## DISC5 AWARDS

9/24/2026

**$52,425,400** GWG RECOMMENDED

**$52,425,400** CIRM TEAM RECOMMENDED

**$52,500,000** AMOUNT AVAILABLE

**$0** BOARD APPROVED

| | APP # | TITLE | BUDGET REQ | GWG Recmd | CIRM Recmd | SCORE (MEDIAN) | Mean | SD | Score Range Low | Score Range High | Number of GWG Y | Number of GWG N | Resubmission | Previous CIRM Funding |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DISC5-19989 | Advancing next-generation models for the development of thymoregenerative medicines | $2,500,000 | Y | Y | 92 | 92 | 2 | 89 | 95 | 15 | 0 | N | Y |
| | DISC5-20068 | Decoding regulatory sequence function during human brain organoid development | $2,500,000 | Y | Y | 91 | 88 | 11 | 50 | 92 | 14 | 1 | N | N |
| | DISC5-20026 | Leveraging iPSCs and a deep learning-based virtual cell model to identify therapeutic targets for ALS | $2,500,000 | Y | Y | 90 | 90 | 3 | 85 | 95 | 14 | 0 | N | Y |
| | DISC5-20172 | Phoenix WNTears: A Platform for Selectivity Expanding Adult Stem Cell Potency and Plasticity | $2,500,000 | Y | Y | 90 | 88 | 3 | 82 | 90 | 13 | 2 | N | Y |
| | DISC5-19908 | Generating biological age in a stem cell-based complex disease model of osteoarthritis | $2,499,999 | Y | Y | 88 | 88 | 2 | 85 | 92 | 14 | 0 | N | N |
| | DISC5-19839 | Uncovering disease and cell-type-specific constraints on therapeutic delivery using a human CNS tissue discovery platform | $2,500,000 | Y | Y | 88 | 87 | 4 | 80 | 94 | 12 | 3 | N | Y |
| | DISC5-20195 | Decoding Human Pancreatic Progenitor Biology to Enable Next-Generation Stem Cell-based Therapies for Type 1 Diabetes | $2,499,875 | Y | Y | 88 | 86 | 4 | 75 | 90 | 11 | 4 | N | Y |
| | DISC5-20158 | Engineering Nephron Connectivity in Human Kidney Tissue Using Synthetic Signaling Centers | $2,500,000 | Y | Y | 87 | 86 | 2 | 81 | 88 | 13 | 2 | N | Y |
| | DISC5-19638 | Systematic cell-type-resolved single-base resolution functional characterization of neurodevelopmental risk genes | $2,500,000 | Y | Y | 86 | 86 | 1 | 85 | 88 | 14 | 0 | N | Y |
| | DISC5-19747 | Leveraging lessons from normal development to overcome barriers to efficient transplantation of stem cell derived cortical neurons. | $2,500,000 | Y | Y | 86 | 86 | 2 | 80 | 90 | 13 | 2 | N | Y |
| | DISC5-19949 | Neurovascular control of follicle growth using stem-cell derived ovary assembloids | $2,499,997 | Y | Y | 85 | 86 | 2 | 80 | 90 | 13 | 2 | N | N |
| | DISC5-19810 | Impact of Sex-Differences on Intestinal Stem Cell Function in Pediatric Inflammatory Bowel Disease | $2,499,999 | Y | Y | 85 | 86 | 3 | 80 | 90 | 12 | 3 | N | N |

| | APP # | TITLE | BUDGET REQ | GWG Recmd | CIRM Recmd | SCORE (MEDIAN) | Mean | SD | Low | High | Y | N | Resubmission | Previous CIRM Funding |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DISC5-19686 | Allelic Expression States as Determinants of Neurodevelopmental Disorder Risk | $2,500,000 | Y | Y | 85 | 85 | 2 | 80 | 90 | 13 | 2 | N | Y |
| | DISC5-20061 | Characterizing how neurons from human stem cell transplants integrate into spinal neural circuits following injury | $2,499,999 | Y | Y | 85 | 85 | 1 | 83 | 86 | 13 | 2 | N | Y |
| | DISC5-19811 | Mapping Human Retinal Niches to Improve Stem-Cell Therapy for Geographic Atrophy | $2,500,000 | Y | Y | 85 | 85 | 5 | 75 | 90 | 10 | 4 | N | N |
| | DISC5-19893 | Revealing the Mechanistic Architecture Governing CAR-T Cell Potency and Failure at Single-Cell Resolution | $2,499,738 | Y | Y | 85 | 84 | 4 | 75 | 85 | 13 | 2 | N | Y |
| | DISC5-19870 | Foundational Mapping of Splicing-Derived Neoantigen Recognition in Human Sarcoma Using Stem Cell-Enabled Immune Platforms | $2,500,000 | Y | Y | 85 | 84 | 4 | 75 | 93 | 8 | 7 | N | Y |
| | DISC5-19807 | A precision organoid platform to investigate APOE4 dependent cerebrovascular impairments | $2,500,000 | Y | Y | 85 | 84 | 6 | 75 | 90 | 7 | 6 | N | N |
| | DISC5-19957 | Spatiotemporal Control of Human Primordial Germ Cell Fate by the Embryonic Niche | $2,425,796 | Y | Y | 85 | 83 | 3 | 75 | 85 | 9 | 6 | N | N |
| | DISC5-19950 | Temporal AI models for programming therapeutic cell state transitions | $2,499,998 | Y | Y | 85 | 82 | 8 | 60 | 90 | 8 | 7 | N | N |
| | DISC5-19900 | Validating Safety and Efficacy of Extremeophile Stress Tolerance Transgenes in Stem Cells | $2,499,999 | Y | Y | 85 | 82 | 9 | 60 | 95 | 7 | 7 | Y | N |
| | DISC5-20142 | APOE4 compromises BBB integrity through pericyte-microglia cross talk | $2,499,999 | N | N | 84 | 84 | 2 | 81 | 90 | 5 | 8 | Y | Y |
| | DISC5-19775 | Mechanisms driving the tempo of life in human cells | $2,494,573 | N | N | 83 | 83 | 4 | 75 | 88 | 7 | 8 | N | Y |
| | DISC5-19843 | Causes and consequences of age-related myeloid bias | $2,500,000 | N | N | 83 | 82 | 4 | 70 | 85 | 6 | 9 | N | N |
| | DISC5-19705 | Leveraging natural and engineered hematopoietic surface receptor variation for non-toxic stem cell transplantation | $2,481,613 | N | N | 82 | 82 | 4 | 74 | 88 | 6 | 9 | N | N |
| | DISC5-20120 | Systematic Expansion and Decoding of the Cytokine Language for Predictable Stem Cell Fate Control | $2,423,140 | N | N | 80 | 82 | 5 | 75 | 90 | 5 | 10 | N | N |

