in vitro models and patient samples that would provide deeper insight into AD and PD.



- A fully successful project could lead to new insights into druggable targets for AD and, specifically, further validation of serine perturbations as a feasible treatment for neurodegeneration.

Innovation: Evaluate the project for innovation relative to the current state of research

- The focus of the project on metabolic flux rather than steady state is innovative and has the potential to be very impactful. This analysis is developed all the way from single-cell analysis to clinical work.
- The clinical flux analysis program defining metabolic flux alterations in patients with neurodegenerative disease using stable isotope tracing, as described, is very exciting. This will generate innovative data in PD, AD, and MCI.
- The project will deliver innovative regulatable vectors for gene therapy based on serine metabolism.
- Although focusing on glia is not entirely innovative, as others do so as well, the application of metabolic flux analysis to astrocytes and microglia, with oligodendrocytes as a backup option, is innovative.
- The proposal includes multiple cutting-edge technologies. If it all works, it would be remarkable, but there appears to be substantial technological development still needed before these approaches are ready to be tailored and scaled for neurodegenerative disease.
- The project uses state-of-the-art iPSC technology to develop in vitro models for AD and PD. The first step of novelty lies in the use of metabolic flux analysis in vitro and in patients, combined with other multiomic approaches. In addition, the Perturb-seq genetic tool to interrogate pathways that alter metabolic processes related to PD and AD is novel. The aim to integrate in vitro and new patient data has the potential to yield truly novel insights into these diseases.

Rationale: Evaluate the scientific rationale in the proposal

- The rationale for the project is strong, with a focus on two areas: glial cell biology (astrocytes and microglia) and metabolic flux. Microglia have come to the fore in AD research recently, principally based on genetic findings in sporadic AD from GWAS studies, so it will be useful to have strong metabolic profiling data collected in glia.
- The preliminary data are strong, for example the pilot data on lipidome remodeling in sporadic AD astrocytes and the influence of APOE alleles on metabolic flux in iPSC-derived microglia. These data, along with the spatial MS imaging results, give confidence in the group's ability to deliver the project.
- The applicants note the strong convergence of AD GWAS loci and PD-linked genes on sphingolipid and sterol pathways, providing good justification for the metabolic focus.
- There is preliminary evidence of lipidome remodeling and decreased efflux associated with cholesterol ester accumulation in AD astrocytes.
- The data pointing to SDS as a key regulator of systemic serine are intriguing.
- The adaptamer technology for gene therapy is very exciting.
- The clinical study involves a very demanding protocol, so recruitment and retention will be challenging.
- The rationale for taking a metabolic approach to gain deeper insight into AD and PD and their treatment is solid, based on the literature and prior and preliminary data from the team. The combination of in vitro, in vivo, and patient data approaches is excellent and very impressive.



- The feasibility of carrying out the proposed experiments is high based on prior and preliminary data.

Plan & Design: Evaluate the project plan and design

- The project is well designed to deliver the results as planned. The integration of in vitro methodologies, such as metabolic profiling in glial cells, work in organoids, and clinical metabolic flux profiling in AD and PD patients is strong.
- The flow from foundational data obtained in Aim 1 in the cell models through the CRISPRi screen of the top 150 genes to be selected in Aim 2 works well.
- The connection between perturbed lipid biology and calcium homeostasis, through interactions at the ER, is well made and helps place lipids at the center of cellular dysfunction in disease.
- The AAV vector design for the adaptamer technology to generate a therapeutic vector for regulation of systemic serine, targeted to the liver to benefit a CNS condition, is highly novel.
- The assembled team is strong, with a highly complementary mix of expertise in stem cells, glial biology, and clinical neurology. The project management plan, strategic partnerships, and resource allocation are appropriate.
- While these investigators have access to impressive core instrumentation, the sheer volume of samples may strain even these facilities.
- The clinical study is very logistically complex and costly.
- The investigative team is outstanding, with strong, complementary expertise.
- The management and communication plan is good, including project managers, monthly meetings, and consideration of decision-making and conflict resolution frameworks.
- Overall, the project is well planned and is very likely to yield many of the outcomes suggested. Pitfalls and alternative strategies are relevant and clear. The team and expertise are very strong. The budget and timeline are appropriate and clear, and the management and communication plan is feasible.
- The selection and sizes of studies are still somewhat unclear. It would have been meaningful to include a clearer discussion of power analysis, including discrepancies between the various cell lines and patient groups. Some details and discrepancies in choices of cell models between Aim 1 and Aim 2 (organoids and later assembloids) remain, but it is likely that these will be clarified as the project progresses.

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations

- The proposal will work with cohorts from a White, non-Hispanic population and exclude Latino or African American participants. This will reduce heterogeneity in the study, which may confound results in a small study. Notably, cohorts now being collected for future studies do include Latinos.
- The project will work with a clinical sporadic AD cohort balanced between men and women and will include age-matched cognitively normal individuals from the same ethnic group.
- The clinical study explicitly plans to have comparable numbers of men and women and recognizes sex-specific differences in sphingolipid metabolism.
- The experimental design is very clear with respect to disease-specific genetics and disease progression, but somewhat less clear regarding other factors that can affect disease. This may, of course, affect how



generalizable the outcomes are for future treatment development. Still, the size of the studies suggests that the results could be applied to diverse populations affected by disease.

- The team's prior and current research is well anchored in contacts with patient organizations. However, the exact enhancement strategies are not described in great detail.



Application#	DISC4-19334
Title (as written by the applicant)	Advancing Therapeutic Discovery Through Multimodal Analyses of Genetic and Cellular Mechanisms in Neuropsychiatric Disorders
Project Objective & Impact (as written by the applicant)	Drug development for autism and schizophrenia is hampered by disease mechanism knowledge gaps and minimal consideration of genetic heterogeneity across patients. We will address both bottlenecks to improve drug discovery.
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> • Engineer stem cell lines with autism and schizophrenia risk mutations for subsequent phenotyping studies in models of the developing human brain • Characterize molecular and morphological abnormalities in 3D brain assembloids • Investigate neuropsychiatric-relevant dysfunctions in brain cell firing and connection abilities • Identify defects in neurogenesis using "cell villages" of hundreds of patient cell lines • Integrate our molecular and cellular assay datasets to identify and validate drug targets
Statement of Benefit to California (as written by the applicant)	Autism and schizophrenia are neuropsychiatric disorders that collectively affect 3-4% of people. Thousands of Californian families are impacted, and the estimated annual economic costs are in the tens of billions of dollars. Here, we will use stem cell-derived models of the developing human brain to identify disease mechanisms and nominate drug targets. We will conduct these investigations using human stem cell lines that represent the ancestral backgrounds of >75% of California residents.
Funds Requested	\$13,957,175
GWG Recommendation	(85-100): Exceptional merit and warrants funding, if funds are available
Process Vote	<p>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."</p> <p>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."</p>

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	86
Median	90
Standard Deviation	6
Highest	90
Lowest	75
Count	14



(85-100): Exceptional merit and warrants funding, if funds are available	9
(1-84): Not recommended for funding	5

FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

Key Strengths and Weaknesses
<ul style="list-style-type: none"> • This is an exceptional proposal. However, there are few key issues regarding the analyses proposed that should be addressed to make sure the study can deliver the impact expected. • Outstanding team, well written proposal, high significance, proposal an excellent fit with grant mechanism. Weakness in how to handle variability of interneuron density across assembloids, also variability from interneuron subtype density. Weakness in assuming that intra-organoid connectivity resembles that in vivo. • Technical tour de force. • Landmark paper supporting feasibility of aims 1 and 2. • State of the art to get to disease mechanisms in neuropsychiatric disorders (NPDs). • Excels in terms of community engagement and considerations of diversity. • Some aims are high risk and unlikely to be successful, in particular cell villages and electrophysiology.
Significance: Evaluate the project's significance and potential for impact
<ul style="list-style-type: none"> • The project is a technical tour de force, proposing a wide range of analyses to investigate the brain biology underlying autism spectrum disorder (ASD) and schizophrenia (SCZ). The data generated are likely to be an exceptional resource for neuroscientists around the world. • While the analytic depth proposed is impressive, it is not clear how results across these analyses will be integrated to provide a cohesive picture of the pathogenesis of the disorders investigated or translated into clinically meaningful discoveries. • This proposal seeks to address a major challenge in NPDs with known genetic drivers: identifying common phenotypes and mechanisms that are reasonably proximal to symptoms and thus might be targeted with a relatively limited arsenal of therapeutics, as opposed to a one-gene, one-therapy model for each of the dozens and growing number of genetically based NPDs. • This proposal addresses a major gap in psychiatric genetics by moving from genetic discoveries to mechanistic insights. Psychiatric disorders lag behind many neurological conditions due to their complex etiology and genetic architecture. Given the current crude understanding of etiology for most NPDs, the potential impact of this work is substantial. There are currently few proven paradigms to overcome this gap. • These insights could facilitate therapeutic development in a field where there has been limited progress over the past few decades.



- The program would generate a highly valuable functional genomics resource for NPDs.

Innovation: Evaluate the project for innovation relative to the current state of research

- The investigators propose several technical innovations to advance the investigation of brain biology at the cellular and molecular levels.
- While the investigators highlight that they will assess both rare and common genetic variation, the analyses are primarily focused on characterization of rare mutations, with limited integration of their interplay with common variation.
- The investigators highlight the novelty of investigating two disorders to broaden understanding of pathogenesis across the psychopathology spectrum. However, they provide limited justification for why ASD and schizophrenia were selected.
- The investigators highlight the novelty of analyzing samples from participants of Admixed-American (AMR) background. This is an important aspect; however, there is limited discussion of the analytical challenges associated with recently admixed ancestral backgrounds.
- The scale of mutations to be tested is highly innovative.
- Joint analysis of ASD and SCZ is a strength of the proposal. The motivation for this specific selection could have been described more clearly, but both disorders have rare high-penetrance as well as polygenic components, share overlapping genes, and implicate early brain development through different mechanisms.
- The proposal combines high-throughput CRISPR editing across multiple risk genes with large-scale multiomic and spatial proteomics phenotyping.
- The proposed electrophysiology stack is novel and well motivated for interrogating circuit-level dysfunction in NPDs.
- Another strength is the inclusion of both rare and common variants linked to the disorders. However, investigating mechanisms of polygenic contributors to NPD risk represents a major challenge, and some prior attempts using organoids have been less successful. The cell village design, including stimulus-response and molecular quantitative trait loci (molQTL)/molQTL/re-molQTL concepts, is an exciting proposition, particularly for capturing dynamic mechanisms.

Rationale: Evaluate the scientific rationale in the proposal

- The proposal does not include a strong rationale for the dual investigation of ASD and SCZ. The investigators highlight the presence of six high-confidence risk genes overlapping between these disorders, but they do not indicate whether this overlap is greater than expected by chance. Additionally, further discussion of polygenic and symptom overlap would have strengthened the rationale.
- Most analyses are focused on a limited number of rare mutations. While these can provide important insights into brain biology, their translational impact is likely to be limited because such mutations explain disease in only a small number of affected individuals.
- Overall, the proposal is outstanding; the following comments focus on weaknesses.
 - The investigators note that they and others have validated the ability of hCOs to faithfully recapitulate cortical development at the genomic, biochemical, and physiological levels. It may be



more reasonable to state that some aspects of cortical development are recapitulated, as a major missing component—connectivity—is not known to be captured in this system.

- Is there evidence that any aspect of intra-organoid connectivity resembles intra-cortical connectivity?
- The study of aberrant connectivity in organoids or 2D+ cultures can still reveal themes of activity patterns that differ from controls across many gain- or loss-of-function studies of NPD-related genes, and these patterns may reflect some aspects of NPDs. However, the repeated overstatements regarding the fidelity of the assembloid system in modeling cortical development are concerning.
- A major missing foundational dataset is an assessment of how consistent activity patterns in assembloids are across repeated differentiation of the same lines. There is reasonable concern that the relatively subtle alterations produced by many gain- or loss-of-function mutations will fall below the signal-to-noise threshold for detection. The Rett syndrome example, as well as SCN1A and CHD8, are outstanding, but these represent severe neurodevelopmental phenotypes resulting from haploinsufficiency.
- Including two disorders is a strength in terms of rationale. ASD has higher potential to deliver, given more robust prior work. However, it is also important to test the framework in a disorder with a different genetic architecture. This represents a good balance between feasibility and risk.
- The choice of genes is well justified, and there is a clear plan for generating reproducible CRISPR resources.
- Strong preliminary data support Aim 1. Moreover, the group recently published a high impact paper providing proof of principle that cortical organoids can produce mutation-linked signatures, with effects across distinct ASD genes converging with increasing maturation.
- The electrophysiology plans in Aim 2 are exciting, and the applicants provide a strong technical description supporting feasibility. However, these assays can be sensitive to factors such as cell composition, and there is concern about whether they will yield robust and interpretable readouts.
- For the cell village approach, the key question is whether these systems can uncover shared phenotypes across different variants and genetic backgrounds. This aim has less prior support in the literature for these disorders but scores highly in terms of innovation.
- Aim 4 appears somewhat appended to the rest of the proposal. This aim is clearly dependent on the success of the earlier aims in elucidating mechanisms and identifying the most promising targets.

Plan & Design: Evaluate the project plan and design

- The cellular and molecular analyses are described in detail and are expected to advance the understanding of the pathogenic processes affecting brain biology. The timeline proposed and the budget requested are appropriate. The design is supported by preliminary results and the investigators' expertise.
- The investigators propose to analyze samples from AMR individuals. However, limited information is reported regarding how genetic diversity will be modeled. For instance, the investigators should provide detail regarding how local ancestry will be modeled, especially when comparing cases and controls. Indeed, AMR individuals can present a large variation in their ancestry distribution (see Figure 1 in PMID: 32028992). Additional expertise in population genetics may be useful.
- While the investigators aim to integrate polygenic risk and rare mutations, the majority of the analyses are focused only on rare mutations. There is a general lack of detail regarding how common variants will be



integrated into the proposed analyses. For instance, they could consider investigating samples derived from individuals with high vs. low polygenic risk.

- Response QTLs will be investigated in the proposed study. While this is a potentially interesting goal, there is currently very limited evidence that gene-environment interactions contribute to a large proportion of the heritability of psychiatric disorders. Additionally, it would be important to clarify how there is no justification regarding the stimulant conditions selected to test response QTLs.
- The investigators propose to investigate ASD and SCZ to gain insights into shared and disorder-specific mechanisms. However, limited justification and detail are provided regarding the comparisons between these conditions. Additionally, SCZ expertise seems to be limited compared to ASD expertise among investigators.
- Will assembloids be screened for inclusion based on the ratio of excitatory and inhibitory cells, and the distribution of the interneurons across the fusoid (a low throughput endeavor), to reduce variability, and if not, what evidence exists as to the reproducibility of interneuron density and distribution, not to mention subclass composition, across assembloids? Some kind of power analysis or at least discussion taking into account variability of interneuron density/subclass relative to predicted effect sizes of the +/- mutations would be very helpful here, especially in the context of the many very sanguine statements of the power and validity of this approach.
- A related challenge is that many of the genes proposed to study that do result in phenotypes from known or putative haploinsufficiency, such as *Nrxn1*, *Grn2a*, are also associated with highly variable penetrance of NPD-related phenotypes in +/- mutants. The lines are generated on several wild type backgrounds, and there is tremendous enthusiasm for the rigor of that design employing two lines from two individuals each of two general ancestries. It is appreciated that the proposal is not intended to study ancestry as a variable. But the strong likelihood that variable penetrance secondary to genetic background effects independent of sex or ancestry will lower the ability of this design to detect common themes between the two ancestries, or sexes, for some fraction of mutations should be stated.
- To their credit, the applicants state they will focus on modules and regulatory drivers that are most robust (observed for all 4 EUR and AMR backgrounds and both sexes across at least 2 methods). But the number of mutations that have these shared phenotypes in their system may be lower than anticipated, secondary to both signal/noise issues and genetic background effects on penetrance. On a related note, employing the “village culture” approach at this scale is extremely high-risk, albeit potentially high payoff (especially for neurogenesis phenotypes). The challenge is that line differences in growth will sometimes be secondary to line idiosyncrasies rather than to disease-related characteristics.
- Consistent consideration of reproducibility and statistical power.
- At times, the outputs could be more clearly defined, e.g., what constitutes a robust convergent phenotype?
- The applicants represent the global leaders in these fields. However, expertise in schizophrenia is notably missing.
- The budget seems appropriate for this scale of program. There is in-kind matching. The timelines are quite ambitious, but the applicants provide clear plans and rationale so they might be able to deliver this. It is good that there is a clear plan overlapping production and analyses where appropriate.

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations

- The investigators aim to expand the representation of ASD and SCZ research by analyzing samples collected from AMR individuals. This is supported by their previous work with Hispanic communities and the resources available from their institutions. However, there are serious issues regarding how data will be analyzed to ensure reliable findings can be generated from the analyses proposed.



- The application excels in this area. There is credible consideration of population impact throughout the application.
- Explicit consideration of sample sex and ancestry for aims 1 and 2, but lacking detail about cell village donors.
- Strong community engagement plan with significant evidence of prior leadership in this area.



Application#	DISC4-19371
Title (as written by the applicant)	Transplantation of human forebrain assembloids as a platform for therapeutic screening in neurodevelopmental disorders
Project Objective & Impact (as written by the applicant)	We need mechanistic understanding and therapeutics for neural circuit dysfunction in neurodevelopmental disorders including epilepsy and autism. The inaccessibility of human brain tissue is a barrier, while animal models fail to recapitulate human-specific neurodevelopmental processes and limit translation. This project will use human cellular models to identify biomarkers and therapeutics.
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> • Establish in vivo t-hFA platform for modeling E/I dysfunction across neurodevelopmental disorder risk genes • Define molecular, cellular, and circuit mechanisms underlying E/I dysfunction • Correlate in vivo t-hFA circuit and behavioral signatures with patient clinical and EEG biomarkers • Develop and validate ASO therapeutics targeting E/I homeostasis
Statement of Benefit to California (as written by the applicant)	Around 2.8% of Californian children are diagnosed with autism spectrum disorder, 1.6% with intellectual disability, and 1% with epilepsy. We will determine the role of neural circuits in neurodevelopmental disorders and integrate findings with data about molecular and cellular abnormalities as well as validate our findings across diverse cell lines. A better understanding of how human brain circuits are dysfunctional will point us toward better ways to protect, improve, and treat those symptoms.
Funds Requested	\$13,999,999
GWG Recommendation	(85-100): Exceptional merit and warrants funding, if funds are available
Process Vote	<p>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.”</p> <p>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”</p>

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	89
Median	89
Standard Deviation	2
Highest	92
Lowest	85
Count	14



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

FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

Key Strengths and Weaknesses
<ul style="list-style-type: none"> • A major strength is the ability to mimic aspects of mature neural circuitry using human neurons in vivo, and the ability to manipulate the circuitry pharmacologically. There are no major weaknesses, but the low throughput of the transplantation approach and complexity of the readout may preclude examining a sufficient number of genetic backgrounds which may be limiting. • Clear focus and sophisticated tools. If successful, high impact for at least some neurodevelopmental disorders (NDDs). • System can be adopted for other studies to analyze the impact of organoids of any kind on neuronal circuit integration. • Potential translatability of rat EEG to human EGG? • Limited to known common variants. CNVs are not considered. Limited to E/I balance changes that might not be causative of a specific variant but consequential. • Developmental timing mismatch in the transplantation approach is not addressed. • Key strengths: The investigator and the team, strong rationale and preliminary data, comprehensive analysis, potential translation and broad impact.
Significance: Evaluate the project's significance and potential for impact
<ul style="list-style-type: none"> • It has long been hypothesized, but never fully tested, that the genetic heterogeneity of ASD and other neurodevelopmental and epileptic encephalopathies (DEE) converges on a common mechanism: alteration of excitatory/inhibitory (E/I) balance in brain circuitry. This project will test whether engineering gain-of-function (GOF) or loss-of-function (LOF) mutations in four NDD genes in a control human iPSC line produces similar alterations in E/I balance in brain circuitry. • The selected GOF and LOF mutations are all associated with increased risk for NDD, DEE, or ASD, enhancing clinical significance. The model system—human forebrain assembloids (t-FA) transplanted into rat cortex—links detailed molecular and electrophysiological characterization at the cellular level to hypothesized circuit dysfunction via in vivo extracellular recordings of altered neuronal oscillation power spectra, a measure of E/I balance. • The applicants also plan to perform direct comparisons to patient EEG data but do not provide evidence that such direct comparisons are feasible. • The model incorporates assessment of animal behavior as well as design of corrective treatments that could be tested for restoration of E/I balance after systemic or intracisternal administration in transplanted



animals. If successful, this approach could serve as a paradigm for other disease model systems, although its practical impact is tempered by the low throughput of the transplantation approach.

- The core focus of this application is to generate a model system capable of identifying changes in E/I balance, a dysfunction thought to be a common feature of many NDDs. If successful, this would be highly transformative for at least a subset of NDDs.
- The systematic analysis of GOF and LOF comparisons to identify shared E/I dysfunction mechanisms using a multimodal phenotyping pipeline—including molecular profiling, cellular characterization, circuit analysis, and machine learning integration at three and seven months post-transplantation to capture trajectories of change—is comprehensive and is expected to yield new insights and identification of convergent phenotypes across NDD subtypes.
- Development and validation of ASO therapeutic approaches targeting E/I homeostasis will be complemented by small-molecule screening using a co-investigator’s AI platform to predict promising candidates, offering potential for rapid translation.
- The approach is limited to known, common variants. While there is a high degree of overlap among individual NDD risk profiles and a predominance of genes associated with synaptic transmission, the odds ratio conferred by any single risk polymorphism is small. Rare variants and copy number variations, which are more common contributors, are not addressed.
- The in vivo transplanted human forebrain assembloid (t-hFA) platform captures both excitatory and inhibitory elements and is first in class. The systematic GOF versus LOF comparison across four ion channel genes associated with severe NDDs offers an unprecedented opportunity to test some of the longest-standing theories in ASD and NDDs.
- Parallel studies in patient populations and ASO experiments provide an extraordinary platform for development and validation of potential NDD therapeutics.
- Neurodevelopmental disorders (NDDs), including developmental and epileptic encephalopathies (DEE), autism spectrum disorder (ASD), and epilepsy, are highly prevalent and lack effective treatments. There is an urgent need for novel therapeutics grounded in disease pathology.
- E/I imbalance has been proposed as a core driver of NDD etiology. The proposed work will establish a preclinical model to directly examine E/I balance in a human cellular context.
- Successful identification of ASOs targeting ion channel variants may translate into clinical therapies.

Innovation: Evaluate the project for innovation relative to the current state of research

- A major point of innovation is the transplanted assembloid model system. The PI's lab has pioneered the assembloid transplantation approach to build a human circuit connected to the animal’s sensory apparatus. The current model enables the integration of excitatory and inhibitory neurons within the cortex of the animal and thus the emergence of oscillations and more advanced network activity.
- Another innovative approach is the extracellular recording of the transplant enabling assessment of network circuitry (synchronicity, oscillation power spectra, spectral slope (a measure of E/I ratio), coherence analysis, and detection of epileptic spikes). This system will permit detecting the emergence of aberrant circuitry in the transplant. However, the extent to which this extracellular recording system can be compared to a human scalp EEG is an open question.
- The in vivo context allows investigators to integrate molecular features (scRNA-seq), cellular activity (patch clamp), network activity (EEG, extracellular recording) and behavior. The incorporation of all these measures and dimensions using a machine learning approach in a common space will in principle allow the



investigators to discover the mechanism of how divergent genetic and cellular alterations can lead to a similar E/I imbalance and design mechanism-based treatments.

- Tools used are sophisticated and built on existing foundation approaches developed by others. The longitudinal EEG Monitoring was pioneered by the applicant's lab and used in monitoring E/I imbalances in Timothy Syndrome (2022).
- While the approach itself is conceptual not novel (other groups employ transplanted organoids). The applicant's lab has been the pioneer in transplanting assembloids instead of defined organoids.
- The t-hFA platform in it of itself is an enormous achievement.
- The application of patch-seq to transplanted assembloids represents a novel integration of single-cell electrophysiology with transcriptomics within in vivo human neural tissue. The integration of MERFISH spatial transcriptomics, coupled with foundation model informed data integration is cutting-edge.
- The use of developmentally regulated alternative splicing as a therapeutic target for slice-switching ASOs is creative and important.
- Using the human forebrain assembloids transplanted into rats as an in vivo model for functional and behavioral testing.
- Comprehensive analysis at multiple levels for 4 NDD ion channel genes that are potentially druggable.
- The multidisciplinary team provides a unique synergy.

Rationale: Evaluate the scientific rationale in the proposal

- The rationale for the choice of gene variants and their implementation is supported by the literature and by the PI's lab's prior successful use of this approach with CACNA1C to model Timothy syndrome in rats.
- The in vivo approach, although labor intensive, models more mature human circuitry and enables investigation of altered network imbalance in NDDs. The t-FA transplantation strategy is supported by preliminary data suggesting its ability to model more complex circuitry and epileptic seizure behavior. Whether seizures are a direct consequence of E/I imbalance in the transplant could be demonstrated by restoring E/I balance using ASO or small-molecule treatments.
- Based on the emerging consensus that NDD pathology arises from altered neural circuit activity and homeostasis, influenced by genetic and environmental vulnerability, there remains a major knowledge gap in understanding how NDD-associated genes contribute to or drive defects in E/I balance. This gap represents a bottleneck for therapeutic development.
- The model involves transplantation of human forebrain assembloids into rat brains to generate a more complex circuit system and enable behavioral outcome testing.
- The applicants provide proof of concept for this approach in Timothy syndrome, which is currently undergoing final safety studies in preparation for a clinical trial.
- Whether this approach will be successful for loss-of-function variants is unclear.
- Transplantation of human cells into rats inherently introduces a developmental timing mismatch that could affect, mask, or distort readouts and lead to incorrect conclusions. This caveat is not addressed or acknowledged.



- Prior work using ASOs for Timothy syndrome provides proof-of-principle evidence that this platform can identify and validate therapeutics. However, Timothy syndrome is a gain-of-function disorder, and it is unclear how well the approach will translate to loss-of-function variants.
- Preliminary data spanning in vivo electrophysiology, transcriptomics, and behavioral readouts support the construct validity and physiological relevance of the t-hFA platform.
- The transplanted human assembloid model (t-hFA), developed by the PI, has been shown to model epilepsy originating from human cells.
- There are strong preliminary data supporting the overall rationale and hypothesis.

Plan & Design: Evaluate the project plan and design

- Overall, the experiments are well designed, with comprehensive assessment of transcriptomic profiles, electrical properties, circuit properties, and behavior, integrated with machine learning and combined analyses. Potential pitfalls are appropriately discussed.
- One concern is the variability and complexity of the approach. The investigators do not provide an assessment of how experimental variables—such as the number of transplanted and surviving cells, transplant location, cellular composition of the assembloids, or genetic background of the donor line—may affect the tested outcomes.
- The spatial transcriptomic experiments are not well justified, particularly given the lack of information about the morphological and cellular architecture of the transplanted t-FA. The two regions of interest (ROIs) span only a minimal portion of the graft and may not be representative of the whole.
- The comparison between recordings in rats and EEG in patients will rely on baseline power spectral density across frequency bands, spectral slope, gamma power, and evoked or induced gamma activity. This is potentially very exciting, but it would have been informative to provide examples demonstrating the extent to which t-FA recordings and patient EEG can be meaningfully compared across these features.
- The focus on four genes associated with epilepsy and NDD that harbor both GOF and LOF variants is sensible and likely to provide insight into the nature of E/I balance disruptions.
- Patch-seq performed on slices allows direct correlation of molecular and physiological phenotypes within the same cell, which is interesting. However, this approach is biased toward identifying molecular markers in well-defined physiological and morphological cell classes, and this limitation is not discussed.
- The proof-of-concept data (Fig. 9) are unclear. Only 50% of animals exhibited seizure activity in the hyperthermia-induced febrile seizure model using the DEE variant. This raises questions about why the non-responder rate is high and how this will affect interpretation of outcomes for variants that may be milder or more severe. It is unclear whether only responders will be analyzed to identify common E/I patterns, and discussion of pitfalls and alternative strategies (e.g., increasing power, use of additional drugs) is limited.
- The proposal progresses logically and is well organized.
- Overall, the discussion of pitfalls and alternative approaches is reasonable.
- The team is outstanding and exceptionally well suited to carry out this project.
- Despite these strengths, there is no guarantee that this project or platform will represent the long-awaited turning point in the heavily invested, gene-centric approach to NDD therapies.



- E/I dysfunction resulting from genetic mutations will be examined at cellular, molecular, circuit, and behavioral levels (Aims 1–3), with strong preliminary data supporting feasibility.
- Comparison of EEG data from t-hFA models and patients will be performed to identify potential biomarkers using multimodal foundation models.
- ASOs will be designed and screened as potential therapeutics targeting either individual variants or convergent mechanisms across the four genes.

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations

- The PI will work with foundations and advocacy groups to discuss experimental strategy and ethical issues. This is thoughtfully organized and well presented.
- Data will be validated in control hiPS lines from diverse sex and ancestral backgrounds (female Hispanic donor; African American female donor) through matching funds support..
- The project includes generation of a set of isogenic iPSC lines under different genetic backgrounds that can be shared with other investigators.
- The PI has been engaged in education outreach programs and other team members are involved in education of high school and undergrad students through various programs at the applicant institution.
- There is a plan to engage with patient and family advocacy groups.



Application#	DISC4-19291
Title (as written by the applicant)	Reversing age-dependent neurodegeneration by elimination of RNA pollution
Project Objective & Impact (as written by the applicant)	This project addresses the lack of mechanistic insight into how age-dependent RNA misprocessing drives neurodegeneration. Using patient-derived iPSCs and aged induced neurons, coupled with small-molecule screens and xenotransplantation of human 3D induced spheroids, we will identify regulators that restore RNA homeostasis, accelerating discovery for Alzheimer's and Parkinson's diseases, and ALS.
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> • Define aging- and disease-specific RNA pollution signatures in patient-derived neuronal models and human biospecimens • Identify the cellular and metabolic sources that generate RNA pollution in aged neurons and define the functional consequences of their perturbation • Identify regulators of age-dependent RNA pollution using arrayed shRNA screening in fibroblast-derived neurons • High-content small-molecule and ASO screening to identify druggable pathways and evaluation in xeno-models
Statement of Benefit to California (as written by the applicant)	This project will accelerate discovery of new therapies for Alzheimer's disease and related dementias, Parkinson's disease and ALS -- neurodegenerative diseases that impose a major health and socioeconomic burden on California's aging population. Using patient-derived stem cell models and induced neuronal cell models, small-molecule screening, and in vivo validation in human neuronal xenografts, the work will drive therapeutic innovation, and strengthen California's biomedical ecosystem.
Funds Requested	\$13,000,000
GWG Recommendation	(85-100): Exceptional merit and warrants funding, if funds are available
Process Vote	<p>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."</p> <p>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."</p>

SCORING DATA

Final Score: 87

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

Mean	87
Median	87
Standard Deviation	2
Highest	90
Lowest	85
Count	12



(85-100): Exceptional merit and warrants funding, if funds are available	12
(1-84): Not recommended for funding	0

FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

Key Strengths and Weaknesses
<ul style="list-style-type: none"> • Strengths: Use of induced neurons (iNs) to retain signatures of aging; the new concept of an RNA basis for aging; new ways of thinking about Alzheimer’s disease (AD) and Parkinson’s disease (PD), in a way already considered in Amyotrophic lateral sclerosis (ALS); potential to lead to RNA-based therapeutics for AD and PD. • Weaknesses: issues of collating and integration of huge datasets; ascribing all aspects of the cell biology of aging to RNA dysfunction risks a loss of focus. • The proposal has the potential to generate critical data understanding RNA biology's impact on neurodegeneration. • Novel and bold ideas; excellent cell models and population sampling; broad approach; exceptionally strong team with expertise in all areas of the project.
Significance: Evaluate the project’s significance and potential for impact
<ul style="list-style-type: none"> • The project addresses a very significant area: how aging conveys vulnerability to AD, dementia, and neurodegeneration as the main risk factor. This project aims to discover which aspects of aging itself drive risk, focusing on dysfunction of RNA biology, which the investigators have termed “RNA pollution.” • The potential for impact is high, as targeting RNA biology in AD and PD is a new area. RNA biology is well studied in ALS but less so in AD and PD. • If the project leads to new RNA-focused therapies for neurodegeneration, the impact will be very high. • The concept of “RNA pollution” as a unifying framework and convergent aging-driven mechanism is compelling. • The ambition of studying RNA pollution across five diseases is impressive. By necessity, this will compromise disease-specific depth, but it may be justified in order to take such a unique angle. • The central focus of this proposal is that widespread RNA misregulation in aged cells, such as mislocalized RNA-binding proteins (RBPs) resulting in splicing errors and RNA damage, drives pathological sensitivity. This stress is postulated to reduce neuronal resilience, creating vulnerability to neurodegenerative triggers and thereby increasing the risk of AD, PD, ALS, and potentially other age-related neurodegenerative diseases. • The hypotheses and datasets generated relating to RNA vulnerability as a concept have the potential for broad impact in RNA biology, with applications across many disease areas.



- Unique datasets will be generated from very strong sources.

Innovation: Evaluate the project for innovation relative to the current state of research

- The use of an induced neuron (iN) system where aspects of cellular aging are retained in differentiated neurons is very strong, as they showed in their 2025 paper which led to this work.
- The concept of "RNA pollution" is novel and generates new ways of thinking about the basis of neurodegenerative diseases.
- The group has developed two parallel 3D culture systems which they use for specific applications: a thin layer 3D gel for in vitro work and screening and a high density 3D spheroid format for use as an organoid system in vitro and transplantation in vivo.
- The proposal is a fresh reframe away from late stage protein aggregation pathology toward upstream RNA-level dysfunction.
- A testable mechanism model in linking mitochondrial dsRNA release through PKR-dependent ISR activation to downstream RNA processing defects and stress granule persistence.
- The use of induced neurons (iNs) that retain donor age signatures is important and the application of single-cell long-read RNA sequencing is impressive.
- Takes base in innovative, "new" biology with clear implication for human disease.
- Advanced and cutting edge methodology.

Rationale: Evaluate the scientific rationale in the proposal

- The rationale is strong and built upon two areas. First, aging is the biggest risk factor for neurodegeneration, and understanding why that is essential. Second, the group has a recent paper in which they define how mislocalized RNA-binding proteins (RBPs) lead to splice errors, RNA damage, and cellular stress. The role of proteins such as TDP-43 in ALS points to RNA processing as an important factor in age-related disease.
- However, as argued, "RNA pollution" seems to lie at the root of almost all cellular dysfunction, from RNA misprocessing to perturbed mitochondrial membrane potential. This generates a project of enormous breadth, which risks losing focus.
- The proposal is built on compelling foundational work from a 2025 paper on RBP mislocalization, splicing defects, and chronic ISR activation in the absence of external stressors.
- The experimental approach is well designed, with a logical through-line from discovery to functional characterization to therapeutic translation.
- This project takes a multi-omic and functional genomics approach with the aim of restoring RNA "health" and thus protecting cells from disease. The ideas are novel and bold. The comprehensive data generated will be used to identify mechanisms that can be targeted for therapy, and screens for ASOs will be conducted. Thus, the proposal spans from new biological insights to therapy development in a coherent flow, but it is also very demanding in terms of time and resources.
- There is a risk that the initial mapping and search for mechanisms will not yield clear candidates for therapy development.



Plan & Design: Evaluate the project plan and design

- The use of patient sample cohorts that provide access to matched induced neurons (iNs) or fibroblasts, CSF, plasma, and postmortem brain tissue is very powerful.
- The project uses an impressive number of cell lines (>200) across healthy aging, and several neurodegenerative disease types. This is followed by studies of 75 brain tissue samples and 75 biofluid samples. Overall, the project proposes to undertake a very large number of analyses on a very large number of samples, which risks losing focus.
- This is a complete and thorough analysis of the >200 cell lines, including paired-end short-read RNA-seq; single-cell long-read RNA-seq; translation profiling using RNA editing–based mediated profiling paired with long-read sequencing; subcellular fractionation followed by proteomics; enhanced cross-linking immunoprecipitation to comprehensively map RNA N6-methyladenosine (m6A); and spatial transcriptomics. This may be too much.
- The project proposes an enormous amount of data collection and analysis, which may be ambitious to complete on time and on budget.
- The group of PIs is excellent, and the consortium structure is briefly presented and appropriate. However, management of such an enormous program of data collection and collation will be challenging.
- Overall, the approach has a very logical through-line, from discovery to functional characterization to therapeutic translation.
- The investigative team is absolutely world class.
- Team communication and management plans seem reasonable, albeit somewhat generic.
- The project spans identification of RNA pollution biomarkers to ASO screening for therapeutic targets, to small-molecule screening in high-content assays, followed by validation in organoids and xenografts.
- A key strength is access to brain tissues with corresponding fibroblast and/or iPSC lines derived from the same donors, which is a unique resource in which RNA pollution signatures can be mapped. This alone makes the project very compelling and worth financing!
- The sample sizes are commendable.

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations

- The project will use samples of male and females of varied age, but also across diverse ancestries so as to capture demographic heterogeneity. How will this heterogeneity be allowed for in the analysis?
- The project will also incorporate differences in metabolic health, toxin exposure, and socioeconomic context. Will this lead to variability in the datasets?
- Partnerships with patient advocacy organizations and community and public education are well covered with allocated resources.
- The proposal explicitly emphasizes inclusion of underrepresented groups.
- The partnerships and collaboration with patient advocacy and community education are laudable.



- The demographics of the donors from these large datasets are not presented. Well developed education and outreach programs.



Application#	DISC4-19391
Title (as written by the applicant)	Dissecting cell-specific genetic and molecular drivers of amyotrophic lateral sclerosis (ALS) for therapeutic insights
Project Objective & Impact (as written by the applicant)	Objective: We combine machine learning, genetics, neuropathology, and stem-cell-based screening to discover genetic drivers of ALS. Current treatments target known genes like SOD1, but only ~10% of patients have identified drivers. Impact: This work will enable new therapeutic strategies and advance precision medicine for ALS.
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> • Perform single-cell long-read RNA-seq and multiome (snRNA-seq + snATAC-seq) in the motor cortex, spinal cord, frontal cortex and occipital cortex of ALS patients and controls. • Train machine learning models to prioritize and interpret cell-specific genetic drivers of ALS risk and severity including GWAS/WGS from Project MinE. • Conduct a Perturbseq / CRISPRi screen in iPSC-derived cell types with readout of (i) target gene expression; and (ii) expression of cryptic exon targets associated with TDP-43 LOF • Validate targets via immunohistochemistry in patient tissue • Test therapeutic ASO targets in ALS iPSC neurons and glia.
Statement of Benefit to California (as written by the applicant)	Our research will advance understanding of ALS mechanisms and accelerate discovery of new therapeutic targets, benefiting Californians affected by this devastating disease. By developing human stem-cell models and testing new treatment strategies, we aim to drive innovation in precision medicine and strengthen California's leadership in neurodegenerative disease research.
Funds Requested	\$12,999,837
GWG Recommendation	(85-100): Exceptional merit and warrants funding, if funds are available
Process Vote	All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG." Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."

SCORING DATA

Final Score: 85

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

Mean	84
Median	85
Standard Deviation	2
Highest	87
Lowest	80



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

FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

Key Strengths and Weaknesses

- Scientifically sound and well-supported by preliminary data.
- Strong preliminary data.
- High potential to deliver therapeutically relevant insights.
- Several issues in terms of design could be addressed (described below).
- Overall, this is a fundable, well-developed, ambitious proposal led by leading experts in their respective areas of research. The preliminary data support feasibility, and the project is well-positioned for rapid translation given the stepwise study design and consideration of patient subtypes. With attention to the questions noted below (see Plan & Design comments), the potential likelihood of success and future translation with broad applicability for all with ALS is high.

Significance: Evaluate the project's significance and potential for impact

- The proposal targets a real bottleneck in ALS that most patients don't have a known genetic driver.
- The project has significant potential for impact. It tackles a major challenge in ALS therapeutics: the unknown genetic drivers of sporadic ALS and incomplete mechanistic understanding of the disease. Successful completion would represent a paradigm shift in understanding ALS etiology.
- The potential for broader impact is high. The generation of a large-scale, multi-omic atlas from four key CNS regions in ALS and controls would be an important data resource. It would be very valuable for ALS and for neurodegeneration research more widely.
- The pathway to therapeutic development is compelling. The proposal is built on a strong pipeline. The team's preliminary success with ASO validation of targets like CCDC146 and VAV2 demonstrates a proven workflow for translating genomic discoveries into therapeutic candidates.
- A successful outcome would establish a proof of concept for the proposed pipeline which could be applied to other neurological and eventually psychiatric disorders.
- This proposal is highly significant, as it pairs advanced profiling, analytic strategies, and validation approaches with human postmortem tissues and iPSCs to identify genetic drivers (and ultimately treatment targets) for ALS, a fatal, progressive neurodegenerative disease that is mostly sporadic (90% of cases) and with limited treatment options.



- The resulting profiling datasets will be a valuable, sharable resource.
- The stepwise study design leveraging multiomics profiling with machine learning to identify genetic ALS drivers, confirmation of biological relevance in iPSC lines, validation of hits in postmortem tissues, and in vitro assessment of therapeutic targeting potential positions the research well for rapid translation. The consideration of patient subtypes to inform applicability and engagement of consultants interested in therapeutic development further support translational potential.