| | APP # | TITLE | BUDGET REQ | GWG Recmd | CIRM Recmd | SCORE (MEDIAN) | Mean | SD | Low | High | Y | N | Resubmission | Previous CIRM Funding |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DISC5-20042 | Functional dissection of pathogenic splicing defects in neurodegeneration | $2,500,000 | N | N | 80 | 82 | 3 | 80 | 88 | 3 | 10 | N | N |
| | DISC5-19929 | Immunopathogenic Mechanisms of Anti-Hu Paraneoplastic Neurologic Disease | $2,494,912 | N | N | 80 | 79 | 5 | 70 | 87 | 3 | 12 | N | N |
| | DISC5-20198 | Systematic Discovery of Next-Generation Reprogramming Factors | $2,500,000 | N | N | 80 | 79 | 3 | 70 | 80 | 0 | 15 | N | N |
| | DISC5-19717 | Integrating cardiac organoids and in silico models to define the mechanistic roles of sex hormones in Atrial Fibrillation | $2,479,659 | N | N | 80 | 77 | 5 | 70 | 85 | 1 | 14 | N | N |
| | DISC5-20237 | Defining the Neural Stem Cell Nexus: A High-Resolution Proteomic Atlas of Convergent Dysfunction in Autism Spectrum Disorder | $2,499,998 | N | N | 80 | 77 | 6 | 60 | 80 | 0 | 13 | N | N |
| | DISC5-19569 | When “young” cells meet old tissues: mechanistic impact of host senescence on human iPSC-derived musculoskeletal tissues | $2,499,998 | N | N | 75 | 76 | 4 | 65 | 82 | 0 | 15 | N | Y |
| | DISC5-19787 | Unlocking the Brain's Hidden Immune Regulatory Cells: A New Path to Alzheimer's and Parkinson's Cures | $2,498,937 | N | N | 75 | 74 | 7 | 65 | 85 | 1 | 13 | N | N |
| | DISC5-19580 | Population-wide deimmunization of gene therapy cargos | $2,500,000 | N | N | 75 | 73 | 3 | 65 | 75 | 0 | 15 | N | N |
| | DISC5-19851 | 3D Bioprinted Cortical Organoids with Human-Chimpanzee Chimeric White Matter for Exploring Evolutionary Constraints on Human OPC Maturation Capacity | $2,499,999 | N | N | 75 | 73 | 5 | 65 | 80 | 0 | 14 | N | N |
| | DISC5-19822 | Elucidating and targeting -syn and neurodegeneration in patient stem cell models of Parkinson’s disease with AI, chemical biology and robotics | $2,499,996 | N | N | 70 | 72 | 3 | 70 | 75 | 0 | 14 | N | N |
| | DISC5-19844 | Minimal invasive approach for monitoring stem cell and gene therapy | $2,499,989 | N | N | 70 | 70 | 5 | 60 | 80 | 0 | 15 | N | N |
| | DISC5-19646 | Stem cell-based approaches to dissect the neuro-immune axis in aging-associated peripheral neuropathies | $2,499,943 | N | N | 70 | 69 | 2 | 65 | 70 | 0 | 15 | N | N |
| | DISC5-19927 | The Virtual Stem Cell | $2,499,998 | N | N | 70 | 68 | 4 | 55 | 70 | 0 | 15 | N | Y |
| | DISC5-20093 | AI-Guided Model Discovery for human stem cell differentiation | $2,478,299 | N | N | 70 | 67 | 4 | 60 | 70 | 0 | 15 | N | N |