Innovation: Evaluate the project for innovation relative to the current state of research

- Highly innovative; essentially would be a new framework for ALS genetics.
- The project employs state-of-the-art technologies and dry-lab approaches. The level of integration across genomics, computational biology, and stem cell modeling is exceptional.
- It also innovates by focusing on non-traditional genetic variation by focusing on tandem repeats which have proven relevance in ALS.
- The therapeutic validation strategy is innovative in its scope. Using a large panel of familial and sporadic ALS iPSC lines to test ASO candidates ensures findings are not restricted to a single genetic subtype, enhancing translational potential.
- Single nucleus profiling provides important insight into cell-type contributions.
- The combination of a range of innovative approaches and expertise, including advanced multiome and single nucleus profiling, machine learning, ex vivo/iPSC-based biological verification, and validation of therapeutic targeting potential for lead candidates, addresses a critical need in the field to enhance mechanistic insight and identify novel treatment targets.
- The proposal pairs innovative stem cell and genetic research strategies to advance insights, validate findings and biological relevance, and set the stage for translation of novel treatments.

Rationale: Evaluate the scientific rationale in the proposal

- Strong rationale because most ALS is sporadic with substantial heritability, but few genes/targets are known; mechanisms are only partially characterized.
- The hypothesis that deep single-cell profiling combined with genetic modelling will uncover cell-type-specific drivers of sporadic ALS is strongly supported by the team's own preliminary data.
- The rationale for specific experimental choices is generally strong but has gaps. The choice of four brain regions (motor cortex, spinal cord, frontal cortex, occipital cortex) is rationalized by comparing disease epicenters to "upstream" or less-affected regions to understand disease progression. However, the choice of the occipital cortex as a control region is not justified, and the risk of low neuronal yield from some regions is not addressed.
- Feasibility and access to key resources are of key importance. The rationale depends entirely on acquiring 200 high-quality samples from 50 donors from two biobanks. Access and availability are subject to official review.
- The decision to use short-read instead of long-read WGS for the 50 donor genomes is a weakness. For a project emphasizing tandem repeat biology, long-read sequencing is the gold standard for accurate genotyping. The cost difference is marginal relative to the overall budget, and its absence undermines the quality of the core genomic dataset.



- The rationale is strong and leverages leading omics and data science to uncover potential promising targets for continued validation and therapeutic targeting. The premise of validating in patient tissues supports biological relevance, while perturbation analyses and ASO-based rescue experiments in human iPSC models support functional implications and therapeutic potential.
- The preliminary data support the ability to glean meaningful advances using the proposed study design steps, which will be led by leaders in the respective areas.
- The profiling and validation in human tissue, along with the selection of a range of human iPSC lines and cell types (neuron, astrocyte, microglia), will support the ability to glean meaningful results that will be relevant to the ALS community as a whole, while also providing a foundation for similar applications for other neurodegenerative diseases.

Plan & Design: Evaluate the project plan and design

- The end-to-end pipeline is strong and logical.
- Experimental design is comprehensive and quite ambitious in parts. The aims build upon one another. However, the scale of computational modeling (Aim 2b) and functional screening (Aim 3) is immense.
- Power calculations are essentially absent. For example, the n=50 for multi-omics discovery is based on consultation with Target ALS. The statistical methods proposed for tissue validation (Aim 4) appear limited (t-tests, ANOVAs) and do not consider critical covariates.
- Team expertise, leadership, and resources are outstanding. The investigators are all leaders in their fields with complementary, world-class expertise in genomics, machine learning, stem cell modeling, tandem repeat genetics, and ALS neuropathology. Institutional resources are appropriate.
- This reviewer could not find information on data access routes/agreements for sources from other PI/consortia and sparse information on agreements or routes for accessing biological materials.
- There is little information on how the VMIS score is going to be developed, lacking specifics on model integration, benchmarking, and validation. Developing a useful variant score is a very challenging undertaking so the lack of detail and dedicated time and resource for this questions the feasibility of this objective.
- The applicants are leaders in their respective areas of research and roles for the proposed project, and the feasibility of the proposed work is well supported by the preliminary data and prior work of the investigator teams.
- The stepwise study design leveraging multiomics profiling with machine learning to identify genetic ALS drivers, confirmation of biological relevance in iPSC lines, validation of hits in postmortem tissues, and in vitro assessment of therapeutic targeting potential positions the research well for rapid translation. The consideration of patient subtypes will enhance applicability and the engagement of consultants interested in therapeutic development further support translational potential.
- The iPSC lines reflect a range of ALS sub phenotypes by including control, sporadic ALS, and lines with multiple known genetic mutations and by focusing on different cell types (neuron, microglia, astrocyte).
- The proposal is ambitious and well-designed to acquire multi-level evidence based on human tissues, cell models, and validation and initial therapeutic testing. Its focus on multiple ALS subtypes, multiple tissue regions, and multiple cell types increases potential applicability across the field.
- Despite the high potential impact of this proposal, a more refined strategy focusing on brain regions that will be most informative (are novel results anticipated for the occipital cortex or could that be removed?)



would allow for a more thorough, focused investigation while keeping costs similar and enhance likelihood of success.

- Questions: Given that the outcome is to identify genetic drivers associated with ALS risk and severity, how will clinical features be integrated and how are they expected to affect findings? What is the anticipated impact of onset segment (note: proposal indicates “favor limb onset over limb onset” so effect of onset segment is not clear and relevance to design is not discussed). Are differences expected given that postmortem tissues likely all represent late-stage disease?
- Is FTD status known for participants selected for Aims 1 & 2, and how is this expected to affect the analysis results for the different tissues (e.g., frontal cortex)?

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations

- Broader population impact via open science is very strong.
- The proposal fails to address the demographic composition of the crucial 50-donor post-mortem cohort or the existing datasets (e.g. Project MinE). This omission significantly limits the generalizability of the findings.
- Outreach and partnership components are minimal.
- The initial aims are designed to identify genetic drivers of disease and account for factors, such as chromatin accessibility and functionally relevant SNPs, STRs, genes, and enhancers, using samples from controls, C9 ALS, and sporadic ALS participants to support applicability across those with ALS. The final aims are designed to validate the identified targets and design ASO-based compounds targeting these hits to support translation.
- The cohort is designed to capture the spectrum of ALS sub phenotypes, therefore reflecting the diversity of the population, but the small cohort size may not allow complete representation of external factors (e.g., may not capture variability due to environmental/exposures present in other populations/regions or that may emerge in a larger cohort) and little discussion is included on how the sub phenotypes will be considered in the analyses (impact on power?) or impact expected outcomes.
- The iPSC lines reflect a range of ALS sub phenotypes by including control, sporadic ALS, and lines with multiple known genetic mutations and by focusing on different cell types (neuron, microglia, astrocyte).
- Translational aspects of the proposal and letters of support reflect partnerships that will support future translation.



Application#	DISC4-19196
Title (as written by the applicant)	Reprogramming the Spatial Transcriptome for Precision Neurodegenerative Therapies
Project Objective & Impact (as written by the applicant)	In neurodegenerative diseases like ALS, RNA molecules often end up in the wrong place inside cells, but no one knows how to rescue disease by targeting RNA to the right places. We build powerful new tools to track and fix RNA misplacement, uncover how it harms neuronal cells, and pave the way for a new kind of treatment called “spatial RNA medicine” for ALS and other neurological disorders.
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> • Decode disease-specific spatial transcriptome disruptions in human neurons and glia for ALS patient vs. healthy control hiPSCs and in postmortem tissue. • Alter RNA localization to establish causal links to cellular function via high-throughput screening and high-content imaging in ALS patient hiPSC-derived models. • Understand mechanisms linking mis-localized RNAs to ALS. Map altered RNA-protein interactions and investigate how aberrant splicing and RNA modifications drives mislocalization in ALS. • Validate spatial RNA control for disease phenotype rescue. Use AAV-CRISPR-TO to validate targets in vivo and engineer UTRs to rescue neurodegeneration in ALS hiPSC models.
Statement of Benefit to California (as written by the applicant)	California's 39 million residents represent vast genetic and ancestral diversity. This diversity poses challenges, but also opportunities for ALS research. Our project embeds this diversity from inception, ensuring findings will be relevant to all Californians. Using diverse hiPSC lines and patient tissues, we will identify convergent spatial RNA pathologies and potential therapeutic targets, creating a generalizable, mutation-agnostic platform for ALS and related neurodegenerative diseases.
Funds Requested	\$14,000,000
GWG Recommendation	(85-100): Exceptional merit and warrants funding, if funds are available
Process Vote	<p>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.”</p> <p>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”</p>

SCORING DATA

Final Score: 85

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

Mean	82
Median	85
Standard Deviation	4



Highest	86
Lowest	70
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	8
(1-84): Not recommended for funding	6

FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

<p>Key Strengths and Weaknesses</p> <ul style="list-style-type: none"> • Strengths: Great team, innovative approaches, potential to yield data of wide interest to the research community. Weaknesses: Wider relevance of RNA mislocalization to disease unknown; may be difficult to correct if mistargeting is widespread in ALS. • The application seeks to identify actionable therapeutic targets specifically in spatial mislocalization of RNA. • There is much interdependency within the aims - in particular, Aims 3 and 4 depend on findings from Aims 1 and 2. Preliminary data for ALS specific RNA mislocalization would be helpful to assess feasibility. • The investigators mention an AI-guided spatial medicine machine learning model trained to predict UTR motifs but insufficient detail on these plans is provided.
<p>Significance: Evaluate the project's significance and potential for impact</p> <ul style="list-style-type: none"> • Successful completion of this project would significantly advance understanding of human disease by defining the causal role of RNA localization in neuronal health and neurodegeneration, with a particular focus on ALS, a disorder that currently lacks well-validated and actionable therapeutic targets. By moving beyond descriptive transcriptomic profiles, this work establishes the spatial transcriptome as a dynamic and manipulable system that directly influences cellular function and survival. • The investigators aim to deliver an accessible data browser containing a comprehensive, subcellular-resolution spatial transcriptome of ALS. They will also generate ready-to-use hiPSC-derived neuronal and glial lines engineered with CRISPR-TO machinery. The integrated technology platform, combining programmable RNA localization with high-resolution subcellular RNAseq, represents a pipeline that can be readily applied to other neurodegenerative diseases characterized by RNA dysregulation. • The likelihood that a successful outcome of this project would rapidly advance new therapeutic or biomarker development is high. The proposal is explicitly designed to move beyond discovery toward actionable outputs by combining mechanistic studies with early-stage validation of therapeutic strategies. Aim 4 focuses on validating high-priority targets in a neurodegenerative mouse model and identifying UTR-zipcoded spatial RNA medicine candidates to optimize therapeutic RNA delivery.



- The investigative team has a strong track record of translating academic discoveries into clinical-stage programs and plans to leverage expertise from the institution's laboratory for cell and genetic therapy in regulatory strategy and process development to define a clear path toward IND submission.
- Subcellular atlas of RNA localization will provide an important knowledge base for neuroscientists. The aspect of RNA biology is poorly understood but is likely to be of great importance.
- Work may lead to new therapeutic approaches to modify RNA mislocalization in Aim 4. The concept of therapeutics based on correction of RNA localization is innovative.
- ALS represents a major public health burden, with no curative therapies and limited understanding of core disease mechanisms.
- There is broad consensus that disrupted RNA metabolism, including aberrant RNA processing and spatial mislocalization, is a central feature of ALS biology.
- The proposal aims to identify actionable therapeutic targets specifically related to RNA spatial mislocalization, rather than genes merely correlated with ALS pathology.
- A key strength is the focus on causality, seeking to determine whether RNA mislocalization directly contributes to neurodegeneration.
- Therapeutic realignment of RNA and development of "RNA medicine" is a novel approach.
- Despite strong evidence implicating RNA mislocalization in ALS, its precise causal role remains debated. While this is the driving reason for this proposal RNA mislocalization often emerges alongside other convergent processes such as proteostasis failure, impaired nucleocytoplasmic transport, and mitochondrial dysfunction thus making it difficult to determine whether it represents an initiating driver of disease or a downstream amplifier of neurodegeneration.

Innovation: Evaluate the project for innovation relative to the current state of research

- Traditional omics approaches tell which genes are expressed, but leave a critical gap in understanding where RNA transcripts actually function within cells and how their misplacement contributes to disease. This proposal offers a new way of thinking about RNA localization, moving it from a passive observation to a programmable and druggable feature.
- The project introduces a new therapeutic approach - spatial RNA medicine. This includes engineered RNA medicines (e.g., therapeutic mRNAs with custom-designed UTRs to guide them to cell locations where they are needed most) and methods to correct or manipulate naturally occurring pathological RNAs to restore proper function. By combining fundamental insights into RNA behavior with targeted intervention, this work offers a novel framework for studying and treating neurodegenerative diseases.
- This project uses an interdisciplinary approach, integrating human iPSC-derived neurons and glia, neuron-glia-astrocyte tri-cultures, post-mortem tissue analysis, high-res spatial transcriptomics, programmable RNA perturbation, in vivo disease models, and advanced computational analytics. These technologies are integrated into a single, iterative platform, where mapping, functional perturbation, mechanistic exploration, and therapeutic validation continuously inform and strengthen one another.
- Several innovative stem cell and genetic research approaches are employed, including CRISPR-TO, an inducible system that targets specific RNAs to defined subcellular compartments, high-resolution APEX-seq and MERFISH to achieve subcellular spatial transcriptomics at nanometer to sub-micron resolution, and AI-guided spatial medicine engineering to predict UTR motifs that direct transcripts to specific cellular compartments.



- RNA localization in disease has not been systematically addressed beyond the paradigm of SMA.
- Clinical input, cell biology and bioinformatics approaches are integrated into the proposal.
- APEX-seq and CRISPR TO are innovative. Spatial RNA therapeutics are all new technologies. 3D neuron astrocyte microglia models are not totally innovative, but are state of the art.
- The proposal seeks to establish a direct causal link between RNA localization and disease-relevant functional outcomes, moving beyond descriptive studies.
- The concept of spatial RNA medicine, in which therapeutic interventions realign RNAs to appropriate subcellular compartments, is novel.
- The use of CRISPR-Transcript Organization (CRISPR-TO) is a major innovative component, enabling programmable repositioning of endogenous RNAs and direct experimental testing of RNA localization–function relationships.
- Application of CRISPR-TO to ALS-relevant RNAs is particularly exciting and innovative.
- The proposed “Decode–Perturb–Explain–Validate” framework is logical and appropriate but is not itself highly novel.
- The primary innovation lies in the biological questions and experimental tools.

Rationale: Evaluate the scientific rationale in the proposal

- The proposal’s foundation relies heavily on the assumption that RNA mislocalization is a hallmark and driver of ALS pathology. A more solid evidentiary basis would have strengthened the case for the study’s approach.
- The rationale is robust and ambitious. hiPSC neurons, glia, and 3D co-cultures ensure physiological relevance and enable targeted genetic manipulation. CRISPR-TO allows for programmable endogenous RNA repositioning as a unique platform to interrogate spatial function at subcellular resolution. Complementary high-res APEX-seq and MERFISH enable precise RNA mapping, while proximity labeling and isoform-aware analytics reveal mechanistic links between RNA-binding protein mutations and pathology.
- The preliminary data is impressive and show the team’s expertise in deploying cutting-edge techniques and constructing physiologically relevant neural co-cultures.
- The preliminary data, however, primarily establish technological feasibility rather than directly addressing RNA mis-localization in ALS. There are no data confirming the existence or relevance of RNA spatial defects in ALS models. For a proposal centered on elucidating the causal role of RNA mis-localization in ALS, more direct preliminary evidence in this specific context would be valuable to justify the experimental strategy.
- Focus on ALS and the role of RNA mislocalization - in principle this could be important but there is no evidence that it is key to neurodegeneration. The example of SMA may be unusual. Is a single mRNA likely to be key, as for b-actin, or is this a rare case?
- SMA provides precedent for the concept and it is clear that there is aberrant RNA processing in ALS. RNA mislocalization in astrocytes or microglia has not really been investigated.
- Preliminary data in the retina shows that CRISPR TO can redirect beta actin mRNA to the perinuclear region resulting in photoreceptor dysfunction. Preliminary data to validate APEX-seq is strong (proximity



label of RNA) but not yet validated for many target areas- presynaptic terminal post initial axon segment. The RNA fluorescent reporter does not seem to be validated extensively but is key to the screen.

- 3D stem cell models with disease mutations and isogenic controls are appropriate.
- It is well established that RNA mislocalization is a major etiological feature of ALS.
- The investigators propose 1) profile RNA mislocalization using spatial transcriptomics in postmortem ALS brain tissue and 2) extend these analyses to hiPSC-derived neuronal and glial models from donors.
- This approach is well suited to identifying disease-associated spatial RNA signatures.
- The proposal includes functional testing of mislocalized RNAs in neurons and glia.
- Mechanistic studies are planned to identify pathways affected by mislocalized RNAs in both In vitro cellular systems and In vivo mouse models.
- The progression from discovery to functional and organismal validation is conceptually strong.
- However, there is substantial interdependency among aims especially for Aims 3 and 4 which depend on findings from Aims 1 and 2.

Plan & Design: Evaluate the project plan and design

- Aims 1 and 2 are well-developed, with clear experimental design and anticipated outcomes.
- Greater detail would strengthen the rationale and feasibility of Aims 3 and 4. Aim 3 is somewhat difficult to follow and lacks sufficient detail to distinguish it from Aim 2. Clarification on its unique methodological approach would be beneficial. For Aim 4, additional information on the in vivo mouse model is needed, such as the number of animals to be used, their age at treatment, whether both sexes will be included, and the use of an appropriate control group.
- Questions: It is unclear what subcellular compartment the candidate RNA will be targeted to – is it based on the subcellular localization of the RNA in control cells in Aim 1? How many candidate RNAs will be tested? In the anticipated results, the proposal suggests that repositioning a subset of in vitro candidates may result in improved or worsened behavioral outcomes. Is the goal of the aim therapeutic correction or simply to observe a biological response?
- Potential pitfalls and alternative approaches are adequately addressed; however, a detailed timeline for the project is missing, making it difficult to evaluate the budget and feasibility.
- The PI and Co-I's are clearly leaders in the field and well-qualified to execute the proposed studies. Roles and responsibilities are clearly described, with defined procedures for meetings, decision-making, conflict resolution, and publication. The inclusion of a central Project Manager adds valuable oversight and coordination to the effort.
- The project assumes RNA localization in post-mortem tissue reflects living state, which may well be wrong, and the proposal does not address this issue. End stage disease relevant to pathogenesis?
- AI technology to query 3'UTR function may or may not work and changing 3'UTR might affect multiple aspects of RNA processing
- Do trilineage organoids show appropriate relationships between cells? Does TDP43 loss affect so many RNA that it will be impossible to correct with a few changes?



- Preclinical assessment of RNA redirection using functional rescue experiments could provide proof of concept for the therapeutic approach.
- This is an outstanding team of technical innovators with great achievements and relevant experience. Good project management and technical staff.
- Communications within project groups and across them on a regular basis. Budget and timeline appropriate.
- The overall methodological rigor of the proposal is high across Aims 1-4.
- Use of spatial transcriptomics is particularly strong for identifying precise subcellular RNA localization.
- Functional assays are appropriately planned in both neuronal and glial cell types.
- The proposal mentions AI-guided spatial medicine approaches, including machine learning models to predict UTR motifs associated with RNA localization. However, insufficient methodological detail is provided to fully evaluate the AI component.
- The postmortem brain cohort is relatively small (n = 10), though typical for spatial transcriptomic studies.
- No explicit project timeline is included, limiting assessment of feasibility.
- Demographic, clinical, and genetic background information for the cohort is not provided.
- Investigators are organized into working groups, but the role of the Data Working Group across specific aims is not clearly defined.

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations

- The project proposes using hiPSCs with three genetic ALS backgrounds (C9orf72, TARDBP mutation, SOD1 mutation); however, the study could be further strengthened by incorporating additional models, such as sporadic ALS and FUS mutation. Additionally, while the proposal mentions the use of postmortem patient tissues, it does not provide demographic details about the tissue bank, making it difficult to assess whether the samples are diverse and representative of the broader ALS community.
- The developed tools and platforms (e.g., spatial transcriptomics, programmable RNA repositioning), form a generalizable pipeline that can be extended to validate findings across populations and neurological diseases. This design strategically positions the research for far-reaching impact not only for ALS, but also across a broad spectrum of diseases characterized by RNA mis-localization, such as Huntington's disease, spinal muscular atrophy, and select forms of Alzheimer's disease.
- The team's strengths are further underscored by a demonstrated track record of partnership, innovative educational outreach, and inclusive engagement, making science accessible to students, researchers, and community members. Long-standing relationships with patient and clinical organizations reinforce this commitment, ensuring two-way communication and real-world relevance in both the design and dissemination of research findings.
- Genetically diverse cell models will be used.
- Designed to extend to different forms of ALS and FTD.
- Good track record of outreach and education in clinical and basic science.



- The proposal is designed to address ALS broadly, encompassing both genetic and sporadic forms.
- The CRISPR-TO–based strategy has the potential to be applicable across multiple ALS subtypes.
- The work is not limited to a single mutation or pathway, increasing potential impact.
- Investigators demonstrate strong engagement in public outreach related to regenerative and translational neuroscience.
- If successful, the project could enable a new class of spatially targeted RNA therapeutics relevant to ALS and potentially other neurodegenerative disorders.



Application#	DISC4-19226
Title (as written by the applicant)	The immune system of the human brain: A platform for neuroimmunotherapies
Project Objective & Impact (as written by the applicant)	Immune signals (cytokines) maintain health and drive dysfunction in the brain; how they do so is largely unknown. Our team will systematically dissect how cytokines influence human brain cells and test groundbreaking, cytokine-based therapeutic strategies to reverse neurodegenerative diseases such as Alzheimer's Disease (AD).
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> • Create a cytokine response dictionary that defines how cytokines impact the transcriptome and proteome of neurons, microglia, astrocytes, and other key cells in the human brain. • Investigate how cytokines shape specific functional states of neurons and microglia using human stem-cell-derived models and ex vivo brain tissue slices. • Study the impact of aging on neuroimmune interactions, focusing on how aged neurons and blood-brain barrier cells influence immune cell function and contribute to neurodegeneration. • Evaluate how human genetic variability (e.g., APOE genotype) and prior immune challenges, such as viral exposures, alters human microglial responses to cytokines. • Develop gene and cell therapy platforms for targeted delivery of cytokines to neurons and microglia in the brain. • Engineer and transplant human microglia optimized for efficient amyloid clearance and neuroprotection into humanized AD models.
Statement of Benefit to California (as written by the applicant)	Alzheimer's Disease (AD) is (i) a major (and growing) cause of disability and death in people over the age of 65 and (ii) costs families and health systems in California tens of billions of dollars a year. The proposed work builds on new, encouraging preliminary data that cytokines - signals released from immune cells - can reverse several amyloid pathologies in the brain that contribute to AD. Successful cytokine-based therapies could prevent or slow AD progression in >700,000 Californians.
Funds Requested	\$14,000,000
GWG Recommendation	(85-100): Exceptional merit and warrants funding, if funds are available
Process Vote	<p>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."</p> <p>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."</p>

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	80
Median	85
Standard Deviation	10
Highest	88
Lowest	-
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	8
(1-84): Not recommended for funding	6

FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

Key Strengths and Weaknesses

- There are major concerns due to the lack of power calculations and the absence of key information on experimental designs.
- The approach in Project A, using human brain slices treated with cytokines and profiled by multiomics, is quite innovative and has the potential to generate very useful foundational data on transcriptomic and proteomic responses to cytokine treatment in the human brain. Additionally, the use of AAV vectors and engineered T cells as therapeutic delivery systems in Project C is an innovative translational strategy that could open new avenues for immune-based interventions in AD.
- However, there are significant concerns regarding statistical rigor, particularly issues of pseudoreplication in Aim 1, insufficient sample size justification across many of the 18+ proposed assays, feasibility of data integration in Aim 2, and poor coordination of measurements across the three projects. These issues may dampen the quality and utility of the data generated by this grant.
- Overall, this is a scientifically ambitious proposal that addresses critical knowledge gaps in neuroimmunology and neurodegeneration and is highly significant to the field. While much of the broader scientific rationale and tool development is supported by preliminary data or prior publications, the scope and nuance of the questions being addressed, along with the analytical approaches proposed, raise concerns about feasibility and interpretability within the proposed timeframe.

Significance: Evaluate the project's significance and potential for impact

- Project A is intended to generate foundational datasets for understanding the effects of cytokines on molecular profiles and cellular functions in a human context. If successful, these datasets will be useful for interpreting data from Project B and C and other external snRNA-seq data from AD brains. However, a notable limitation is that the selection of cytokines investigated in this study is quite limited.
- Project B investigates how environmental, genetic, and aging factors influence microglial responses and function, examining the ways in which these factors shape microglial biology in the context of AD.



However, except for the Census-seq, the other components will not provide clear causal factors that could translate into actual drug targets for AD directly.

- Project C aims to develop therapeutic delivery systems using AAV vectors and engineered T cells to target the brain. The investigators particularly focus on IL-33, a molecule they have studied. If successful, the efficacy of IL-33 could potentially be tested in a clinical setting in the future.
- With immunotherapies gaining momentum in the treatment of brain disorders, the application is timely and well-positioned to capitalize on emerging insights into cytokine-mediated regulation of brain resilience and disease.
- Addresses an important knowledge gap by linking cytokine exposure to defined cellular states. Despite the identification of many microglial transcriptional states through large-scale sequencing, it remains unclear whether these states arise from intrinsic programs or environmental signals, and what their functional consequences are. Cytokines represent strong candidates, and this resource will be helpful to the field.
- Genetic studies strongly implicate immune-related genes in AD neuropathology. This study integrates several emerging mechanisms that are relevant to immunological pathology of AD (and other neurodegenerative diseases), including cytokine signaling across brain-resident and border-associated cells, immune–stromal interactions, and the functional consequences of genetic risk, with additional exploration of immune memory.
- This proposal examines the immune system as an important explored path for therapeutics in AD that deserves further attention.
- The time is right to explore because of significant advances in single-cell omic technologies, advances in AAV and cell therapy platforms, and the ability to capitalize on resources and lessons learned from immunotherapies in other disease areas.
- Strength: they are exploring three independent therapeutic modalities – AAV mediated gene therapy, engineered T cell therapy, and microglial replacement. Helps to hedge against failures in a single approach, but is extremely ambitious, thereby reducing feasibility.
- Weakness: the in vivo testing relies on only the 5xFAD mouse. They may want to consider including other models that replicate other pathophysiologies to increase translational relevance.
- Regardless, there will be incredible resources generated. The human brain cytokine ‘response’ dictionary and systematic characterization of cytokines’ effects on neuronal function will generate valuable and broadly useful community resources. It will be important to ensure that these are deposited in public repositories and that they adhere to FAIR principles.

Innovation: Evaluate the project for innovation relative to the current state of research

- A notable innovation is the ability to investigate transcriptomic responses in ex vivo human brain slices in a cell-type-specific manner. If established, a comprehensive catalog of gene signatures induced by cytokines would be very useful for drug discovery.
- Census-seq with 50 iPSC lines is an innovative approach to find causal factors for cellular phenotypes under various conditions (cytokine challenge). The result could help interpret GWAS findings for AD and target discovery.
- While the grant lists machine learning as an innovation, the approaches used are very standard, and there are no particularly innovative components. Additionally, it's not clear that the model developed will be used in any of the aims.



- The use of AAV vectors and engineered T cells as therapeutic delivery systems in Project C is an innovative translational strategy that could open new avenues for immune-based interventions in AD.
- Applies cutting-edge technologies to address emerging questions and knowledge gaps, including human ex vivo tissue assays (Aim 1), curated age- and disease-relevant iPSC models (Aim 3), cell “village” and xenotransplantation systems (Aims 4–6), and innovative brain-targeted immunotherapeutic strategies such as AAV-mediated cytokine delivery and brain-targeting T cells (Aim 5).
- Strong potential to generate high-value datasets with broad utility for the field, including the proximity proteomics data for the 'neurotransmission proteome' (Aim 2), a human cytokine interactome for brain-resident cells (Aim 1), and extensive sequencing datasets that will serve as a valuable community resource.
- While microglial xenotransplantation has been proposed and tested in general and AD-specific rodent models (e.g., PMID: 37541210), the development of brain-targeted T cells and modified microglia offers a particularly innovative and promising proof-of-concept approach in brain immunotherapy.
- The cytokine responsive concept for human brain cell types is quite exciting.
- The combination of single-nucleotide RNA sequencing with proximity proteomics in ex vivo human slices is quite novel.
- The adaptation of synthetic biology approaches – synNOTCH receptor that sense amyloid or brain-specific antigen and locally deliver cytokine payloads is very cool.
- Strength: the multimodal integration across scales although there's not much discussion of the challenges of applying ML to relatively small datasets. The integration of these datasets will be its scientific exploration in its own right.

Rationale: Evaluate the scientific rationale in the proposal

- Individual aims address relevant interesting questions regarding immune response in the context of AD models. However, the overall grant lacks a coherent scientific rationale for bundling these disparate projects together. The proposal reads as loosely related studies rather than a unified research program with clear synergistic potential.
- For example, in Aim 3.1 (Project B), it appears that the cytokines in SASP candidates do not overlap well with the cytokines investigated in Project A. How will the results deliver synergistic findings with those from Project A and Aim 3.1? In general, these foundational datasets from Project A are rarely utilized in a meaningful way to interpret the data from Project B and C.
- As another example, within Project A, Aim 1 generates transcriptomic/proteomic data from human brain slices treated with >10 cytokines, while Aim 2 only tests five cytokines of them in iPSC co-cultures. To assess the translatability of the co-culture system, the same set of cytokines should be used in both aims. Notably, Aim 2 does not include IL-33 among its tested cytokines, despite IL-33 being the central therapeutic molecule in Project C, which is a significant missed opportunity.
- This is also related to a lack of synergy in the study design, but it is not clearly defined how new cytokines identified in Project A and Project B will inform Project C.
- The proposal inadequately incorporates extensive molecular findings from actual AD patients, particularly the wealth of data available through initiatives like AMP-AD. This represents a critical missed opportunity to ground experimental design in human disease biology and to provide a framework for interpreting results from AD models. For example, cytokine selection should utilize knowledge from snRNA-seq and proteomics data from AD brains.



- The approach is strongly supported by both the investigators' previous and ongoing studies, as well as within the broader field. Existing epidemiological, genetic, and functional data underscore the significance of the questions addressed in this proposal.
- Investigators bring extensive expertise and a strong track record in neuroimmunology, neurodegeneration, neurobiology, and genetics. Their prior work demonstrates that cytokines are both biologically relevant to neurodegenerative pathophysiology and promising therapeutic targets.
- The PI and co-Is have an outstanding track record and compelling preliminary data demonstrating both tool development and its utility in addressing these complex questions. The proposed experiments are ambitious and complex; while likely to generate valuable results, feasibility remains a potential concern.
- Strong rationale supports human-focused studies of cytokine biology, as rodent models do not fully recapitulate human immune responses. Evidence shows that cytokines influence non-immune brain cells, such as neurons and astrocytes, with important effects on brain function and behavior.
- Numerous previous studies, particularly in late-onset AD and GWAS loci have implicated the immune system in AD.
- They have good data to support the technical feasibility of their proposed methods. These include maintaining ex vivo human brain for over 10 days (Fig. 2), functional neuronal surface proteomics (Fig. 3), cytokine-induced changes in MEA network activity (Fig. 8), and evidence of the microglia village system recapitulating features of in vivo states (Fig. 13).
- The proposal relies heavily on iPSC-derived cell types including iMicroglia, iNeurons, and iAstrocytes. The known limitations and caveats are discussed with the strategy of incorporating ex vivo slices, and xenotransplantation is a good cross-validation approach.

Plan & Design: Evaluate the project plan and design

- Aim 1.1 appears to treat each nucleus as a separate sample; doing so would lead to pseudoreplication and, potentially, false discoveries. Even with a stringent p-value cutoff, this approach is insufficient; more replicates and a pseudo-bulking approach are necessary. Consequently, establishing a reliable and comprehensive 'cytokine responsive' dataset will be challenging.
- From what is described in Aim 2.2.1 and Aim 2.2.2, it will be difficult to align the data to create a unified dataset for machine learning (e.g., different time points between 2.2.1 and 2.2.2). They mentioned augmenting data points but provided no details on how to do it.
- There are at least 18 experiments planned throughout the grant, but only half of them mention sample size and even fewer are accompanied by formal power analyses. This is concerning for the study's ability to detect meaningful differences and produce statistically robust conclusions.
- Some aims will conduct similar phenotyping batteries, for example, Aims 2.1, 2.2, and 3.1. However, these appear to be performed independently without coordination protocols. This lack of standardization and cross-aim communication will limit the ability to reconcile and compare results. A similar issue of uncoordinated readouts is present in Aim 5.1 and Aim 1.
- Leadership and teams have the appropriate expertise and resources for conducting the proposed research. The budget and timeline are appropriate.
- Resources and environmental support are outstanding. Investigators have access to extensive scientific and clinical infrastructure, both through the framework of this proposal and within their home institutions.



- Potential pitfalls and alternative approaches are described; however, some key technical and experimental considerations are not fully addressed (see numbered list below). These could be reasonably integrated into the existing experimental framework.
- A key concern with amyloid-targeting therapies is off-target effects, including ARIA. Because this proposal similarly targets extracellular amyloid-laden structures (such as plaques and vasculature), similar adverse effects could occur, yet this is not addressed. The investigators should consider monitoring for ARIA and assessing vascular changes in animals undergoing xenotransplantation or other therapies.
- Similarly, endothelial cells respond to various cytokines, including IL-33, but are not explicitly examined in this proposal. Data collected from existing experiments will likely provide information, but additional studies examining the impact of cytokines and therapies on vasculature should be considered, especially given the role of vasculature dysfunction in AD.
- The proposal does not fully address key aspects of cytokine use, including concentration, duration, and baseline levels, which are critical given the potential for biphasic responses that could produce opposite effects. Important details such as the tissue or culture half-life of cytokines, their source, and the influence of cell-type-specific posttranslational modifications are also unclear, raising concerns about reproducibility and interpretation of results.
- Potential feedback regulation of cytokine signaling is also not discussed. For example, IL-33 can elevate the expression of soluble ST2, a scavenging receptor that limits its own activity, which could confound interpretation.
- Transplantation experiments are planned in immunodeficient rodent models, but the effects of this background on the neuroimmune mechanisms under study should be considered. While appropriate for proof-of-concept, future work may benefit from humanized models or immunocompetent rodents with clinically relevant immunosuppression.
- As with all the proposals, these are inherently expensive and time-intensive experiments. There are some aim dependencies (Aim 6 microglial replacement) and the scope is extremely ambitious.
- The project will be conducted by a world-class team with the right expertise in their respective areas. Given the sheer scope of the proposal, more thought could have been taken to articulate a detailed management structure, communication plans, and milestone decision points.

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations

- The statement for the broad applicability of the findings is very generic. It merely mentions that the findings could extend to other brain diseases with immunocomponents.
- The grant plans to receive feedback from patients through clinicians, but it appears that patients' voices have not been reflected in the current study design.
- Addresses genetic and environmental heterogeneity and how underlying genetic risk variants may impact therapeutic approaches. Specifically, the experiments described in Aim 4 aim to investigate genetic and environmental factors that influence microglial pathology in AD.
- As age is a significant risk factor for AD, maintaining age-related epigenetic profiles is an innovative and potentially impactful strategy (Aim 3). Although its influence on experimental outcomes is not yet known, this approach directly addresses a key limitation of iPSC-based systems in studying age-related diseases.
- Applicants leverage the CIRM AD collection containing APOE genotypes (2, 3, 4) across both sexes. This is important as APOE status is critical in determining whether patients may be at risk for developing severe off-target effects of anti-amyloid treatments.



- Epidemiological evidence links prior immune challenges, such as injury or infection, to increased AD risk via poorly defined mechanisms. This application uses an innovative xenotransplant system to model these interactions and assess effects on cytokine responses, addressing a key gap in how genetic risk (e.g., APOE genotype) and immune exposures drive pro-inflammatory neurodegeneration.
- While there are diverse tissue sources proposed in the application, there are no specified power calculations to detect ancestry specific effects in the cytokine response or genetic variability analyses.
- There is also very limited discussion of sex-specific effects despite AD disproportionately affecting women.



Application#	DISC4-19444
Title (as written by the applicant)	Utilizing advanced iPSC organ-chip models of ALS and AI technology to discover subtypes, biomarkers and novel therapeutic targets
Project Objective & Impact (as written by the applicant)	We do not know the cause of ALS and so have no target to develop new treatments. This proposal seeks to address this by using recent advances in iPSC derived motor neuron modeling of ALS, novel organ chip technology and AI to help discover subtypes of the disease through a new ReasonALS knowledge engine. This will allow new precision health drug trials for this lethal disease.
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> • Develop and scale isogenic spinal cord- and cortical organ-chips with an integrated complete neurovascular unit. • Integrative multi-omics discovery of ALS subtypes, biomarkers and therapeutic targets using the Answer ALS repository and iPSC-derived organ-chip models • New ALS patients will be enrolled and iPSC lines will be generated for correlation with clinical and postmortem data • Establishment of an AI based hub to enhance our ability to predict ALS subtypes and make the data accessible to the community
Statement of Benefit to California (as written by the applicant)	ALS is perhaps the most serious neurological disease leading to paralysis and death normally within 3 years and is devastating to everyone involved. This research is focused on discovering biomarkers and new causes of the disease using iPSC and engineering technology. This will provide employment for lead CA scientists, generate critical data to allow ALS research to progress in CA and ultimately could help the many patients in CA that are affected.
Funds Requested	\$13,000,000
GWG Recommendation	(1-84): Not recommended for funding
Process Vote	<p>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.”</p> <p>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”</p>

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	5
Highest	92
Lowest	70
Count	13



(85-100): Exceptional merit and warrants funding, if funds are available	3
(1-84): Not recommended for funding	10

FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

Key Strengths and Weaknesses
<ul style="list-style-type: none"> • Ambitious, well-resourced, compelling vision, but feasibility risks remain regarding whether organ-chip complexity will produce a reproducible subtype signal. • The proposal directly addresses a critical barrier in ALS research: the limited mechanistic understanding of sporadic ALS, which accounts for more than 85% of all ALS cases and lacks clear, known genetic drivers. By prioritizing sporadic disease, the study has the potential to substantially broaden current disease models that are heavily biased toward familial ALS. • The use of organ-on-a-chip systems represents a particularly novel and forward-looking aspect of the proposal. These models offer the potential to recapitulate key aspects of ALS-relevant cellular interactions and microenvironmental features that are not captured in traditional 2D culture systems. Their inclusion positions the project to generate mechanistic insights that may be highly relevant for future translational and clinical studies. • A potential concern arises from the preliminary bulk RNA-seq data, which show relatively limited transcriptional differences between patient-derived and control iPSC motor neurons in 2D culture. However, the investigators appropriately acknowledge this limitation and propose to address it through the use of single-cell and spatial transcriptomic approaches, which are more likely to resolve subtle, cell-type-specific, or state-dependent expression changes that may be masked in bulk analyses. • The development of a rapid autopsy program, in combination with collaborative partnerships, is an important asset. This effort is expected to reduce postmortem intervals, improve tissue quality, and expand access to high-value postmortem samples.
Significance: Evaluate the project's significance and potential for impact
<ul style="list-style-type: none"> • The proposal tackles a major ALS bottleneck: sporadic ALS. • There is a significant unmet need in sporadic ALS (85% of all ALS cases), and sequencing/omics of postmortem tissue will further strengthen the ALS data repository. The authors mention ALS subtypes that have been previously discovered, and it is unclear how this project will further build upon that understanding. • The omics data resource will be enormous and shareable for further evaluation of molecular pathways in ALS. The postmortem data would be especially useful. • There is a dearth of animal models for ALS. Organ-on-chip models could provide testable systems for efficacy studies.



- Overall, it remains unclear how the proposal would advance biomarker and therapeutic target discovery. The potential molecular pathways and signatures that could lead to disease, or any hypotheses regarding potential biomarkers, are not clearly articulated.
- The proposal directly addresses a critical barrier in ALS research: the limited mechanistic understanding of sporadic ALS, which accounts for more than 85% of all cases and lacks clear genetic drivers. By prioritizing sporadic disease, the study has the potential to substantially broaden current disease models that are heavily biased toward familial ALS.
- The investigators provide strong foundational support for the proposed work. Prior efforts from this group include the development of large-scale iPSC collections, access to postmortem tissue, and comprehensive genomic and transcriptomic analyses of ALS cases. These existing resources and demonstrated expertise significantly de-risk the proposed studies.
- If successful, the project has considerable potential to advance the field by identifying novel molecular pathways and disease subtypes underlying sporadic ALS. Such insights could inform new therapeutic targets and contribute to precision medicine approaches by linking molecular signatures to disease heterogeneity.
- While the scope of data generation is impressive, it remains somewhat unclear how directly these discoveries will translate into actionable therapeutic strategies. The proposal would benefit from a clearer articulation of how candidate pathways or molecular signatures might be prioritized for downstream functional validation or therapeutic development.

Innovation: Evaluate the project for innovation relative to the current state of research

- Integration of orthogonal omics and AI into subtype discovery is a strength.
- Postmortem and iPSC omics have been previously described, and ALS subtypes have been previously discovered in two separate New York Genome Center papers cited by the authors.
- There is technical innovation in the organ-on-a-chip models, and these could advance capabilities for testing therapeutic efficacy. However, potential efficacy endpoints are not well defined.
- The project proposes innovative stem cell-based organ-on-a-chip models, omics analyses, and machine learning-based computational models, which are largely novel.
- Although omics analyses of postmortem samples have been previously described, the scale of the data combined with novel analytical methods has the potential to uncover new targets.
- 10x xenium spatial data for postmortem samples are exciting.
- The application is innovative across multiple dimensions, including experimental platforms, scale, and integrative analytical approaches.
- The use of organ-on-a-chip systems represents a particularly novel and forward-looking aspect of the proposal. These models offer the potential to recapitulate key aspects of ALS-relevant cellular interactions and microenvironmental features that are not captured in traditional 2D culture systems. Their inclusion positions the project to generate mechanistic insights that may be highly relevant for future translational and clinical studies.
- The breadth of multi-omics profiling, including genomics, transcriptomics, chromatin accessibility, and proteomics, provides a comprehensive framework for dissecting disease mechanisms. This approach is well suited to uncover both mechanistic drivers and candidate biomarkers across different molecular layers of ALS pathology.