| | APP # | TITLE | BUDGET REQ | GWG Recmd | CIRM Recmd | SCORE (MEDIAN) | Mean | SD | Low | High | Y | N | Resubmission | Previous CIRM Funding |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | DISC5-19876 | Efficient Somatic Cell Reprogramming through AI-Guided Transcription Factor Control (FateFactor) | $2,499,981 | N | N | 70 | 64 | 9 | 48 | 75 | 0 | 15 | N | N |
| | DISC5-20094 | Autoimmunity and Personalized Medicine: Can Blood Stem Cells Predict Future Disease? | $2,500,000 | N | N | 65 | 66 | 6 | 55 | 75 | 0 | 15 | N | N |
| | DISC5-19805 | Elucidating the impact of deleterious coding variants on serious mental illness phenotypes | $2,499,995 | N | N | 65 | 64 | 7 | 50 | 70 | 0 | 15 | N | N |
| | DISC5-19543 | Engineering Human Limb Gastruloids for Limb Morphogenesis and Congenital Malformation Modeling | $2,499,997 | N | N | 50 | 47 | 8 | 35 | 60 | 0 | 15 | N | N |

| Application# | DISC5-19989 |
|---|---|
| **Title**<br>(as written by the applicant) | Advancing next-generation models for the development of thymoregenerative medicines |
| **Project Objective & Impact**<br>(as written by the applicant) | The thymus is essential for producing T cells that protect against infections and maintain immune tolerance. Most knowledge comes from animal studies, leaving gaps in our understanding of human thymic function. This research will address these gaps, creating new models to study thymic defects and test new therapies, ultimately benefiting patients with immunodeficiencies and autoimmune diseases. |
| **Specific Aims**<br>(as written by the applicant) | • Identify and validate signals that promote sustained expansion of human thymic epithelial cells<br>• Identify and validate factors controlling differentiation of human thymic epithelial progenitors into functional epithelial subsets. |
| **Statement of Benefit to California**<br>(as written by the applicant) | This research will greatly advance our ability to study the human thymus, an organ critical for training the immune system to fight foreign bodies, infections, and cancers. It will create a roadmap for growing tissue in the lab, generating new tools to study thymic defects, and enabling the testing of regenerative therapies. These studies thus have broad relevance, potentially benefiting patients with immunodeficiencies, autoimmune diseases, and other conditions impacted by thymic dysfunction. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>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: 92**

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

| | |
|---|---|
| **Mean** | 92 |
| **Median** | 92 |
| **Standard Deviation** | 2 |
| **Highest** | 95 |
| **Lowest** | 89 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 15 |
| **(1-84): Not recommended for funding** | 0 |

# FINAL COMMENTS

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

**Key Strengths and Weaknesses**

- This is an excellent proposal that is well-designed and leverages exciting new models for human thymic cell differentiation/organoids and primary human thymus single-cell datasets. While the implications for rare thymic development defects and treatment-induced needs to boost T cell reconstitution are clear, the potential for benefit with aging is less well discussed/developed. Overall, enthusiasm is high.
- This is a well-written proposal with strong preliminary data supporting feasibility.
- The significance of the overall aims of the project, the strength of the preliminary data and the approach outlined in the plan, and the quality of the investigators are cause for high enthusiasm.

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

- This proposal is focused on a significant goal -- to define the intrinsic and extrinsic mechanisms that govern expansion, differentiation, and functional specialization of human thymic epithelial progenitors (TEPs) and thymic epithelial cells (TECs).
- If successful, the work will not only advance foundational knowledge for human thymus regeneration but will also have broad implications for potential new therapies, in vitro mechanistic work, and as new sources of human thymus for humanized mouse models.
- The ultimate goal of this proposal is to establish a robust in vitro platform to study the human thymus and accelerate the discovery of novel regenerative therapies. If successful, the project will significantly advance our understanding of thymus biology, enable the generation and expansion of thymic epithelial progenitors (TEPs) in vitro, and open new avenues for thymus regeneration in vivo.
- Given the central role of the thymus in immune competence, these advances have broad implications for aging, immune deficiency, and regenerative medicine across multiple disease contexts.
- Success of this project has the potential to provide key biologic insights into factors regulating expansion of thymic epithelial cells (TECs), and factors controlling differentiation of TECs into functional subsets.
- The mechanistic knowledge gained from successful project completion could inform development of future therapeutics for restoring thymic function in subjects with congenital or acquired (e.g., following cytotoxic therapy) immune deficiency, treating or preventing autoimmune disease, and reversing thymic involution. These have potential implications for health span and lifespan.

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

- While FOXN1 is a well-established key regulator of thymic development, there remain open questions about how its expression is regulated. This will be addressed here in hPSC-derived human thymus stromal models.
- Besides interrogation of basic biology, this proposal also aims to advance approaches to thymic regeneration, which has relevance for translation to the clinic.

- Novelty is apparent in the combination of approaches used, which has been informed by work with both human and mouse thymus and thymic organoids. Key single-cell datasets on thymic cells across ages is a novel resource that will be impactful.

- This proposal brings together two investigators with complementary expertise in thymus biology and regeneration. The Principal Investigator has developed one of the first protocols to differentiate thymic epithelial cells from hPSCs, while the Co-Investigator has established robust methodologies for generating thymic organoids. Their combined expertise, together with state-of-the-art stem cell, organoid, and functional genomics approaches, positions the team strongly to address this important challenge.

- Key innovative aspects include:

1. The establishment of scalable, human stem cell–derived thymic stromal platforms capable of supporting sustained TEP expansion and differentiation into functional thymic epithelial cells (TECs).