Rationale: Evaluate the scientific rationale in the proposal

- The authors state that they will be able to discover ALS subtypes in organ-on-a-chip models. Although these models are more mature than 2D models, iPSCs are epigenetically reset and do not contain the inflammatory microenvironment of ALS tissue. Transposable elements are deregulated by epigenetic changes in ALS, and the feasibility of detecting these changes in the Answer ALS repository has been shown.
- Overall, the scientific rationale, along with the preliminary data, is well developed.
- The proposal aims to correlate organ-on-a-chip and postmortem data, but the risk in this approach remains high, since these represent different microenvironments and likely different epigenetic profiles due to reprogramming.
- The scientific rationale and methodological rigor are strong. The experimental designs across Aims 1–4 are well justified and logically structured, with appropriate use of contemporary technologies to address the central hypotheses.
- A potential concern arises from the preliminary bulk RNA-seq data, which show relatively limited transcriptional differences between patient-derived and control iPSC motor neurons in 2D culture. However, the investigators appropriately acknowledge this limitation and propose to address it through the use of single-cell and spatial transcriptomic approaches, which are more likely to resolve subtle, cell-type-specific, or state-dependent expression changes that may be masked in bulk analyses.

Plan & Design: Evaluate the project plan and design

- Good design choices that directly address known shortcomings of 2D iPSC models.
- The design is well done. The organ-on-a-chip model is defined. The multi-omics approach is comprehensive and well defined. Postmortem data generation is well planned.
- No clear plan is described for testing a particular hypothesis, a defined set of genes or transcripts of interest, or stress response signatures that may tie discovered signatures to disease in organoids and postmortem tissue. It remains unclear how we will know whether organ-on-a-chip models are appropriate disease models for biomarker discovery or therapeutic testing.
- Timelines, budget, and expertise are appropriate.
- The preliminary data provided across aims convincingly demonstrate technical feasibility for tissue processing, sequencing, organ-chip implementation, and large-scale genomic analyses.
- The proposal clearly outlines expected outcomes, potential limitations, and alternative strategies, with a high likelihood of successful execution.
- The inclusion of a custom ALS-focused Xenium spatial transcriptomics panel represents a major strength, as it enables targeted validation of key findings within anatomically and cellularly defined contexts.
- The development of a rapid autopsy program, in combination with collaborative partnerships, is an important asset. This effort is expected to reduce postmortem intervals, improve tissue quality, and expand access to high-value postmortem samples.
- Given the large number of assays and datasets proposed, it is sometimes difficult to clearly distinguish between completed preliminary work and future data generation. While this does not constitute a major weakness, improved clarity in distinguishing these elements would enhance the readability of the proposal.



- The strategy for integrating proteomic data with transcriptomic and spatial datasets is underdeveloped. Although the investigators appropriately acknowledge known discrepancies between RNA and protein abundance, the proposal does not sufficiently describe how these differences will be analytically reconciled. Furthermore, while single-cell proteomics is mentioned, methodological details and integration strategies, particularly with spatial transcriptomic data, are lacking.

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations

- Explicitly incorporate environmental and social determinants.
- Sporadic ALS occurs in a heterogeneous population and provides an opportunity to recruit a varied population.
- The patient community and advocacy voice are represented.
- The autopsy program can be designed to increase diversity.
- Working closely with the Alpha Clinic to leverage their outreach programs is helpful.
- The biobank serves as a central resource for this application and represents a major strength, with over 1,200 individuals spanning diverse genetic, ethnic, and environmental backgrounds across California and the broader United States.
- Although recruitment efforts for underrepresented populations are currently limited, the investigators demonstrate a clear commitment to improving diversity within the cohort, which is essential for identifying broadly relevant disease mechanisms.
- The emphasis on single-cell and spatial profiling approaches is well aligned with the goal of extracting maximal biological insight from even modestly sized cohorts. Given the size of the biobank resource, the investigators are well positioned to identify molecular signatures associated with distinct ALS subtypes.
- The rapid autopsy program is likely to further enhance recruitment efforts and cohort diversity, while simultaneously improving tissue quality and downstream data reliability.



Application#	DISC4-19319
Title (as written by the applicant)	How does Parkinson's Disease (PD) impact brainstem and limbic system neurons outside the motor system?
Project Objective & Impact (as written by the applicant)	Degeneration of dopaminergic neurons has been extensively studied in Parkinson's Disease (PD), yet debilitating non-motor cognitive, autonomic and neuropsychiatric symptoms can be severe and even precede the classical motor symptoms by years. Here, we focus on developing stem cell models for understanding the pathogenesis of non-motor symptoms in PD and related forms of dementia.
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> Define cellular vulnerabilities and candidate resilience factors in select brain regions in PD and DLB. Develop and mechanistically characterize iPSC-derived neuronal models of non-motor symptoms in PD and DLB. Validate PD-associated cellular pathways and develop therapeutics to target disease pathology and protect vulnerable neuron populations.
Statement of Benefit to California (as written by the applicant)	This research will further our understanding of the underlying causes of Parkinson's Disease and Dementia with Lewy Bodies, which affect an estimated 93,928 Californians, their families, and caregivers. By using patient samples and cellular models, this project will guide new therapeutic strategies aimed at treating non-motor symptoms with the goal of improving quality of life for patients, and potentially extending to Alzheimer's disease, which is commonly characterized by Lewy body pathology.
Funds Requested	\$12,999,478
GWG Recommendation	(1-84): Not recommended for funding
Process Vote	<p>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."</p> <p>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."</p>

SCORING DATA

Final Score: 80

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

Mean	80
Median	80
Standard Deviation	1
Highest	81
Lowest	75
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	14



FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

Key Strengths and Weaknesses

- Strengths: Focus on non-motor PD symptoms, including dementia; AAV work directed at tropism; understanding the intrinsic biology of resilience and vulnerability as a route to therapy; generation of new protocols to differentiate neuronal types.
- Weaknesses: Over reliance on the KOLF line rather than a diverse iPSC set; lack of novelty in certain places (e.g.: TFEB); the focus on non-motor is welcome, but the uniqueness may be oversold.
- Overall, this is a relevant and potentially impactful project addressing a clear gap in the field, but with some technical and conceptual risks related to model development, lack of preliminary data and reliance on end-stage tissue.

Significance: Evaluate the project's significance and potential for impact

- The remit of the proposal is a focus on non-motor symptoms in PD, likely caused by dysfunction of non-dopaminergic neurons. These include depression, dementia, and memory impairment caused by dysfunction or death of neurons in limbic and cortical regions. Increased understanding of the cell biology underlying non-motor aspects of PD would be valuable.
- The development of new iPSC protocols to differentiate neurons from understudied regions of the brain, such as the amygdala, hippocampus, and hindbrain, would be very powerful. Of these, hindbrain developmental biology appears to be the most understood, and differentiation protocols are the most advanced.
- The project is very ambitious and will generate a very large omics dataset, which will be a valuable contribution to the field.
- Understanding why some neuron types are more or less vulnerable in Parkinson's disease would be significant.
- The pathophysiology of non-motor symptoms in PD and dementia with Lewy bodies (DLB) has not received as much attention and has not been studied extensively using human systems.
- The snRNA-seq dataset from PD and dementia with Lewy bodies patients will provide a unique database with information from the amygdala as well as other less-studied subcortical regions.
- It is unclear whether organoids will accurately model the pathological environment and basis of selective vulnerability in the adult PD brain.
- Neuroprotection (neuronal resilience) approaches have largely failed to translate into the clinic.
- Understanding the basis of cell-type-specific vulnerability would be significant.



- This project has a clear focus on understanding the non-motor symptoms—dementia, depression, hallucinations, sleep disorders, and orthostatic hypotension—in PD and dementia with Lewy bodies, for which there is a clear need for further study.
- These symptoms represent a major unmet clinical need, lack effective treatments, and the field also lacks good human cellular models that capture the neuronal populations most affected.

Innovation: Evaluate the project for innovation relative to the current state of research

- The new iPSC protocols to differentiate neurons for the amygdala, hippocampus, and hindbrain are highly innovative. These protocols can be shared and would be a valuable contribution to the field.
- The AAV directed evolution work is a strength of the proposal and is highly innovative. The PI has generated vast libraries of AAV variants to enable this directed evolution work.
- The plan to develop a single-cell atlas from brainstem and limbic regions is novel.
- A diverse set of protocols will be used to establish regionally specific neuronal identities.
- The basis of the midbrain–hindbrain boundary is well established during neural patterning, as are iPSC-based protocols to drive this identity.
- The use of AAV variants to direct cell type-selective infection in Aim 3c is an innovative approach to stem cell disease modeling and therapeutics.
- This study aims to dissect the selective neuronal vulnerability of these neurons in PD and DLB, with the goal of identifying therapeutic targets that restore cellular processes in neurons susceptible to Lewy body pathology, with a focus on the brainstem and limbic regions.
- The focus on resilience rather than aggregate clearing is interesting and potentially impactful.
- The proposal uses good methodology but does not go beyond the current frontline.

Rationale: Evaluate the scientific rationale in the proposal

- The rationale to understand the cell biology of vulnerability in non-dopamine neurons is strong. Ideas such as that differences in lysosomal activity or pH may underlie vulnerability or resilience are a good rationale for the work.
- The rationale to tackle this question from a range of angles (iPSC-derived neurons, scRNA-seq and spatial transcriptomics) is strong.
- Aim 1 represents a rational approach to develop a limbic/brainstem database. Aim 2 will use a diverse set of iPSC-based models to then study neuronal vulnerability to perturbations and energetics. Aim 3 will focus on genetic risk factors and various approaches to elicit neuroprotection in vitro.
- The protocols proposed are generally very feasible with proof-of-concept data available in the published domain as well as provided by the applicants for specific assays.
- The project builds on the well-established literature that Lewy body pathology, characterized by alpha-synuclein aggregation, accumulates selectively in specific neuronal populations in the brainstem and limbic system.

Plan & Design: Evaluate the project plan and design



- The analysis of the cell biology techniques to be used to assess, for example, lysosomal function is covered in great detail. As with other aspects of such a massively ambitious project, it may be unlikely that all components can be achieved.
- The groups are well placed to undertake the proposed work, and the consortium structure and experimental integration across groups are well described.
- The AAV work is well planned and strong. Generating cell-type-specific gene expression systems through directed evolution of tropism and the use of cell-type-specific promoters will be innovative and valuable.
- In many places, the novelty of the work is overstated. For example, it is generally accepted that lysosomes become less acidic with ageing, so this is not a new hypothesis. Similarly, discussion of TFEB covers well-trodden ground.
- There is an overreliance on the KOLF iPSC line. This line has become very widely used, but it is not perfect and may carry CNVs relevant to neuronal function, as reported recently in Cell Stem Cell. The authors of that paper strongly advocate the use of multiple patient and healthy control derived lines. It would be preferable to work with a mix of isogenic engineered lines, PD patient lines, and healthy controls to better capture genetic differences in PD.
- The plan to validate clinical disease models (Aim 1d) seems unlikely to succeed given the small number of donors (40) to be used for snRNA-seq data generation.
- Several aspects of the proposal seem underdeveloped and hard to follow. For example, it is unclear what experiments will actually be performed in Aim 2a–b. Aim 2a describes procedures to direct iPSC development toward hindbrain identity. For Aim 2b, it appears that cells will be generated for use in other aims, but no actual experiments are described within this subaim. It is unclear what endpoints will be used to define success or failure.
- Pitfalls and alternative approaches are described.
- The PI lacks some experience coordinating projects of similar scale. The co-investigators have relevant expertise in iPSC-based models, single-cell genomics, and AAV tools.
- A communication plan involving monthly Zoom meetings and yearly mini-symposia is described.
- The project relies heavily on single-cell transcriptomic analysis of postmortem patient tissue and cell models to identify disease-associated pathways. While this approach is appropriate, it should be acknowledged that postmortem material largely represents end-stage disease, which may limit conclusions about early pathogenic mechanisms, and that the actual number of neurons recovered will be low. In contrast, the cell models are based on iPSCs, in which cellular age is erased during reprogramming.
- An interesting and potentially high-impact component is the development of hiPSC-based models of limbic and distal brainstem neurons, as robust human models of these regions do not currently exist. The approach to generate these organoids is grounded in developmental biology findings from one of the applicants. However, these models are not yet fully established or validated, and as with all iPSC systems, cellular rejuvenation remains a limitation when studying late-onset neurodegeneration.
- The planned use of CRISPRi perturbation and Perturb-seq screening is well aligned with the mechanistic goals of the project and represents a strong methodological component, but it lies in the high-risk/high-gain domain.
- The AAV work is compelling but premature.

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations



- Outreach events to the Parkinson's community, as well as lay education and outreach activities, are all well covered.
- The iPSC lines are generally focused around KOLF (one male line), which limits diversity. Notably, engineered isogenic lines based on a female line (CW02170) will also be used, which is positive. However, a much wider and more representative set of iPSC lines should be included.
- The postmortem brains collected for study are representative of the applicant institution's patient population, which serves a diverse community.
- A reference male and female iPSC line will be used as controls. Variants of the male line that have been previously engineered by CRISPR to bear familial PD-linked variants will be used. The group plans to develop similar female lines as needed but does not plan to use these routinely.
- Postmortem samples are available in large numbers from both sexes. The racial diversity of samples is not provided, but sampling encompasses patients from two disparate regions.
- The team is involved with PD foundations and support groups as part of their outreach efforts. One core team member is a physician who directly cares for dementia patients, including those with PD and DLB.
- There is heavy reliance on a single iPSC line.



Application#	DISC4-19227
Title (as written by the applicant)	Multi-omic Mapping of the Human Autonomic Nervous System to Accelerate Therapeutic Discovery for Dysautonomia
Project Objective & Impact (as written by the applicant)	Our project addresses the lack of dysautonomia therapeutics by creating an unprecedented cellular and molecular atlas of the human autonomic nervous system. Donor-matched human iPSC-derived complex organoid models will elucidate disease mechanisms, while a cutting-edge AI model will integrate comprehensive tissue and cellular data for therapeutic target prediction and validation in organoids.
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> • Generate a comprehensive, spatially-resolved multi-omic atlas of the human autonomic nervous system paired with a donor-matched hiPSC biobank from healthy and dysautonomic donors. • Model neuro-cardiac interactions using AIM 1 hiPSC-derived cardiac organoids. • Evaluate the myelination potential of hiPSC-derived oligodendrocytes from AIM 1 healthy and dysautonomic donors. • Establish a functional perturbation–measurement–modeling loop to decode neuro–glia–immune interactions in the autonomic nervous system. • Develop a multimodal AI model of the human ANS to enable in silico predictions of novel therapeutic targets for autonomic nervous system diseases.
Statement of Benefit to California (as written by the applicant)	Over 5 million Californians are affected by dysautonomia, which is incurable and associated with reduced quality of life, loss of workforce participation, and increased medical costs from symptomatically managing a chronic condition. By improving our understanding of the physiology and pathology of the human autonomic nervous system, our project aims to identify targets to reduce disability, restore workforce participation, and deliver significant economic and health benefits to California.
Funds Requested	\$14,000,000
GWG Recommendation	(1-84): Not recommended for funding
Process Vote	<p>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.”</p> <p>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”</p>

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
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Median	80
Standard Deviation	5
Highest	90
Lowest	70
Count	13
(85-100): Exceptional merit and warrants funding, if funds are available	2
(1-84): Not recommended for funding	11

FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

<p>Key Strengths and Weaknesses</p> <ul style="list-style-type: none"> • Outstanding team with an innovative set of approaches to an important but poorly studied problem. • To date, no comprehensive molecular-resolution atlas of the human autonomic nervous system exists, particularly one integrating spatial and proteomic information. • Understanding the molecular and cellular diversity of the ANS is essential for identifying disease-relevant pathways and mechanisms underlying dysautonomia. • Key strengths: Outstanding team members, the importance and urgency of the Autonomic Nervous System (ANS) profiling. • Weaknesses: Lack of details in Aim 1 and 4, choices and relevance of the cell types in Aim 3. • The inclusion of a large number of tissues increases biological coverage but also introduces analytical complexity. • The proposal includes donors with heterogeneous diagnoses (e.g., Parkinson’s disease, multiple sclerosis, ALS), yet it is unclear how co-morbidities and disease-specific effects will be modeled or stratified.
<p>Significance: Evaluate the project’s significance and potential for impact</p> <ul style="list-style-type: none"> • The autonomic nervous system is relatively understudied. • Milder forms of autonomic nervous system (ANS) dysfunction are relatively common but vary greatly in severity and causative underlying pathology. • This project aims to generate a single-cell transcriptomic atlas of the human ANS, which could be useful for the broader community.



- Cardiac autonomic dysregulation is therapeutically relevant, though most severe autonomic dysfunction is secondary to other chronic diseases such as diabetes, multiple system atrophy, MS, and PD. The proposed concept that an ANS therapy could be applicable across these diseases seems unlikely.
- The ANS is integral to homeostasis, and disorders of the autonomic nervous system have diverse impacts on health across multiple organ systems. Despite this, it has not been studied in much depth with contemporary analytical techniques. These studies will advance our knowledge of ANS function in health and disease.
- The ANS is integral to homeostasis, and disorders of the ANS have diverse impacts on health across multiple organ systems. Despite this, it has not been studied in much depth with contemporary analytical techniques. These studies will advance our knowledge of ANS function in health and disease.
- Aims 4–5 are designed to identify biomarkers and potential targets for intervention in disorders with ANS dysfunction, in particular cardiac dysautonomia. How quickly new therapeutics will emerge from the program is unclear, though all the core team members have extensive experience in translation.
- ANS dysfunction is a common and clinically significant feature of a wide range of disorders, including primary dysautonomias as well as neurodegenerative diseases such as Parkinson’s disease, multiple sclerosis, and amyotrophic lateral sclerosis.
- Because the ANS regulates essential homeostatic functions including cardiovascular control, thermoregulation, gastrointestinal motility, and immune modulation, its dysfunction contributes substantially to morbidity and reduced quality of life.
- Despite the pervasive influence of the ANS, there is a striking lack of functional genomic, spatial, and proteomic datasets characterizing autonomic neurons, glia, and their target tissues in humans.
- The proposed multiomic atlas addresses a major unmet need by providing a molecularly resolved framework for understanding autonomic circuit organization and dysfunction.
- By integrating central and peripheral components of the ANS, the project has the potential to uncover shared molecular mechanisms underlying diverse dysautonomia phenotypes.

Innovation: Evaluate the project for innovation relative to the current state of research

- The proposal includes an array of innovations, including generating a multi-omic human autonomic atlas; studies of neuro-cardiac interactions; genetic and functional perturbation; automated live-cell imaging; and other methods.
- There are no comprehensive single-cell databases of the human ANS.
- The approach is multidisciplinary in that it focuses on using iPSCs to study effects on different cell types and systems.
- The team will investigate iPSCs from various donors relevant to ANS dysfunction, including those with PD, MSA, and other non–PD-related autonomic dysfunction.
- The ANS is understudied, and although it is not entirely correct that there have been no single-cell atlases of the ANS, this team’s efforts will be far more comprehensive than anything available today. Combined innovations in cell-based modeling, omics, and high-content screening technology will significantly advance our understanding of ANS function in disease.
- Investigators will employ sophisticated omics technologies using state-of-the-art technology.



- Deep visual proteomics provides state-of-the-art analysis of protein expression at the single-cell level. Modeling of ANS cardiovascular interactions is very innovative, as are studies of autonomic ganglia and axonal transport. The proposed AI models link transcriptomic and proteomic data with imaging outputs.
- To date, no comprehensive molecular-resolution atlas of the human autonomic nervous system exists, particularly one integrating spatial and proteomic information.
- The proposal combines single-cell transcriptomics to define cellular diversity, spatial transcriptomics to preserve anatomical and circuit context, and proteomics to capture post-transcriptional regulation and signaling states.
- Matched hiPSC lines derived from donors will be used to model individual genetic backgrounds and enable functional validation.
- Advanced experimental platforms will be employed to interrogate autonomic circuit function.
- The integration of these approaches is not particularly novel but is a logical strategy for dissecting human ANS biology.

Rationale: Evaluate the scientific rationale in the proposal

- Generation of iPSC lines from each donor used for ANS profiling is a good strategy, though whether the causal genetic changes will be apparent when studying ANS organoids is unclear.
- The experimental plan for Aim 4 seems underdeveloped, and the justification for choice of assays is unclear.
- The therapeutic rationale for rescue of dysautonomia takes a broad approach and yet is likely to be very disease specific.
- The team does not study Schwann cells, which seems like a major oversight given their critical role in ANS function.
- The rationale for a detailed molecular and cell biological characterization of the human ANS is very strong. The aims are well integrated, though not interdependent, and move from patient data to cell models to the molecular level.
- The core team's laboratories have developed all the sophisticated technologies that will be required for these studies. Clinical data support a critical involvement of ANS pathology in all conditions under study.
- Analysis of human tissue and cell models will provide important validation. The models are sophisticated and likely to yield robust data. Perturbation screens are well considered.
- The core team comprises internationally recognized experts in human tissue collection and processing, omics, stem cell-based models, and cardiology, and their preliminary results and track records provide a high level of confidence in the overall feasibility of the proposal.
- Understanding the molecular and cellular diversity of the ANS is an important step for identifying disease-relevant pathways and mechanisms underlying dysautonomia.
- Cell-type-specific transcriptional and proteomic changes, rather than gross anatomical abnormalities, are increasingly recognized as drivers of autonomic dysfunction.
- The proposal plans to survey an impressive 28 distinct tissues from 30 donors, providing broad coverage of central, peripheral, and target tissues relevant to autonomic regulation.