2. The implementation of a CRISPR-based gain-of-function screen in FOXN1 reporter hPSC-derived TEPs, enabling unbiased identification of signaling pathways regulating FOXN1 activation and thymic epithelial fate.

3. The systematic interrogation of thymic epithelial specification using a mechanistic framework that integrates signaling inputs, spatial organization and cell-cell interactions.

- This project establishes scalable, human SC-derived thymic stromal platforms capable of supporting both sustained TE progenitor (TEP) expansion and differentiation into functional TECs, which are not achievable with animal models or primary human tissue alone.

- The project integrates organoid culture, genetic engineering, and functional genomics in human TEC progenitors, enabling causal discovery approaches that are not feasible in current systems.

- By coupling FOXN1-centered reporter models with CRISPR-based gain-of-function screening, this work identifies activating regulatory pathways that are inherently more amenable to pharmacologic or biologic modulation, creating a direct bridge from discovery to therapeutic development.

**Rationale: Evaluate the scientific rationale in the proposal**

- Use of hPSC to derive TEPs and TECs is a strong approach to address unmet needs for regenerating thymus tissue.

- The preliminary data are compelling, and the expertise of the group is well matched for the proposed work.

- The scientific rationale is generally sound and supported by strong preliminary data demonstrating feasibility of key components of the proposed system, including hPSC-derived TEP differentiation, FOXN1 reporter line generation, and thymic organoid culture systems.

- The team presents strong preliminary data, including successful in vitro differentiation of TEPs from hPSCs, establishment of a FOXN1 reporter line, and derivation of primary thymic organoid cultures from both mouse and human tissue. They also demonstrate genome editing capability in these systems and provide single-cell RNA sequencing datasets of human thymic stroma across developmental stages, which serve as a valuable reference atlas for the proposed studies.

- A key strength is the establishment of a 3D air-liquid interface system capable of supporting T cell development from hematopoietic stem cells, providing a functional readout for thymic epithelial activity.

- The proposed research is based on sound scientific rationale. Prior CIRM support was critical in supporting research on methods to differentiate hPSCs into TEPs. These methods form the basis for the research proposed in the current application.

- Preliminary data is extensive and strongly supports the rationale and feasibility of the current project. Transplantation of hPSC-derived TEPs into athymic nude mice yields functional TECs that recruit and select T cells that are functional and have a diverse T cell receptor repertoire.

- The organoid culture system first established with mouse thymic tissue has now been extended to human thymic tissue obtained from pediatric donors at the time of cardiac surgery. But the TECs obtained from latter organoid system are not all positive for key thymocyte markers emphasizing the need to identify additional factors critical for full and efficient differentiation as proposed.

- Genome engineering using CRISPR-CAS system has been achieved in murine thymocytes and this provides basis for exploiting this method in human thymocytes. This will allow identification of genes key for the full differentiation of target cells.

- Likewise, scRNA-seq has been performed on human thymus tissue to identify the diverse cell types making up the thymic stroma. This should allow identification of key genes for development and function.

**Plan & Design: Evaluate the project plan and design**

- The proposal aims to dissect the signals required to support long-term growth of undifferentiated progenitor cells and their ability to produce diverse functional TEC subtypes.

- It is unclear if reduced thymic capacity, which begins declining quite early in life, occurs to promote host fitness in some currently unknown way. While it is easy to envision how declining thymic function has detrimental effects for production of naive cells with capacity to respond to new threats, perhaps there will be currently unforeseen detrimental impacts of therapeutically boosting thymic function in older age (e.g., autoimmunity? loss of immunological memory?). This should be considered.

- The proposed work is logically structured. Aim 1 focuses on identifying signals that regulate TEP expansion using both primary and hPSC-derived systems. This includes systematic testing of cytokines, growth factors, and extracellular matrix components, complemented by co-culture experiments with non-epithelial thymic populations.

- The inclusion of age-stratified stromal donors (neonatal, infant, adult) is a particularly strong aspect and will enable interrogation of age-dependent functional decline. If differences in TEP-supporting capacity are observed, scRNA-seq-based analyses will be used to identify candidate mediators, which will then be functionally validated.

- Aim 2 focuses on identifying factors that enhance FOXN1 expression and delineating signaling pathways that guide differentiation into specialized TEC subsets.

- While highly promising, some aspects of the experimental design would benefit from further clarification, including the definition of intermediate differentiation states, whether specific growth factors will be tested alone in combination or sequentially, and how efficiency of inducible systems will be assessed (i.e., what is the frequency of cells responding to Dox?).

- A key concern relates to the potential silencing of inserted constructs, even when targeted to the AAVS1 locus, particularly in differentiated cellular contexts. The applicants appropriately acknowledge this limitation and propose alternative strategies, including methyltransferase inhibitors and alternative CRISPRa-based approaches.

- The proposed inclusion of thymic mesenchyme and other supportive cell populations is a major strength. However, additional consideration of developmental stage (e.g., neonatal versus adult mesenchyme) in co-culture experiments in Aim 2 may further enhance biological relevance and interpretability.

- The project is extremely well designed and is highly likely to yield meaningful results. Each of the project's aims and sub-aims are described in detail in the application and combine to give confidence in providing important, useful and novel data on the critical questions addressed.
- Pitfalls are identified for each aim/sub-aim and alternative approaches identified should the proposed methods fail to yield results, e.g., if factors added to the differentiation media are ineffective the use of small molecule chemical libraries is a viable alternative.
- The budget appears to be appropriate, but given the ambitious goals of the project, There are some concerns about the timeline being adequate.
- The project team is very strong, with an established (since 2014) and effective collaboration between these two labs that bring complementary strengths. Their track record of significant accomplishments to date gives confidence (but of course not a guarantee) of success in bringing the proposed project to successful completion. The staffing for the project and the resources and environment are superb. Importantly, the access to human thymic tissue has been assured.
- A detailed management plan and team communication plan for the collaboration is provided in the application.

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

- Appropriate.
- The proposed study demonstrates strong consideration of population diversity and biological variability. The team plans to validate findings across multiple hPSC lines and primary tissue donors, enhancing robustness and reproducibility.
- Sex-based differences will be explicitly considered and the inclusion of donors across a developmental spectrum (pediatric to adult) will enable systematic evaluation of age-dependent differences in thymic progenitor function and niche support.
- The inclusion of donors with diverse HLA haplotypes and genetic backgrounds strengthens the generalizability of findings and ensures broader applicability across populations.
- The applicants have taken differences in sex and in genetics (HLA haplotype) into account in designing their studies so that results will be broadly applicable.
- The applicants point out that target diseases disproportionately affect minority populations in California which enhances potential applicability of their discoveries.
- The application describes the outstanding mentoring record of the investigators and outlines their involvement with a number of relevant groups and organizations that will assure effective outreach, partnership and educational efforts.

| Application# | DISC5-20068 |
|---|---|
| **Title**<br>(as written by the applicant) | Decoding regulatory sequence function during human brain organoid development |
| **Project Objective & Impact**<br>(as written by the applicant) | The majority of genetic traits that are associated with diseases is located in regions of the genome that are difficult to study, because they regulate genes during human body development, and they are often far away from the genes they regulate. This project will provide new powerful tools to study how the sequences work, by enabling the use of stem cell-derived organoids for these studies. |
| **Specific Aims**<br>(as written by the applicant) | • Define poised and active CREs in each cell type during brain organoid development<br>• Establish Dual-TSS-MPRA in sorted organoid cell populations<br>• Develop single-cell-Dual-TSS-MPRA and demonstrate its utility to characterize CREs during organoid development |
| **Statement of Benefit to California**<br>(as written by the applicant) | The proposed project will contribute to the State of California's strong stem cell research focus by developing new methods that will enable researchers to use stem cell-derived organoid differentiation as a model to study genetic variants that affect human neurodevelopment or contribute to other disorders. Insights from these studies will provide better genetic diagnostics and help identify genomic targets for new regenerative medicine cures for genetic disorders that affect many Californians. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

**Final Score: 91**

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

| | |
|---|---|
| **Mean** | 88 |
| **Median** | 91 |
| **Standard Deviation** | 11 |
| **Highest** | 92 |
| **Lowest** | - |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 14 |
| **(1-84): Not recommended for funding** | 1 |

# FINAL COMMENTS

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

| **Key Strengths and Weaknesses** |
|---|
| • This proposal presents a highly innovative and technically sophisticated approach to studying regulatory sequence function during human neurodevelopment, supported by strong preliminary data, a compelling interdisciplinary strategy, and the potential to create a broadly useful platform for functional interrogation of noncoding disease-associated variants in human organoid systems.<br>• Strength: This is a detailed and technically sophisticated project that will provide high-resolution maps of accessible and transcriptionally active cis-regulatory elements (CREs). While there are many potential technical caveats that need to be considered, the experience of the group, which has established many of the tools, will provide feasibility and will generate data that will advance the field in a critical manner.<br>• This project capitalizes on the power of organoid models and will be the next step toward gaining an understanding of gene dysregulation in virtually all developmental disorders.<br>• Weakness: Experimental details are scant, although the group has published many of the approaches.<br>• This is an exceptional functional genomic approach and the first application of single-cell MPRA to brain development. |
| **Significance: Evaluate the project's significance and potential for impact** |
| • This proposal addresses a central gap in understanding how cis-regulatory elements (enhancers) control gene expression during human development, particularly in complex brain tissue. By enabling functional testing of thousands of regulatory sequences in human stem cell-derived organoids during differentiation, it has reasonable potential to advance foundational knowledge of gene regulation in relevant developmental contexts.<br>• The proposal introduces a broadly applicable framework for high-throughput, cell type- and stage-specific analysis of regulatory DNA in developing human tissues. This could significantly improve how enhancer function is studied, though the advance is primarily methodological rather than conceptual.<br>• The approach is well positioned to generate mechanistic insight into how sequence variation alters enhancer activity and transcriptional programs, informing interpretation of disease-associated variants. However, the path to direct therapeutic or biomarker development is largely indirect.<br>• Human organoid models offer a unique opportunity to understand distal gene regulatory elements during human development.<br>• Elucidating the role of these regulatory elements is critical, considering that the majority of developmental trait- and disease-associated gene variants occur in CREs, which play a role in regulating gene expression during differentiation.<br>• The proposal provides a comprehensive and unbiased dissection of the genetic regulatory logic of brain development. |
| **Innovation: Evaluate the project for innovation relative to the current state of research** |