- Parallel generation of hiPSCs from the same donors enables direct linkage between in vivo molecular states and in vitro functional modeling.
- The use of specialized neuro-cardiac cell systems derived from hiPSCs is particularly well suited to studying autonomic control of cardiac physiology and dysautonomia-related cardiovascular symptoms.
- It is unclear whether the proposal aims to identify shared autonomic signatures across diseases or disease-specific autonomic pathologies, and how these possibilities will be analytically distinguished.

Plan & Design: Evaluate the project plan and design

- In Aim 1, they will collect samples from patients with PD, multiple sclerosis, or amyotrophic lateral sclerosis... What others? Which diseases will be the focus, and why? How many diseases and how many patients will be studied? This is not clearly proposed. More detail and specificity are needed for disease types and proportions. How many hiPSC lines will be generated?
- In Table 3, they show that available iPSC lines include cells from donors with PD and dysautonomia. Is this proposal going to use all of the iPSC lines in Table 3, in addition to generating more iPSC lines? How about familial dysautonomia iPSC lines?
- The research plan in Aim 2 is outstanding and feasible, as the team has a wonderful track record. They propose to test PD-relevant stressors. It is also important to study non-PD dysautonomia in their cardiac model, for example using familial dysautonomia iPSC-derived autonomic neurons.
- Aim 3 is a little bit confusing. It is not clear why they propose to use a brain slice model rather than a shiverer autonomic neuron model.
- In addition, it is not clear which neurons will be used for the myelination studies. While the team has pioneered the myelination capability of iPSC-derived oligodendrocytes for disease modeling such as autism, it is difficult to justify the focus on brain neurons and tissues for myelination defects in dysautonomia.
- Perhaps it might be worthwhile and more relevant to dysautonomia if the team focused more on other disease-relevant cell types, such as satellite glial cells or Schwann cells.
- In Aim 4, it might be more informative if they had specific plans for generating neuronal and glial subtypes.
- Some aspects of the research plan seem unfocused.
- It appears that the bank of available tissue for Aim 1 is not yet available.
- The number of planned donors appears to be very small (only 10–15 healthy donors and no definitive plan for the number of donors with dysautonomia).
- Pitfalls and alternative strategies are discussed for each aim.
- The team is appropriate; however, there is little evidence that these investigators have worked together previously or are currently working on ANS-related projects.
- A communications plan using Slack, shared IT infrastructure, and regular meetings is in place.



- The project has well-defined aims that, while ambitious, are highly likely to yield significant outcomes. Tissue sampling will provide a comprehensive ANS atlas. iPSC and omics data will come from the same patients.
- There is reliance on postmortem material without consideration of potential limitations.
- The plan is very ambitious, but the resources and capabilities of this group of investigators provide some reassurance that the goals will be reached in a timely fashion.
- This is a highly experienced group of investigators with high competence in the management of complex projects.
- Project management, including appointments for overall management and coordination of data management, is well described.
- The proposal focuses on comprehensive profiling of autonomic neurons and glia, a relatively understudied cellular population with high relevance to disease.
- Generation of large-scale, multimodal omics datasets and a matched hiPSC resource represents a major strength and will be broadly valuable to the field.
- The inclusion of a large number of tissues increases biological coverage but also introduces analytical complexity.
- AI-driven integrative analyses are proposed to enable cross-layer inference and identification of candidate perturbations.
- However, some enthusiasm is diminished by limited methodological detail, including donor recruitment and cohort composition, criteria for tissue inclusion, and timing and coordination of multi-omic data generation.
- The proposal includes donors with heterogeneous diagnoses (e.g., Parkinson's disease, multiple sclerosis, ALS), yet it is unclear how comorbidities and disease-specific effects will be modeled or stratified.
- Additional clarity on how autonomic signatures will be disentangled from disease-specific pathology would strengthen the approach.

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations

- With ANS dysfunction predicted to affect over 5 million Californians throughout their lives, there is an urgent need for research that reflects the full spectrum of the state's population. The team will work with a dysautonomia support network, an institutional community outreach team, and an international patient advocacy organization.
- ANS dysfunction is predicted to affect a large proportion of the population at some point in their lives.
- The planned iPSC donor diversity (gender and race) is not provided.
- The proposal will interrogate genotypes from diverse backgrounds.
- The team has an excellent track record of patient outreach, engagement, and education. Interaction with target patient advocacy groups has already been initiated.
- The sampling strategy includes individuals across a range of ancestries, genetic backgrounds, and both sexes, enhancing generalizability.



- Active partnerships with patient advocacy organizations support meaningful engagement and dissemination.
- Collaboration with an expert in narrative medicine will help translate complex findings into accessible and impactful messages for patients and the public.
- If successful, this project will generate a foundational resource that could transform understanding of dysautonomias and inform diagnostic and therapeutic development.



Application#	DISC4-19452
Title (as written by the applicant)	Developing novel organoid-derived cellular therapies to restore hippocampal circuit function in mesial temporal lobe epilepsy (MTLE)
Project Objective & Impact (as written by the applicant)	We will identify mechanisms of hippocampal cell-type vulnerability in mesial temporal lobe epilepsy (MTLE) using human brain tissue-based analyses and biophysically realistic computational modeling. By leveraging novel cell-targeting tools and organoid models, we will develop and test precise cell replacement strategies to restore network function and guide new therapeutic approaches.
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> • Identify changes in cell type composition and connectivity that correlate with cognitive dysfunction in patients with mesial temporal lobe epilepsy (MTLE) • Develop a biophysically realistic model of hippocampal circuit dysfunction • Isolate and validate hippocampal cell types from mixed organoid populations • Test the ability of organoid-derived cell populations to restore hippocampal function in models of MTLE
Statement of Benefit to California (as written by the applicant)	Mesial temporal lobe epilepsy (MTLE), the most common cause of drug-resistant focal epilepsy, affects ~100,000 Californians. This project leverages human hippocampal tissue reflecting the state's diverse population to advance stem cell-based models and cell replacement strategies that restore function, driving precision neuroscience and reinforcing California's leadership in regenerative medicine and translational neurotherapeutics.
Funds Requested	\$12,965,718
GWG Recommendation	(1-84): Not recommended for funding
Process Vote	<p>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."</p> <p>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."</p>

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	85
Lowest	70
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	2



(1-84): Not recommended for funding

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FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

Key Strengths and Weaknesses

- This reviewer found this proposal to be overall fundable due to the close integration of modeling and experiments, which was much tighter than in the majority of applications.
- Key strengths: The investigator team, the use of patient samples, the focus on hippocampus. Key weaknesses: Lack of validation of in vitro models, small sample size and the use of a single iPSC line.
- Very strong application up to the isolation of interneuron subclasses from organoids then transplanting them, which seems highly unfeasible both from the perspective of isolating adequate numbers of the subclasses, and of obtaining adequate survival post-transplant when they are isolated by FACS.

Significance: Evaluate the project's significance and potential for impact

- Mesial temporal lobe epilepsy (MTLE) is the most frequent focal epilepsy in adult patients. Available treatments are not always effective and patients also suffer from cognitive decline. Therefore, developing more effective treatments is necessary. The proposed project aims to develop a cell replacement therapy for MTLE to restore hippocampal circuit homeostasis-suppressing seizure and restoring cognitive function.
- It is potentially highly significant to develop evidence-based cellular replacement that restores hippocampal circuit function in MTLE.
- A clinical trial of TLE in which around 30 patients have been transplanted with mixed medial ganglionic eminence derived interneurons is ongoing. Conceivably a method to enrich for interneuron subgroups for transplantation could improve outcomes, especially if combined with evidence for seizure or imaging based characteristics that support use of a subclass.
- The modeling aspect from the very deep phenotyping data could be transformative.
- The greatest significance of this project actually goes beyond the target condition itself - if you can merge live tissue acquisition with detailed and plausible computational models, you will have demonstrated a new form of research that could be cloned for several diseases and offers a potential solution to developing therapies for diseases with highly intertwined molecular, electrophysiological and connectivity components.
- While the cell transplants used in the study are largely for validation that the selected cell types control the phenotype, that is also a method under consideration as a treatment, so anything that could optimize that would be beneficial.

Innovation: Evaluate the project for innovation relative to the current state of research

- The use of organotypic slices to inform a modeling of hippocampal activity with input from in vivo studies (EEG, MRI, PET) is highly innovative.



- By utilizing a computational model to make the effects of cell type proportion and connectivity patterns more evident, you have a rapid screening mechanism that will likely save many years of wet bench experimental work. Importantly, the integration with the computational model appears to be at least potentially feasible, although there are some concerns about overfitting.
- Everyone claims that their project is integrative, multimodal, multinomic, etc. But this proposal actually confronts a very significant historical divide in the literature between dynamical systems and electrophysiological understanding of the brain versus traditional molecular biology and human data acquisition and behavioral measures.
- The combinatorial use of stem cell derived organoid, mouse and patient tissue to identify specific cell types contributing to MTLE.
- Develop a computational model for hippocampal circuits.
- There is a team of both clinicians and basic scientists with complementary expertise.

Rationale: Evaluate the scientific rationale in the proposal

- Very high enthusiasm for the rationale of this study. A challenge is that the rationale depends on surgical resection-derived organotypic slices being adequately representative of the pathology contributing to cognitive symptoms. If not, then fairly little will be gained from the proposal.
- Cell transplants are one of the few viable therapies for MTLE, so identifying the cell types and connectivity patterns that are most affected in the human disease, and then attempting to restore those in computational and experimental human cellular models. is a straightforward way of addressing the disease and highly rational, though ambitious.
- The main hypothesis is that a loss of one specific and some cell types in the adult hippocampus contribute to the cognitive decline of MTLE-HS patients. However, this rationale is not strongly substantiated by the available data.
- A mouse intrahippocampal kainic acid IHKA model will be used to model MTLE. However, the hypothesis that interneuron subtypes which are human-specific or human-enriched may show disease-related phenotypes only in human specimens cause concerns of the utility of this mouse model.
- It is not clear whether the assembloids will exhibit specific cell loss as seen in patients.

Plan & Design: Evaluate the project plan and design

- On a practical level, the series of milestones makes complete sense - defining cell types of interest in humans, checking for altered hippocampal connectivity, combining those in a computational model, checking if mainstream mouse models recapitulate this, then developing and transplanting relevant cell types in model systems. It's ambitious but also highly logical.
- Overall strong design.
 - Aim 1M4 - Moderate enthusiasm for associating cognitive deficits with scRNA seq and connectivity data. Outstanding team for conducting the statistics but may be hard to make more selective associations other than the finding that more disrupted pathology correlates with lower neurocognitive scores.
 - Power analysis would be helpful that indicate how cognition will be ranked, how many subjects will be tested and how many expected to be in various levels of neurocognitive function (or predicted



frequency distribution of scores for the whole subject group), and what effect size in the scRNA seq and connectivity (how is this defined?) would be needed to identify a significant association.

- Aim1M5 - It is unclear whether seizure frequency will be correlated with pathology in the mouse. Dozens of papers have tried to address this issue, albeit without the level of MTLE pathology analysis to relate the mouse phenotypes. It seems like a missed opportunity to not also conduct some level of neurocognitive testing in the mice, before and then after kainate, and potentially cross validate a human pathology - neurocognitive association with that modeled in mouse.
- Aim 4 - The proposed 'cell reader' construct will, as shown, pick up both PV and SST-fated medial ganglionic eminence derived-interneurons when they are at the migratory stage. Post migration, it would theoretically enrich for PV. However, there may be limited utility in transplanting post-migratory interneurons, since they would leave a clump of cells unlikely to more than perhaps enhance inhibition very locally.
- There is also concern that cell viability after transplant will be limited after sorting any type of neuron past around DD60, (proposed is DD 100+) and whatever cells do survive are likely to have been at the progenitor stage during the sort.
- Even for the grant mechanism that encourages high risk high payoff studies, the lack of evidence that cells, especially the interneurons expressing a cell reader construct introduced by AAV at DD100, can be harvested at a usable scale and transplanted, then survive in vivo or on a hippocampal organoid, is deeply concerning and probably unfeasible.
- Applicant states they "predict that slices transplanted with GE inhibitory populations will be resistant to generating epileptiform activity, and further hypothesize that replacement of SST+ inhibitory neurons will be more effective than mixed inhibitory populations, or PV+ inhibitory neurons alone." No evidence for this is presented, or to this reviewer's knowledge exists in the literature, that human stem cell derived PV+ or inhibitory neurons can be selectively generated or isolated in a manner viable post transplant.
- Obtaining key parameters from humans with a disease and then understanding their effects more fully with a computational model is a valuable approach for diseases often represented by animal models which have major limitations in the context.
- Most studies with a computational component, it is clear that they've been tacked on, but in this case it serves a central purpose, which means you can actually take advantage of areas where simulations are strong and wet experiments are weak. Pulling this off requires a group of people with enough mutual understanding of what each other does on a technical level - that is rare.
- The applicant's response to the question about low-dimensional solution space was convincing. However, the applicant's response pointing to cancer lines as a source of likely effect sizes was not convincing. If data on expression from the actual disease aren't available (it's best to include targeted assays in this search), then the applicant should search for data from the closest human disease.
- In this reviewers' opinion the transplantation aim was 'moonshot' but tolerable due to the overall importance of the modeling and experimental work. But for other reviewers, it was a complete dealbreaker.
- The results from patient samples will vary depending on the surgery and affected areas. This concern also applies to proposed bar-coded RV tracing experiments, which require the whole hippocampus. Given this variability, the number of samples (total 16 across 4 years) will be small to draw meaningful conclusions.
- A bio-realistic computational model with single cell resolution will be generated to build a human hippocampal circuit. It is not clear whether sufficient data can be generated to train the model to have predictive value for MTLE. Some cells are already lost in patient MTLE samples. This is also a concern for in silico experiments if the cell types interested are not present.



- It is not clear how variable are the Hc-GE assembloids and there is no preliminary data to characterize and quantify all the cell types. This is essential for all downstream analysis proposed in Aim 3. A single iPSC line is also a limitation.
- KA will be used on human hippocampal organotypic slices to induce epilepsy-like activities, however, the origin of these human tissues are already from patients with pathology. How these will reflect MTLE is not clear at all.

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations

- This study would be very much the first of its kind; point being there's not really a white male bias, there's just no data of this kind. There's at least equal attention to female models, and the iPSCs will likely have ~20 % Hispanic origin given prior history. There are moderate outreach efforts for minority researchers.
- This project is really operating at a fairly abstract level where this reviewer is not sure that environmental factors will play into the things they're measuring. It is unclear if there's any data to support that one way or the other. Sex could theoretically factor in, but they are balanced in that. Frankly there aren't a lot of options for this brain tissue from living younger people, and so you kind of have to accept whatever you get.
- Patient samples will be collected from sites at [redacted hospital name] and [redacted hospital name] has patients across gender, age, genetic backgrounds, socioeconomic and geographic groups. One limitation is that only a single male iPSC line will be used. Mouse MTLE models will also be used. There is a plan to engage with patient advocacy groups, but no specific plans for patient outreach.



Application#	DISC4-19278
Title (as written by the applicant)	Cellular drivers and cell therapies elucidated from stem cell models for brain cancer
Project Objective & Impact (as written by the applicant)	By analyzing human brain-tumor samples for proteins regulated primarily at the level of translation, and by applying emerging technologies in target identification, lineage tracing and therapy engineering, we will transform our understanding of brain cancer biology and identify robust new therapeutic targets to enable effective cellular therapies to improve outcomes.
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> • Identify proteins regulated primarily at the translational level in human brain cancers. • Functional characterization/validation of proteins that change at the translation level and previously unannotated cancer-specific peptides to inform stem-cell biology and to identify neoantigens. • Use lineage tracing in brain tumor samples and iPSC- and patient-derived organoid models to identify cells underlying formation, growth, and response to therapy. • Apply all-human, high-throughput methodologies to develop effective CAR-T cell therapies for glial brain tumors. • Define patient engagement, drivers of trial participation, and experiences with clinical trial counseling.
Statement of Benefit to California (as written by the applicant)	Our studies define the translome and proteome of the most common and malignant brain tumors that arise in patients in California. Our studies promise to improve our understanding of brain tumor biology, particularly stem cell biology. We use this knowledge to develop improved cellular therapies for patients. We also engage patients receiving cellular therapies to help both providers and patients to improve the course of therapy for these diseases.
Funds Requested	\$14,000,000
GWG Recommendation	(1-84): Not recommended for funding
Process Vote	<p>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.”</p> <p>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”</p>

SCORING DATA

Final Score: 79

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

Mean	78
Median	79
Standard Deviation	3



Highest	80
Lowest	70
Count	14
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	14

FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

<p>Key Strengths and Weaknesses</p> <ul style="list-style-type: none"> • Strengths include unmet need, team of investigators, and discovery science. Weaknesses include disjointed aims and lack of focus in studies across brain tumors. • Key strengths: the research team, the broad coverage of multiple brain tumor types and its potential impact. • Key weaknesses: lack of preliminary data and rationale for particular models to be used.
<p>Significance: Evaluate the project's significance and potential for impact</p> <ul style="list-style-type: none"> • The idea that more targets can be identified in these tumors is provocative. Identifying the transcriptome in brain tumors is highly impactful. • Brain tumors remain among the most lethal cancers and are not effectively targeted with current therapies. • Current approaches to characterize brain tumors and individual tumor-derived cell populations have focused on transcriptional profiles. However this is a significant limitation as protein levels are not always correlated with mRNA levels. • The ribosome profiling and other related data will be made available to the community and will provide a valuable resource. • Identification of neoantigens for potential targeting by CAR-T cell therapy is a strength. • Complementary single cell proteomic approaches are not proposed which somewhat limits the potential impact. • The development of new CAR-T cell therapies based on the identification of neoantigens expressed in brain tumor cells could have a major impact on the field. • Heterogeneity is one of the hallmarks of brain tumors, which are devastating and lack effective treatments. The proposed project aims to identify protein drivers of brain tumors, and to develop a CAR-T treatment. • If successful, the project will lead to potential new targets for therapies.



Innovation: Evaluate the project for innovation relative to the current state of research

- The proposal includes a novel technology for identifying membrane peptides specific to tumors. Unmasking the translome is highly novel for brain tumors.
- The project focuses on two major gaps in our knowledge of brain tumors, notably their heterogeneity and tumor-specific protein/neoantigen markers.
- Ribosome sequencing provides a means to examine specifically the translated mRNAs.
- The fully humanized organoid models of tumor growth combined with the lineage tracing will provide an innovative platform to validate hits from the proposed database.
- A main strength of the application is its focus on proteins underlying brain tumor pathology.
- Another strength is the use of direct patient samples or patient-derived organoid models that maintain the microenvironment.
- The application of new technology to identify membrane peptides specific to tumors is innovative.

Rationale: Evaluate the scientific rationale in the proposal

- Aims 1–3 are outstanding. Aim 4 is somewhat weak. It is not clear how discoveries from Aims 1–3 will overcome challenges to CAR T therapy, which include trafficking, persistence, and tumor-induced immunosuppression. These challenges are ineffectively modeled in ex vivo systems.
- The rationale for the focus on translated mRNA and protein expression is fundamentally sound.
- The complementary data focused on identification, validation in human models, and targeting with CAR T cells represent a well-reasoned plan supported by the team’s expertise.
- Aim 5 is somewhat thematically distinct from the other, more discovery-focused aims.
- The experimental plan seems feasible given the extensive expertise of the PI and co-investigators.
- The choice of three different types of brain tumors, representing the major adult and pediatric malignant and adult non-malignant brain tumor types in California, will have a broad impact.
- In many experiments, there is a lack of justification for why a specific model will be used and a lack of description of how many samples will be used.

Plan & Design: Evaluate the project plan and design

- Aims 4–5 seem to be very disconnected from the exciting themes of discovery science throughout the proposal.
- The new, proposed technology allows for specialized ribosomal sequencing but is limited to genetically modified cell lines. As such, it will not capture native expression within the intact tumor.
- Pitfalls and alternative approaches are considered.
- The budget appears appropriate given the scope of the work outlined.



- The team is appropriate. The PI is an expert in brain tumors and a leader in the field with relevant expertise in human stem cell models. Individual co-investigators have expertise in specific aspects of the research plan.
- A communication plan based on Slack and Google Apps is described. The steering committee will meet biweekly.
- The transcriptome and proteome will be performed using bulk cells, and candidate hits will be validated in in vitro cell models through expression and loss-of-function/gain-of-function studies. Different cell models, not all of which are patient derived and some of which rely on transplantation into mice, will be used for different tumor types, but there is no justification or description of why particular models are used.
- Aim 3 will focus on lineage analysis using in vitro models. This aim diverges significantly and does not seem to contribute to the overall goal of the proposal.
- The human organoid tumor transplantation model will be established as an in vitro model for testing CAR-T treatment in a high-throughput manner. Although PBMCs will be combined into the model, it does not fully reflect the tumor microenvironment. In addition, a major limitation is that iPSC-derived cortical organoids are immature in nature, which differs from patient conditions.