- The proposal demonstrates good integration of functional genomics, genome engineering, single-cell sequencing, and stem cell-derived organoid models. The combination of locus-specific CRISPR-based reporter integration with MPRA and single-cell readouts during differentiation represents meaningful cross-disciplinary synergy, although most individual technologies are established.
- A key innovation is the development of a framework for functional interrogation of regulatory DNA directly within developing human tissues rather than static systems. The proposal also introduces Dual-TSS-MPRA and its single-cell adaptation, enabling simultaneous measurement of enhancer activity and transcription initiation at base resolution during organoid development. While conceptually impactful, the framework largely extends emerging approaches in functional genomics.
- A technical strength is the use of locus-specific reporter integration to minimize position effects and reporter silencing. Integration of single-cell MPRA with organoid differentiation and targeted 5' scRNA-seq is innovative and could provide a valuable platform for studying developmental regulatory elements. Several components remain technically complex and not yet validated at the proposed scale, particularly for longitudinal single-cell analyses in organoids.
- The applicants developed a reporter strategy that allows measurement of enhancer activity on the target promoter as well as enhancer transcription initiation at base resolution. This is a very sophisticated model and a novel approach.
- The proposal represents a state-of-the-art application of bulk and single-cell MPRA to human organoids toward uncovering cell type-specific, genetically regulated gene expression.

**Rationale: Evaluate the scientific rationale in the proposal**

- The scientific rationale is strong and well justified. The proposal addresses an important limitation in studying human enhancer function during development, particularly the difficulty of testing regulatory elements in dynamic developmental systems rather than static cell lines.
- The proposal is supported by good preliminary data, including the development of Dual-TSS-MPRA. These data support the feasibility of the core platform, although important technical risks remain around reporter stability, library complexity, and single-cell sensitivity at scale.
- The experimental approaches are generally appropriate. The integration of organoids, genome engineering, single-cell transcriptomics, and MPRA technologies is appropriate for studying enhancer function during human neurodevelopment. Nevertheless, the overall strategy is technically ambitious, and successful integration of multiple developing methods may be difficult.
- As CREs are mainly active during development and cell differentiation, it is logical to use organoids to understand their role. The rationale is straightforward.
- Aim 1 will use standard omics approaches to define poised and active CREs in brain organoids on a cell type-specific basis.
- The establishment of a Dual-TSS-MPRA approach in sorted organoid cell populations is interesting and should yield highly informative results.
- The establishment of a single-cell Dual-TSS-MPRA that provides information about cell-to-cell heterogeneity is an important extension.
- The rationale is clearly written and applies an innovative new scMPRA approach to complex human organoids for the first time.

**Plan & Design: Evaluate the project plan and design**

- The project is logically structured and aligned with the goal of linking regulatory sequence to function during differentiation and is likely to generate mechanistic insights. The integration of multi-omic profiling with functional perturbation is a strength, though technically complex.
- The main risks are identified, including reporter silencing, position effects, limited library complexity, and single-cell detection sensitivity, and reasonable mitigation strategies are provided.
- The scope is likely excessively ambitious for a 3-year project, given the need to simultaneously develop and optimize multiple technically demanding components. Moreover, the interdependence of these components introduces substantial execution risk.
- The team is a strength of this proposal, with complementary expertise in the proposed methodologies. The communications plan is appropriate.
- Experimental details are missing. For example, what organoids will be used? Dorsal- or ventral-directed or self-assembled organoids? Considering the vast amount of work that is conducted using these organoids, a better description would have been helpful.
- Any type of organoid contains multiple neuronal and progenitor states and cells at different maturation stages; thus, rare cell types could disproportionately drive effects in single-cell readouts. How is this controlled for?
- At later time points, cells in the same organoid will differ along a developmental timeline. How can state-specific regulation be distinguished from stochastic promoter behavior?
- Delivery of MPRA libraries might be uneven. How is this controlled?
- In single-cell Dual-TSS-MPRA, each construct may be observed in only tens of cells, leading to potentially incorrect directionality estimates. This should be discussed.
- There are no major score-driving weaknesses.

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

- There is some consideration of genetic background and sex. The focus on non-coding regulatory variation, including variants linked to NDDs, supports potential broad relevance. The team is committed to training and outreach.
- There is a strong plan for genetic findings to benefit trans-ancestry analyses.

| Application# | DISC5-20026 |
|---|---|
| **Title**<br>(as written by the applicant) | Leveraging iPSCs and a deep learning-based virtual cell model to identify therapeutic targets for ALS |
| **Project Objective & Impact**<br>(as written by the applicant) | ALS is a complex disease and there is a pressing need for new therapeutic strategies that rescue multiple forms of ALS, particularly those with unknown genetic etiologies. Our study will use a deep learning-based virtual cell model and iPSCs to identify and validate new therapeutic targets with broad efficacy on ALS patient neurons. |
| **Specific Aims**<br>(as written by the applicant) | • Aim 1. Train the STATE deep learning model on ALS patient iN perturbation datasets.<br>• Aim 2. Identify and validate new broadly-efficacious therapeutic targets on ALS iNs. |
| **Statement of Benefit to California**<br>(as written by the applicant) | California is the state with the most ALS patients in the country. The new therapeutic targets identified in this study may lead to effective treatments for all forms ALS, including the sporadic population. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

**Final Score: 90**

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

| | |
|---|---|
| **Mean** | 90 |
| **Median** | 90 |
| **Standard Deviation** | 3 |
| **Highest** | 95 |
| **Lowest** | 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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel’s discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in

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

| **Key Strengths and Weaknesses** |
|---|
| • Strengths: First, significance—there is no cure for ALS, and this could help many patients. Second, innovation—using AI is new and powerful. Third, rationale—there are strong preliminary data. Fourth, population impact—the project includes diverse patient cells across both sexes, ages, and genetic forms.<br>• Weaknesses: First, the timeline is too tight—only 15 months for testing. Second, the Data Project Manager is not described. Third, AI accuracy is modest—60% in cancer cells and unknown in ALS. Fourth, it is unclear what will be learned if the AI fails. |
| **Significance: Evaluate the project's significance and potential for impact** |
| • The proposal directly addresses the "n=1" problem that has limited ALS therapeutic development; identifying broadly efficacious targets across diverse patient backgrounds could materially change the field's drug-development economics and potentially its success.<br>• ALS has no curative therapy and very limited mechanism diversity in clinical therapeutics; successful broad targets would be transformative.<br>• Outputs (ranked therapeutic targets, validated ASOs, mechanistic insight via transcriptomic readouts) are immediately actionable for downstream development.<br>• The proposal builds on prior CIRM- and R01-supported infrastructure that materially de-risks execution: an ALS biobank, the GENEVA-ALS platform, and the Tahoe-100M and STATE foundations.<br>• Significance is primarily limited to ALS.<br>• The proposal would be strengthened by an explicit articulation of what level of model accuracy or hit rate would be considered sufficient to advance specific candidates to preclinical work.<br>• The project will develop a new in silico model of ALS, "STATE-ALS," capable of predicting how genetic and chemical perturbations affect cellular function. This is highly significant, as no model is known to integrate these large datasets, including public datasets potentially relevant to ALS.<br>• New therapeutics could be discovered using the models advanced in this project.<br>• This project tackles a major ALS bottleneck: finding therapies that work across many genetic backgrounds. Most screens test only a few patient lines, missing broadly effective targets. STATE-ALS, a virtual cell model trained on iPSC-derived neurons from diverse ALS patients, will predict which perturbations restore healthy cell states. Success would provide a scalable platform to identify targets for sporadic and familial ALS, greatly accelerating drug discovery. |
| **Innovation: Evaluate the project for innovation relative to the current state of research** |
| • STATE is a virtual cell model enabling the prediction of responses to perturbation in cancer cells. It was able to predict perturbations not in the training dataset and at single-cell resolution.<br>• Here, learning will be transferred from STATE, a foundation model (pretrained on Tahoe-100M, ~100M cells, ~1,100 perturbations), to a neuron-specific ALS model. |

- GENEVA-ALS cell-village-in-a-dish reduces batch effects and is a methodological innovation for handling iPSC heterogeneity in screens.
- Multimodal training that incorporates Project MinE WGS data (8,835 cases, 2,550 controls) into the predictive model adds value to target ranking through actual genome-level changes.
- The ASO-based validation strategy for top hits is conventional once targets are nominated.
- This project is highly synergistic across computation and experimentation.
- The project is innovative in its integration of approaches, virtual cell modeling, iN technology, and CRISPR screening.
- The proposal is highly innovative. STATE predicts transcriptomic responses to perturbations at single-cell resolution, capturing biological heterogeneity. The proposed approaches will enable in silico screening across diverse patient backgrounds, overcoming a key hurdle in ALS drug development.

**Rationale: Evaluate the scientific rationale in the proposal**

- Preliminary GENEVA-ALS data are excellent: runs across 4 control, 5 C9ORF72, 2 TARDBP, and 5 sporadic ALS lines, with VAV2 and CCDC146 ASOs already validated as suppressing TDP-43 cryptic exon inclusion across patient backgrounds.
- A genome-wide CRISPRi screen in TARDBP iNs already exists for cross-referencing STATE-ALS predictions, which strengthens the target-prioritization workflow.
- Integration with genetic data (Project MinE rare-variant burden, GWAS) provides well-justified multimodal target ranking further correlated with disease genetics.
- The proposal leans heavily on TDP-43 cryptic-exon readouts as the primary efficacy metric, which is a relatively narrow definition of phenotype.
- STATE's ~60% DEG accuracy and AUPRC of ~0.6 are honest but modest; whether these transfer to ALS iNs at acceptable fidelity is uncertain and is the central scientific risk.
- The preliminary data support the approach. There are many donors and genotypes considered in the preliminary work, which speaks to the broad applicability of the innovative technique.
- The aims are tied to significant training data and could lead to new discoveries within that data for ALS patients.
- The rationale is strong and well supported. Preliminary data show VAV2 and CCDC146 suppression improves survival and restores TDP-43 function across C9, TDP-43, and sporadic ALS iNs. Genetic analyses link CCDC146 to patient survival. STATE already works in cancer cells with high accuracy. Preliminary GENEVA-ALS data correctly predict known drug effects.

**Plan & Design: Evaluate the project plan and design**

- The two-aim structure is appropriately scoped for three years, with defined timing (months 1–21 for Aim 1; months 22–36 for Aim 2).
- Pitfalls and alternatives are well articulated.
- The decade-long collaboration with prior R01 success is a strong signal for team functioning.