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations

- The design uses patient-derived tumor tissues to account for genetic factors affecting brain tumor biology.
- The applicant states that patients from diverse racial backgrounds will be used to generate cell lines for this project.
- The PI and Co-Is are actively involved in outreach associated with brain tumor and stem cell research.
- The teams have been actively engaged in patient outreach and advocacy and formed partnerships with multiple organizations and foundations and industry.



Application#	DISC4-19214
Title (as written by the applicant)	Personalized Paradigms for Discovery and Therapeutics in Glioblastoma
Project Objective & Impact (as written by the applicant)	Progress against GBM is limited by several bottlenecks: 1) Disease heterogeneity defies "one size fits all" approaches; 2) Current culture paradigms for discovery and screening often neglect the stroma; and 3) Current paradigms also ignore key structural/mechanical features of the GBM microenvironment. Success will yield new invasion-limiting strategies, improving patient prognosis and survival.
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> • Develop and validate stromal hiPSC three-dimensional (3D) GBM models to model tumor cell invasion. • Develop engineered 3D GBM models to incorporate vessels and white matter-like tracts to further model tumor cell invasion. • Identify and validate drug targets that halt GSC invasion. • Validate the platform's potential for developing personalized anti-invasive therapy for GBM.
Statement of Benefit to California (as written by the applicant)	Our research will benefit the people of California by producing new GBM treatments, thus improving patient outcomes and reducing health care burdens. While the California-specific incidence of GBM is not well-defined, over 12,000 new GBM cases are diagnosed each year in the US. California's strong cancer research infrastructure, supported by initiatives like its Cancer Registry and the world-class UCSF brain tumor center, will greatly speed dissemination and translation.
Funds Requested	\$12,999,997
GWG Recommendation	(1-84): Not recommended for funding
Process Vote	<p>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."</p> <p>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias."</p>

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	80
Median	75
Standard Deviation	6
Highest	88
Lowest	72
Count	15



(85-100): Exceptional merit and warrants funding, if funds are available	5
(1-84): Not recommended for funding	10

FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

<p>Key Strengths and Weaknesses</p> <ul style="list-style-type: none"> Strengths include unmet need, team of investigators, resource creation that could be broadly applied to help the field. Weaknesses include lack of consideration for all essential microenvironment elements and lack of validation data demonstrating evolutionary concordance between patients and their tumoroids. The investigators use and develop state-of-the-art engineering methods recapitulating critical processes in glioma progression. The proposal has an overly large engineering part to make patient adaptation and drug screening feasible.
<p>Significance: Evaluate the project’s significance and potential for impact</p> <ul style="list-style-type: none"> GBMs are highly heterogeneous and also evolve to exploit in vivo contexts that enhance their survival and growth. Both of these characteristics contribute to their being extremely challenging to model in vitro—a step that could be foundational to higher throughput therapeutics development. This proposal seeks to develop in vitro models of GBM survival, growth, and invasion using hydrogels (Aim 1). The hydrogels will be augmented with oligodendrocytes and endothelial cells, bioprinted using tomographic volumetric additive manufacturing (VAM), or computed axial lithography (CAL) (Aim 2), and will be cultured with established GBM-related cell lines modified by CRISPR-I screens to study potential drug targets (Aim 3), and then this system will be applied to individual patient-derived lines. This is a highly significant problem, and given the strength of the applicant team and environment, the project is reasonably likely to lead to clinically impactful findings. The proposal is potentially very impactful as it attempts to create a new resource for GBM to more faithfully recapitulate its heterogeneity with stromal cells and blood vessels. A successful project would lead to (i) improved protocols for glioblastoma models recapitulating disease-specific features, (ii) proof-of-concept, creating personalized models, and (iii) leads for drug targets to inhibit glioma invasion. These three outcomes could have a significant impact on understanding the disease and developing treatments.
<p>Innovation: Evaluate the project for innovation relative to the current state of research</p> <ul style="list-style-type: none"> It is highly innovative to pull together the separate expertises in hydrogel culture, organoid, GBM culture, measuring invasiveness, data analysis and management. The development of a tool that more faithfully recapitulates GBM heterogeneity is innovative. The team has collaborated and has complementary experience.



- The project uses highly novel approaches for materials engineering and engineered in vitro models (bioprinting). These could establish a platform that provides new insights into GBM. The project further uses state-of-the-art organoid and stem cell technology to generate relevant components of the tumor microenvironment, specifically stromal cells. Finally, it uses state-of-the-art omics technologies and gene editing to investigate targets for halting glioma invasion.

Rationale: Evaluate the scientific rationale in the proposal

- The rationale for this proposal is outstanding. It is fair to question whether the known and unknown factors that different GBMs use to grow and avoid immune attack can be adequately recapitulated in vitro, but this is a reasonable, if extremely ambitious, goal for a discovery-driven project.
- The proposal aims to develop heterogeneous tumoroids, and there are substantiating preliminary data. However, there are concerns that myeloid cells are not fully considered as critical components of the model. While macrophages are mentioned, it is unclear how they will be integrated.
- The rationale for establishing in vitro models that faithfully recapitulate tumor stroma and enable invasiveness studies is particularly strong for glioblastoma. The motivation to include both self-assembled and more advanced bioprinted methods is sound, as invasion commonly occurs along vessels or white matter tracts. There is a substantial engineering effort in materials and printing methods (Aims 1 and 2) required to support relevant cell populations and sizes.
- The prior and preliminary data strongly support the team's ability to develop new engineered matrices. There is also strong competence in iPSC differentiation, but there is uncertainty regarding donor matching between iPSCs and GSCs. The lack of preliminary data for co-cultures of GSCs and stromal cells makes it difficult to estimate how much engineering will be required to recreate physiological structures, whether bottom-up or top-down. This lack of co-culture preliminary data is a major weakness of the proposal.
- The bioprinting methods are interesting and novel approaches, but it is unclear what spatial resolutions are required to study the proposed processes. The preliminary data show very large feature sizes.

Plan & Design: Evaluate the project plan and design

- The plan is outstanding and appropriately detailed despite its complexity.
- The design of the first two aims is largely well conceived and well designed. However, there are concerns with Aims 3 and 4, as the use of the model to predict individual outcomes is largely exploratory and cannot be undertaken without a validating cohort. For example, a dataset demonstrating that these tumoroids model evolutionary biology in patients under standard-of-care conditions would be highly substantiating.
- The project follows a structure of developing and studying: (1) randomly mixed stromal cells; (2) printed vascular and white matter tract structures to study glioma stem cell (GSC) invasion; and (3) targeted invasion assays adapted to patient-derived materials. Pitfalls and alternative strategies are discussed but could, in several cases substantially delay the project, including if matrices are unsuitable and require adaptation, or if full iPSC-derived stromal integration is not successful.
- The selection and sizes of studies are still somewhat unclear. It would have been meaningful to include a clearer discussion of power analysis. GBM is highly diverse. While a selection of GBM lines is suggested in Table 1, it is unclear how these represent the diversity of the disease. The team appears to plan to use stromal iPSC-derived cells that are not isogenic, but there is no clear discussion of how donor-to-donor variability will impact outcomes.
- For macrophages, non-isogenic models are likely to be problematic, and additional complicating factors in cellular interactions may arise with this strategy. Later in the proposal, it is stated that 10 patients will be



used in the first aims. This is then expanded in Aim 4, where 50 newly diagnosed patients are selected for establishment of personalized models in which invasiveness will be investigated.

- It is unclear what timelines are proposed for enrolling these newly diagnosed patients and whether patient-specific iPSC-derived stroma will be included.
- Overall, the team and expertise are very strong. The budget is clear and appropriate. The timeline is somewhat questionable if certain alternative strategies must be adopted and given the complexity of implementing patient-specific models. The management and communication plan is feasible.

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations

- The experimental design is unclear regarding disease diversity. The teams' prior and current research and other activities are well anchored in contacts with patient organizations.



Application#	DISC4-19364
Title (as written by the applicant)	Engineering Stem Cells to Treat Demyelinating Diseases
Project Objective & Impact (as written by the applicant)	This project addresses the lack of effective approaches to repair myelin damage in the central nervous system. By uncovering the molecular programs that enable glial regeneration and engineering human cells with enhanced repair capacity, it aims to advance new treatments for demyelinating diseases such as multiple sclerosis.
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> • Identify the key molecular signals that allow nerve-supporting cells to repair damaged myelin • Engineer human cells to boost their ability to survive and repair myelin damage in the brain and spinal cord • Test the safety and effectiveness of these engineered cells in animal models of myelin injury
Statement of Benefit to California (as written by the applicant)	This research will advance regenerative therapies for diseases that cause myelin loss, such as multiple sclerosis, which affects thousands of Californians. By developing human cell-based strategies to repair nerve damage, the project will promote innovation in the state's biomedical sector and lay the groundwork for future clinical treatments.
Funds Requested	\$13,000,000
GWG Recommendation	(1-84): Not recommended for funding
Process Vote	<p>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.”</p> <p>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”</p>

SCORING DATA

Final Score: 70

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

Mean	68
Median	70
Standard Deviation	7
Highest	75
Lowest	-
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15



FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

Key Strengths and Weaknesses

- The significance of the project hinges on the assumption that Schwann cell repair logic is generalizable and can be safely translated to oligodendrocytes in chronic CNS lesions.
- Strengths: The project addresses a major unmet need, with an appealing vision of generating CNS-remyelinating oligodendrocytes. The use of synthetic biology and the development of a distributable vector “toolkit” are strong features. The overall flow of the project through the design of the aims is logical.
- Weaknesses: The lack of immunosuppression or use of immunocompromised mice is a major limitation. In addition, the proposal does not adequately consider whether the fundamental biological differences between Schwann cells and oligodendrocytes may be insurmountable.

Significance: Evaluate the project’s significance and potential for impact

- The project seeks to make a major advance in therapies for multiple sclerosis by engineering oligodendrocytes or oligodendrocyte precursor cells (OPCs) to repair myelin in the CNS. This would represent a major advance for the field.
- The outcome of the project would be a toolkit (e.g., a set of gene vectors) in which key genes are overexpressed or knocked down to drive OPCs toward a repair phenotype.
- Cell therapy for myelin disease represents a potentially significant therapeutic strategy for demyelinating disorders.
- It is unclear how this project will optimize or engineer cells to maximize their ability to migrate the large distances needed to treat multifocal diseases such as multiple sclerosis.
- The rationale for the idea that modifying oligodendrocyte lineage cells to express Schwann cell genes will improve function is unclear.
- There are no relevant preliminary data, and the concept is poorly rationalized.

Innovation: Evaluate the project for innovation relative to the current state of research

- Cross-lineage repair competence transfer as a conceptual framework is very innovative.
- The project is highly innovative in its aim to engineer oligodendrocyte precursor cells (OPCs) to repair myelin in vivo.
- The project builds on the group’s previous innovative work generating Schwann cells and oligodendrocytes from stem cells.



- The use of synthetic biology—combining regulated gene expression and gene knockdown vectors under the control of feedback or feedforward regulatory elements—to rewire the expression program of OPCs toward a repair phenotype is highly innovative.
- The potential for Schwann cell-mediated repair of CNS disease has been proposed for many years. However, this project provides an innovative set of approaches to identify mechanistic differences that may underlie why Schwann cells possess greater repair potential than oligodendrocyte progenitor cells.
- The protocols for induction of Schwann cells are innovative.
- This project utilizes CRISPR-based screens, single-cell omics, and both in vitro and in vivo approaches.

Rationale: Evaluate the scientific rationale in the proposal

- Feasibility is an issue.
- Is the Schwann repair logic portable to CNS glia in the chronic lesion environment without unintended effects?
- The rationale is strong: Schwann cells can repair myelin in the PNS, but oligodendrocytes cannot repair myelin in the CNS. The concept is to understand how Schwann cells do it and transfer that ability onto OPCs and oligodendrocytes. The risk is that the differences between the cell types may be too great.
- The rationale is supported by preliminary data to understand how gene expression changes over time Schwann cells as they mature and become able to repair. The preliminary data ensures that the project is feasible.
- The vision is to activate pro-repair programs in OPCs in response to lesion-specific cues, achieving spatial and temporal control of remyelination activity. That is a strong rationale.
- Not logical - why not transplant Schwann cells? The use of an immunocompromised model is an unresolved issue.
- The preliminary data suggest that they can reprogram mature cells to a repair-like state. Yet, the work proposed in Aim 2 is instead focused on enhancing the repair potential of OPCs. This is a fundamental disconnect that reduces enthusiasm.
- The applicant provides preliminary data indicating that they have established hiPSC-based protocols to drive SC cultures, based on their similarity of their transcriptome with primary SCs and following engraftment in rat sciatic nerve.
- It's unclear if the induced SCs can form both non-myelinating cells in vivo, also unclear if these cells express c-Jun in vitro and following engraftment.
- In Aim 3, it is unclear if the two different constructs will be used, as they appear, interchangeably. Examples of human iPSC-derived OPC cultures for transplant are not provided, nor how many donors will be used. It appears that immunocompetent mice will be used for xenografts. Details of the immunosuppressive regimen are not provided. It is unclear how the animal model and xenografts will be combined to avoid rejection of transplanted cells by the host immune system.

Plan & Design: Evaluate the project plan and design

- The aims follow clear logic, with appropriate methods at each stage.



- The project is well designed across three aims: first, to understand how repair pathways are enabled and activated in Schwann cells; second, to implement a design to convey this ability to OPCs and oligodendrocytes in vitro; and third, to test this approach in preclinical validation.
- The work in Aim 1 is well laid out, first using RNA-seq to identify repair pathways in Schwann cells and then perturbing or boosting them. This approach enables assembly of a toolkit of genes to be knocked down or overexpressed.
- The in vivo work will use an inducible knockout background to prevent endogenous repair by oligodendrocytes. Engraftment will be tested in vivo, followed by evaluation in a multiple sclerosis model.
- The lack of immunosuppression or use of immunocompromised mice is a major limitation.
- The major biological differences between Schwann cells and oligodendrocytes may be so substantial as to be insurmountable.
- A strength is that three strategies are proposed to enhance OPC survival and repair activity within in vitro injury models. Oligodendrocytes will be tested in both 3D neuron–glia co-cultures and more physiological organotypic slice cultures.
- The PIs are all highly expert in their respective areas, and the management and communication plan is appropriate.
- Aim 1 largely focuses on Schwann cells, while Aim 2 focuses on OPCs. The experimental linkage between these aims is somewhat tenuous, giving the impression that they are conceptually separate.
- While images are provided for the quantitative assays proposed in Aim 1, there is a lack of proof-of-concept data demonstrating the reproducibility and sensitivity of these assays.
- Some pitfalls and alternative approaches are provided.
- The team includes experts in their respective fields. The PI is an expert in hiPSC-based modeling, two co-investigators are experts in basic glial and oligodendrocyte biology, and another co-investigator brings clinical experience relevant to the translational aspects of the proposal.
- Biweekly, monthly, and quarterly team meetings are outlined in the management plan.

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations

- Diversity is built into the experimental inputs.
- Outreach activities, workshops, and engagement with patient advocacy groups and patient organizations are proposed.
- The applicants state that they will use hPSC models derived from genetically diverse donors, including lines with well-characterized HLA types and sex-specific backgrounds, and that this diversity is incorporated into the proposed comparative studies of Schwann cell and oligodendrocyte repair programs. This is excellent and contrasts favorably with over-reliance on KOLF cells.
- The applicants also state that the proposed iPSC-based models will include donors of diverse genetic backgrounds and both sexes. However, the plan describes only three lines, with no mention of sex.
- Two co-investigators have established partnerships with clinical and MS-focused organizations. The involvement of the PI and other co-investigators in these activities is not explicitly described.



Application#	DISC4-19350
Title (as written by the applicant)	Deciphering Neuro-Immune-Vascular Mechanisms in NeuroHIV employing Stem Cell Models and Circuit Analysis
Project Objective & Impact (as written by the applicant)	The project will address the lack of human-relevant models that limit discovery in brain health disorders including neuroHIV. Using stem-cell-based platforms and trans-species multi-omics to define mechanisms of resilience and vulnerability, it will identify causal pathways, biomarkers, and therapeutic targets that will advance understanding and treatment of these debilitating disorders.
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> • Define how the blood-brain barrier (BBB)-immune interface enables HIV infiltration of the CNS. • Determine how BBB dysfunction drives microglia switch to a neurotoxic phenotype. • Define the downstream cellular and functional consequences of the microglia switch. • Integrate multi-scale modeling to identify biomarkers and therapeutic targets that predict or block progression of NeuroHIV.
Statement of Benefit to California (as written by the applicant)	The project will advance brain-health research for Californians with HIV, who experience early aging-related disorders. Using specimens from Californians and inclusive study populations, the project will integrate multi-omics, 3D BBB/CNS models, and humanized mice to identify mechanisms driving cognitive decline and accelerate equitable regenerative-medicine strategies for underserved communities that will translate well to the clinic and to people with other neurodegenerative disorders.
Funds Requested	\$12,999,521
GWG Recommendation	(1-84): Not recommended for funding
Process Vote	<p>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.”</p> <p>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”</p>

SCORING DATA

Final Score: 68

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

Mean	67
Median	68
Standard Deviation	4
Highest	70
Lowest	-
Count	13



(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	13

FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

Key Strengths and Weaknesses

- Strengths: This proposal addresses a critical comorbidity affecting people with HIV (PWH). Elucidating the underlying mechanisms and identifying druggable targets have the potential to significantly reduce disease burden and improve long-term neurological outcomes.
- Preliminary data are provided to support the feasibility of the proposed approaches and demonstrate the team’s capability to successfully execute the planned studies.
- Weaknesses: The proposal is overly dense and lacks a clear, focused narrative. The scientific premise shifts between NeuroHIV, neurodegenerative diseases, TBI, and phenotypes (e.g., superagers and poor agers), without clearly delineating how these areas are conceptually or mechanistically integrated into this project.
- The proposed 3D microfluidic model, while potentially useful for addressing selected mechanistic questions, does not fully recapitulate the three-dimensional neural network architecture or the integrated neurovascular complexity observed in vivo. Rather, it represents a compartmentalized, layered system. Although suitable for certain reductionist analyses, its physiological relevance to the intact CNS may be limited.
- The humanized immune–glia model appears underdeveloped, with several conceptual and technical gaps that are not adequately addressed.
- While preliminary data are provided to demonstrate feasibility, the absence of peer-reviewed publications from the PI’s laboratory specifically related to this model or to NeuroHIV raises concerns.
- It is likely that the project delivers some biomarkers and differences between pooragers and superagers in PWH. The likelihood that it progresses towards a novel therapeutic is lower.
- The preliminary data are unclear on whether the models can fully recapitulate the intricate processes needed to mimic HIV entry into the CNS and neurodegeneration related to HIV
- NeuroHIV is an important clinical problem but the proposal seems like an extension of ongoing work. Relevance to neuroaging and neurodegeneration in the general population is not clear.

Significance: Evaluate the project’s significance and potential for impact

- This proposal seeks to define the host and viral determinants that drive neurodegeneration, neuronal injury, and cognitive impairment in people with HIV (PWH). Despite the widespread use of combination antiretroviral therapy (ART), nearly half of PWH continue to experience persistent neurocognitive impairment, collectively referred to as HIV-associated neurocognitive disorders (HAND).



- These deficits significantly compromise quality of life, functional independence, and workforce participation, and they impose substantial long-term healthcare and societal costs. Thus, the project addresses a major and unresolved clinical problem in HIV medicine.
- The proposed studies are significant because they move beyond descriptive observations of neuroinflammation and instead aim to delineate the specific host–pathogen interactions that lead to synaptic injury, neuronal dysfunction, and progressive cognitive decline.
- A major strength of this proposal is the innovative use of advanced translational models that more faithfully recapitulate human neurobiology. The investigative team has developed sophisticated blood–brain barrier (BBB) and neuronal culture systems, as well as a human glia–reconstituted mouse model that permits in vivo interrogation of human-specific neuroimmune responses.
- These platforms provide complementary mechanistic and functional readouts, enabling rigorous evaluation of both viral factors and host immune pathways in a physiologically relevant context. The integration of human-derived cellular systems with in vivo modeling enhances the feasibility and translational relevance of the proposed aims.
- Importantly, the team also proposes to extrapolate mechanistic insights gained from HIV neuropathogenesis to other neurodegenerative conditions characterized by chronic neuroinflammation and synaptic dysfunction. This cross-disease applicability broadens the overall impact of the project and positions the findings to inform a wider spectrum of CNS disorders.
- Overall, this proposal addresses a critical gap in understanding the mechanisms of HIV-associated neurological disease—an area that cannot be adequately modeled using conventional two-dimensional cell cultures or traditional small animal systems alone. By leveraging innovative human-relevant models and a strong multidisciplinary team, the study is well-positioned to generate mechanistic discoveries; however the meaningful translational potential is minimal.
- A successful outcome could be very impactful for understanding viral entry of HIV into the brain, where currently it's inaccessible to most anti-viral treatments. It could also deliver an impactful understanding of neurodegeneration associated with HIV
- The project would have a broader impact if it is successful in delivering a method for a physiological and immunocompetent organ chip model of the BBB fully based on iPS. Specifically, if this model allows the emulation of viral entry into the brain, the switch to reactive microglia and progression towards a neurodegenerative state. This is very far beyond the state of the art today.
- The hypothesis that understanding of neurodegeneration in HIV can be extrapolated to other neurodegenerative diseases is more speculative and will not be verified, even with a successful project. However, the increased understanding of PWH disease progression is powerful enough if it can be delivered.
- It is likely that the project delivers biomarkers and differences between pooragers and superagers in PWH. The likelihood that it progresses towards a novel therapeutic is lower.
- Neuro-HIV remains a significant challenge despite retroviral therapy. Thus the health span of people with HIV is still compromised. The concept that this syndrome can tell us something about loss of cognition during normal aging is not strongly articulated here. Many aspects of NeuroHIV pathology are unique to viral infection of the CNS. The application would be stronger if it did not make claims of relevance to neurodegeneration and stroke.
- Applicants are already funded to study neuro HIV and BBB.
- Not clear if data generated will be relevant to conditions other than HIV.



- The project may identify blood markers of CNS immune attack and BBB breakdown. It is not clear how these biomarkers might be actionable. Not clear that markers related to superager/poor ager phenotypes will emerge from the study. Not clear how computational analysis will lead to novel therapeutics in the foreseeable future.

Innovation: Evaluate the project for innovation relative to the current state of research

- This proposal is innovative in that it moves beyond traditional reductionist approaches to HIV neuropathogenesis by integrating human-relevant, multicellular neurovascular models with humanized in vivo systems to dissect virus–host interactions at cellular and molecular resolution.
- Rather than relying solely on conventional two-dimensional cultures or small animal models that lack human-specific neuroimmune complexity, the study leverages advanced blood–brain barrier platforms, differentiated neuronal populations, and human glia–reconstituted mouse models to interrogate synaptic injury, neuroinflammation, and cognitive impairment in a mechanistically rigorous and translationally relevant manner.
- The emphasis on identifying discrete host and viral determinants that drive synaptodendritic damage represents a conceptual advance, shifting the field from descriptive observations of neuroinflammation toward causative, targetable pathways.
- The inclusion of plasma and cerebrospinal fluid (CSF) samples from people with HIV (PWH), including cognitively resilient “SuperAgers” and cognitively impaired “PoorAgers,” adds a potentially novel translational dimension by attempting to link clinical phenotypes with mechanistic modeling.
- However, the reproducibility and interpretability of such samples may be limited by intra-individual temporal variability and inter-individual heterogeneity, which could complicate mechanistic conclusions unless adequately powered and rigorously controlled.
- Overall, while the project incorporates some innovative components—particularly in model integration and mechanistic focus—there remain conceptual and methodological weaknesses that may temper its overall impact unless further strengthened.
- The project uses state-of-the-art in vitro models – organ-on-chip (iPSC-derived) and humanized mice in combination with patient samples and high-end analytics. The final part of the project aims to use AI/ML to advance the evaluation of treatments within a holistic NVU model predicting outcomes for inflammation-driven neurodegeneration.
- Overall, this demonstrates an excellent use of technologies, with strong synergy between stem cell research and state-of-the-art analytics. A weakness is the comparably low number of iPS donors suggested. The main innovation is the development of a functional, immunocompetent iPS-derived NVU model.
- Many groups are pursuing the role of microglia in neurodegeneration. The study of individuals with varying responses to NeuroHIV is novel, but the claim that this somehow relates to CNS resilience in the wider population is not well supported.
- The project integrates PIs with expertise in HIV, immunology, and computational biology. Expertise in basic neuroscience or other neurodegenerative disorders is lacking.
- Cellular models of BBB are not innovative, neither are humanized mice described here. BBB model is a commercially available system in wide use.

Rationale: Evaluate the scientific rationale in the proposal



- The rationale for this study is grounded in the hypothesis that peripheral immunogens and inflammatory mediators alter blood–brain barrier (BBB) integrity, leading to barrier disruption and facilitating the infiltration of infected monocytes and activated T cells into the central nervous system (CNS). This process promotes viral seeding and the establishment of persistent, latent HIV reservoirs within the CNS that are not effectively eliminated by combination antiretroviral therapy (ART).
- In addition, HIV infection within the CNS triggers sustained neuroinflammation characterized by microglial activation and amplification of pro-inflammatory signaling pathways. This chronic inflammatory milieu exerts neurotoxic effects, resulting in neuronal dysfunction, neuronal loss, and synaptodendritic injury.
- All these are well-established and published by others in the HIV neuro field. Interestingly, the lead PI is not an established NeuroHIV investigator and has no publication(s) to support the proposed model.
- The scientific framework and division of the four aims are overall sound and rational, with clear milestones and an excellent use of the co-Is' competencies. The hypothesis of microglial involvement in neurodegeneration, in general and for PHW in particular, is well supported in the literature.
- The two main experimental systems, BBB-on-Chip and humanized mice, are used throughout aims 1-3 in complementary activities and would be excellent for these investigations if it can be validated that they recapitulate human processes.
- The BBB-on-chip is based on a commercial platform, which is easy to use and can achieve higher throughput than many organ-on-chip platforms. . A number of protocols are reported to be used to generate iPS-derived neural and neurovascular cells; many of these protocols exhibit limited immunocompetence, and preliminary data are unclear on whether the model can fully recapitulate the intricate processes needed to mimic neurodegeneration in HIV.
- AIM 4 is a bit less well-defined, relying primarily on multi-omics integration and the establishment of an in silico model of the NVU. The specific cells studied, however, could be interesting.
- Superager paradigm is interesting but is likely to be a complex phenotype that is not easy to deconvolute. To what extent does the phenotype reflect resilience to aging or is it resilience to HIV neuropathology? These are patients with HIV and again the relevance to normal aging in the CNS or to other types of neurodegeneration is not clear. Antemortem body fluids to be used in the study are collected within a year of death, tissues are post-mortem and may not be representative of earlier stages
- It is clear that damage to BBB and immune activation are features of NeuroHIV.
- The proposal to use BBB models and humanized mice is rational and well founded.
- Preliminary data demonstrate availability of all model systems.

Plan & Design: Evaluate the project plan and design

- The team proposes to use a three-lane microfluidic system to model brain architecture, consisting of a neuronal compartment and a BBB compartment separated by an extracellular matrix (ECM) layer. This configuration permits the study of peripheral immune cell infiltration into a lower neuronal layer composed of neurons, astrocytes, and microglia.
- While this platform is technically sound and allows controlled interrogation of BBB permeability and immune cell migration, it does not fully recapitulate the structural and cellular complexity of a brain organoid model. In particular, the vascular channels are not interconnected or embedded within a three-dimensional neuronal network, as occurs in vivo. Rather, the system represents compartmentalized cellular layers arranged in parallel.



- The preliminary data demonstrating HIV infection, migration of viral components, and BBB leakage support feasibility. Moreover, the microfluidic chamber system offers practical advantages, including the ability to perform TEER measurements and live-cell imaging within the same integrated platform.
- The inclusion of a BBB–neuronal system in combination with a humanized immune–glia mouse model is an additional strength. However, the in vivo model raises concerns regarding development and rigor. Although the investigators propose reconstitution of the peripheral immune system using human CD34⁺ hematopoietic stem cells, they also plan intracerebral injection of human astrocytes and oligodendrocyte precursor cells (OPCs) to humanize the glial compartment.
- It is unclear how stable and functionally integrated these human glial cells will be over time, particularly since endogenous mouse astrocytes and glia are not depleted. Based on the timeline presented (Fig. 11), it is also uncertain whether productive HIV infection is established in human glial cells within the CNS before downstream analyses are conducted.
- Several proposed experiments appear insufficiently justified. For example, the use of plasma and CSF samples from “Superagers” and “Pooragers” as immune stimulators is speculative. The composition of inflammatory mediators and immunogens in these samples is likely to vary across individuals and time points, and no clear rationale or characterization strategy is provided.
- Additionally, the figures are presented at a size that makes them difficult to read and interpret. The font size for axis labels, statistical annotations, and numerical values is too small to clearly appreciate the data, effect sizes, and statistical rigor. This significantly limits the reviewer’s ability to independently assess data quality, reproducibility, and the strength of the conclusions. Clear, legible, and properly formatted figures are essential for evaluating the robustness of the preliminary data, and the current presentation represents a notable weakness of the application.
- Overall, while the proposed models may allow the team to address specific mechanistic questions, both systems remain highly artificial and do not fully recapitulate the integrated periphery–BBB–neuronal axis observed in vivo. Strengthening the physiological relevance, validation strategy, and justification of certain experimental components would substantially improve the rigor and impact of the proposal.
- There is significant overlap of funding for this proposal with the PIs’ NIH and DoD funding.
- Overall, the project is well planned, with logical and meaningful approaches and timelines. However, the pitfalls related to the lack of required immunocompetence and immune responses in in vitro models are not sufficiently addressed. It is also unclear how many cell lines will be used. A power analysis is included, but it is not clearly linked to the proposed studies. This uncertainty also applies to the number of plasma donors from people with HIV (PWH) and how these samples will be applied in vitro.
- Overall, the timeline is well described and appropriate, and the budget and its distribution are suitable. Leadership and complementary expertise are well delineated, as is staffing. The communication plans and leadership structure are well described.
- The observations regarding the effects of aged plasma on the BBB are unlikely to be easily interpretable. There may be many changes in proteomic profiles and substantial patient heterogeneity.
- The applicants overestimate the potential of studies using patient plasma.
- The budget and timeline appear appropriate for the research proposed, and the project is achievable within these constraints.
- It is not clear that the project leaders have sufficient expertise in neuroscience or neurodegenerative disorders. For some investigators, recent publication records in the biosketches are unremarkable.

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations



- One aspect of the proposal is the inclusion of induced pluripotent stem cells (iPSCs) derived from Black and Latino individuals, in addition to White donors, to generate a BBB–CNS model that more accurately reflects the demographic composition of the California population. This approach enhances population relevance and the potential generalizability of the findings.
- Inclusion of local organizations is a plus; however, the contribution of these stakeholders to the basic science mechanistic work is unclear.
- The experimental design accounts for inclusion of different genetic factors (sex, ethnicity, etc.), but exact study sizes are unclear. The focus on people with HIV (PWH) has a clear and diverse societal impact, and the results may potentially be extended to other neurodegenerative diseases with broader impact. The team’s prior and current research clearly spans relevant PWH populations and may extend further if extrapolated to other neurodegenerative diseases.
- The applicants note disparities in outcomes of HIV treatment.
- The claims that viral infection in the CNS and its sequelae relate to normal cognitive decline are not strongly argued.
- The team has a good record of outreach in the clinical and scientific communities, particularly with respect to HIV.



Application#	DISC4-19207
Title (as written by the applicant)	Restoring network function to treat classes of genetic neurodevelopmental diseases
Project Objective & Impact (as written by the applicant)	Our overall objective is to develop a toolbox based on biophysical modeling and gene modulation to predict an optimal therapeutic strategy to restore normal circuit function in diverse developmental and epileptic encephalopathies. This will avoid the need for a bespoke therapy to correct each mutated gene and instead ameliorate families of disorders based on shared pathophysiological mechanisms.
Specific Aims (as written by the applicant)	<ul style="list-style-type: none"> • Functional and Molecular Characterization of DEE Organoids • Systematic Characterization of Gene Perturbations in Mosaic Organoids • Biophysical Computational Modeling to Predict Therapeutic Interventions • Validation of Therapeutic Predictions via Precision Gene Modulation
Statement of Benefit to California (as written by the applicant)	Epilepsy affects 3 million in the U.S., including over 425,000 Californians. Our study design explicitly accounts for genetic diversity and population variability by using patient-derived iPSC lines from individuals with different DEE genotypes and demographic backgrounds, capturing variation in sex, genetic ancestry and age where possible. By focusing on convergent mechanisms rather than single variants, one therapeutic approach can treat many disorders, reducing the time and cost of treatment.
Funds Requested	\$12,356,743
GWG Recommendation	(1-84): Not recommended for funding
Process Vote	<p>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.”</p> <p>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.”</p>

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	6
Highest	75
Lowest	-
Count	15
(85-100): Exceptional merit and warrants funding, if funds are available	0
(1-84): Not recommended for funding	15



FINAL COMMENTS

Proposals were evaluated and scored according to the review criteria described in the PA/RFA. Following panel discussion and scoring, GWG members were asked to provide brief, criterion-specific final comments summarizing the strengths and weaknesses that drove their final score. For each of the review criteria, reviewers entered bulleted final comments on strengths and weaknesses, focusing on major score-driving considerations rather than detailed critique. The comments below reflect the consolidated input of multiple reviewers and have been compiled and edited by CIRM for clarity, without altering substantive scientific content.

Key Strengths and Weaknesses

- Finding common themes in distinct monogenetic developmental epileptic encephalopathies (DEEs) is an interesting idea. Weaknesses include cell composition in the organoids, especially related to interneurons, and whether disease gene related activity differences in the context of the aberrant connectivity of the organoid will model real disease for the purpose of drug development.
- It's unclear in the application whether they are studying homozygous or heterozygous mutations.
- In general, there is concern regarding the feasibility of the main approaches to be used. It is not clear how excitation/inhibition (E/I) balance can be detected and whether master modulators can be identified.
- The generalizability of the findings is also limited due to very few genes being used.
- The goal to find common disease pathways is a good one. Limited technical innovation. It's not clear how biophysical modeling will cope with mutations in genes other than those with direct effects on neural physiology e.g. ion channels. The application does not argue strongly that its goal of finding common pathways will be achieved.

Significance: Evaluate the project's significance and potential for impact

- There is high enthusiasm for the premise that since the pathological effects of neurodevelopmental disorder (NDD) causing mutations generally start long before clinical phenotypes are identified. Also, despite some progress in premorbid genetic testing, focusing on neurotransmission-related phenotypes could have more clinical impact than focusing on disease gene direct molecular function per se.
- DEEs are a collection of severe neurodevelopmental disorders leading to progressive impairment in cognition, behavior, and neurological function. The main goal of the proposed research is to identify convergent targets, namely the cell type-specific gene regulatory networks (GRNs), for developing therapeutic interventions.
- The proposed study will generate a collection of scRNA-seq data and electrophysiology data from cortical organoids.
- Looks at correcting neural networks to fix developmental disorders-seeks common solution to a range of diseases.
- Potential for broader outcome across multiple developmental disorders, but this may be very difficult to realize. Translational Aim 4 still targets correction of specific genes.
- The concept is that these studies will uncover common networks underlying multiple developmental disorders. However, it seems likely that we will still wind up with a myriad of multiple gene targets rather than a few key networks.



- Targeting GRNs as a therapeutic approach has been proposed for many disorders, including neurological disorders. A new aspect of the proposal is to target cell type specific GRNs. The translatability into the clinic is not clear.

Innovation: Evaluate the project for innovation relative to the current state of research

- The application uses very much cutting edge stem cell culture and recording techniques.
- The proposed project aims to identify convergent or master GRNs that control E/I balance across diverse DEEs using human cortical organoids as a model system.
- New computational models will be used to identify cell type-specific GRNs.
- The multidisciplinary team has clinical experts, stem cell biologists, genomicists, electrophysiologists, translational/regulatory experts and computational neuroscientists.
- The application shows limited innovation. Aim 1 is just model generation. In Aim 2 Perturb-seq may further confuse organoid results. No applicant preliminary data are presented for this technique. Other groups have already applied this approach in cortical organoids, but it is complicated.
- Unremarkable set of approaches for cell work. The biophysical model is interesting, and preliminary data supports the notion that it might be able to predict physiological outcomes from those genes with known biophysical functions. Its broader applicability remains uncertain, and how it will be applied for other classes of neurodevelopmental genes is not clear.
- Does snRNA-seq data not already exist for cortical models of many developmental disorders?

Rationale: Evaluate the scientific rationale in the proposal

- Overall scientific rationale is strong in concept, but there are challenges using the organoid system as proposed. A potential problem lies with the lack of evidence that essentially any connectivity within an organoid meaningfully models that connectivity within the cerebral cortex. Neurons will connect and various network properties will emerge from any system in which neurons are grown together in a healthy culture.
- In addition, neurons bring in different neurotransmitters, such as GABA and Acetylcholine, that will also influence the pattern of excitatory activity in any neuronal culture system that is reasonably high density and again, healthy. It is not surprising that making such cultures with neurons whose mutations directly affect specific aspects of neuronal synaptic formation or transmission will have phenotypes. This has been repeatedly demonstrated in "2D+" and organoid cultures. But, since those phenotypes are evolving within what seems to be a highly artifactual, intra-organoid connectivity, how those phenotypes can be used to design circuit-level therapeutic interventions seems questionable at best. It's hard to see how the aberrant connectivity in an organoid will be an improvement over the cheaper and more accessible 2D+ approaches (neuronal subclasses, + human or rodent astrocytes, microglia, endothelial cells).
- There is great enthusiasm for the goal of identifying circuit-level phenotypes that can be used to screen for therapeutic intervention opportunities without being concerned about the many developmental pathologies that unfolded upstream of those phenotypes. But the reviewer would show more enthusiasm for screening a greater number of NDD-related mutations without conducting the extensive gene expression and other characterizations that are more aligned with traditional studies focusing on how a given mutation alters a cascade of developmental programs to ultimately produce neuropsychiatric phenotypes. Stated simply, if a particular target gene improves network function in organoids from one or more mutations, how does the extensive cellular and transcriptomic evaluation of the organoids affect the translatability of that finding?



- The main rationale is that by identifying convergent mechanisms as targets for restoring neural functions one can develop a treatment that can apply to multiple disorders.
- While using patient derived iPSC based models is considered advantageous compared to conventional animal models, there are considerable limitations of the cortical organoid models, especially the maturation status and the lack of some important cell types.
- Circuit dysfunction is involved in many disorders. Is a common fix likely? Evidence is cited from deep brain stimulation (DBS), transcranial magnetic stimulation, and optogenetics. DBS does not alter PD progression, though it provides significant relief for one set of symptoms. Addressing underlying cellular deficits that characterize many developmental disorders may similarly provide relief.
- Preliminary data support the ability to generate organoids and the applicability of the biophysical model to a subset of neurodevelopmental genes. Insufficient data are provided to assess feasibility of perturb-seq in the applicant's laboratory.

Plan & Design: Evaluate the project plan and design

- The above concerns notwithstanding, there is high enthusiasm in concept, if not in practice, for the approach in Aim 4 of targeting genetic therapeutic interventions to specific neuronal subclasses, pyramidal neurons, or major interneuron subclasses, using AAVs with promoter elements that enrich expression in particular subclasses.
- As written, no evidence is presented that in these organoids, at the oldest stage proposed (DIV120), that somatostatin, parvalbumin or vasoactive intestinal peptide interneurons are present. However, they likely are present, if sparse, and, if so, they are present in densities that might be expected to influence circuit activity if stimulated or suppressed genetically.
- There is a lack of characterization of the cerebral organoids generated from neural stem cells. In Figure 1, it appears that interneurons appeared very early, even before excitatory neurons; this is a significant concern. As excitatory and inhibitory neurons are largely produced by different populations of progenitors, this raises major questions of the feasibility of all subsequent experiments proposed.
- Standard analysis of snRNA-seq from the organoids will be performed. Microelectrode array and whole cell recording will also be done using these organoids. However, there is no preliminary data to show that epileptic like phenotypes can be identified using these physiology approaches.
- Using perturb-seq targeting 23 genes to generate mosaic organoids, cell proliferation and gene expression will be examined by snRNA-seq. This approach is complicated, and there are potential strong non-cell autonomous effects that lead to biased presence and development of cells with different mutations.
- Regarding cortical organoids and CRISPR screens: Mosaic organoids are liable to be highly variable. Where is preliminary data on variability and long-term viability? Donor genotype dictates transcriptional variation, how will this limit outcomes?
- Cortical organoids are a reasonable platform for this study but have limitations that are not really acknowledged.
- Inhibitory interneurons are present in the type of cortical organoid described, but they are not present in numbers seen in cortex because most are generated elsewhere (median ganglionic eminence). They are key players in epilepsy. The proposal seems to overlook inherent variability in cortical organoid models, particularly long term ones.
- Budget and timelines are reasonable.



- The applicant's multidisciplinary team includes a clinician expert in epilepsy, gene editing experts, and computational neuroscience. Extensive experience in organoid technology is not apparent. The management plan looks fine.

Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations

- The research team has a track record of engaging patient advocacy organizations associated with epilepsy. There is a plan to engage trainees in the outreach efforts.
- Good track record of outreach to patient advocacy groups.
- DEE patients suffer from early onset and more severe epilepsy, and effective treatments are lacking. The proposed experiments will use iPSC lines derived from patients and genetically modified iPSCs with different DEE genotypes. There is no clear description on the sex and demographic backgrounds, and ethnic groups of those lines being used.
- Genetically diverse cell models will be used.
- The proposal claims that discoveries will be applicable across a range of disorders.