- Use of FDA-approved positive controls (tofersen for SOD1) and well-defined negative controls strengthens internal validity.
- The data-sharing overview is appropriate; raw and processed scRNA-seq data plus model weights would be valuable community resources.
- The medicinal-chemistry handoff for compound optimization is acknowledged as a future need; this leaves translation past target identification dependent on outside partnerships not formalized in the proposal.
- Aim 2 (validation, mechanism, GENEVA-ALS efficacy assessment) is compressed into 14 months; this is feasible but tight for the time frame.
- The project is overambitious.
- For a DISC grant, it is unclear how the team will know if the model fails to predict novel ALS targets in iNs. The validation of the hits is truly only done in clinical trials. If it does fail, what will be learned?
- The plan is rigorous and well structured, with clear aims, alternative strategies, and a large ALS patient panel (C9, sporadic, TDP-43, SOD1, FUS). Minor concerns include that Aim 1 takes 21 months, leaving only 15 months for validation; the Data Project Manager role is not well described; and the PI has significant effort on other active grants.

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

- The iPSC cohort is intentionally balanced by sex and age decade and spans ALS subtypes (sporadic, C9ORF72, SOD1, TARDBP), which is well matched to the heterogeneity STATE-ALS is designed to capture.
- The ethnic-diversification effort through sample collection is a credible step to make the iPSC cohort more representative of the California population.
- The team holds one of the largest characterized sporadic ALS iPSC cohorts that displays disease phenotypes, which is a real population-relevance asset.
- The PI's leadership role with ALS Network (largest California ALS patient foundation) and team participation in ALS Network events are project-relevant outreach activities.
- The proposal could explicitly state how STATE-ALS will be evaluated for differential performance across sex and ancestry strata.
- The ALS patient panel is well considered and accounts for the genetic, environmental, and other external factors that may influence research findings.
- The team has a strong track record of working with ALS patients and in translational science involving ALS.
- The application addresses population impact reasonably well. The iPSC cohort is balanced for sex and age and includes familial and sporadic lines. Efforts were made to include diverse ethnic backgrounds using California hospital samples.
- Validation across a large patient panel enhances generalizability. Environmental factors are not discussed, but the focus on genetic diversity is appropriate.

| Application# | DISC5-20172 |
|---|---|
| **Title**<br>(as written by the applicant) | Phoenix WNTears: A Platform for Selectivity Expanding Adult Stem Cell Potency and Plasticity |
| **Project Objective & Impact**<br>(as written by the applicant) | Many chronic diseases persist because we cannot reliably shift human tissue stem cells into a repair mode, especially with aging and degenerative disease. This project defines a selective, controllable WNT/FZD7 signal that enables non-genetic reprogramming to expand stem cell regenerative capacity, opening a path to safer, more effective healing in skin and other epithelial tissues. |
| **Specific Aims**<br>(as written by the applicant) | • Determine the role of FZD7 in Wnt-driven epithelial stem cell plasticity during wound repair<br>• Defining the mechanisms that regulate FZD7-driven fate decisions in human stem cells<br>• Map the spatial and molecular responses of FZD7 activation during human wound repair |
| **Statement of Benefit to California**<br>(as written by the applicant) | By defining a non-genetic strategy to expand epithelial stem cell potency, this project will reveal how regenerative capacity can be enhanced for both transplantation and in situ tissue repair. If successful, it will establish a foundation for safer regenerative strategies to improve healing from chronic wounds, epithelial injuries, and age-related declines in repair, while strengthening California's stem cell workforce and accelerating translation to clinical applications. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

**Final Score: 90**

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

| | |
|---|---|
| **Mean** | 88 |
| **Median** | 90 |
| **Standard Deviation** | 3 |
| **Highest** | 90 |
| **Lowest** | 82 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 13 |
| **(1-84): Not recommended for funding** | 2 |

# FINAL COMMENTS

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

**Key Strengths and Weaknesses**

- An outstanding team using a unique model with a single WNT ligand/receptor pair will illuminate with great precision the regulation of an adult lineage with two hierarchical stem cell compartments. The project also has the potential to contribute significantly to improved therapies for severe skin wounds and other regenerative medicine applications.
- This is a highly significant study, and it is largely well designed. It is a potentially meritorious application, but several aspects of the proposed mouse model (Aim 1) and human-to-mouse skin grafts (Aim 3) raise concerns.

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

- Despite efforts to develop cell-based products for wounds and burns, treatment of such injuries remains a major unmet medical need. Better understanding of the production of epidermal stem cells via "repurposing" of hair follicle stem cells should contribute substantially to the development of regenerative medicine skin therapies.
- WNT has long been recognized as a key regulator in the development, maintenance, and repair of multiple tissues. The project should contribute to a more precise understanding of the biological functions of WNT proteins by focusing on a single WNT ligand-Frizzled receptor pair that plays a critical role in skin biology.
- The overall objective of this proposal is to better understand how Wnt signaling acts on hair follicle stem cell (HFSC) potency and then to use the resulting knowledge to enhance tissue regeneration and wound repair.
- Increasing stem cell potency is an important objective, even if part of this potency likely relies on the niche, ECM, etc., which are more directly impacted by disease and age, and could have an impact on regenerative therapy.
- This project has the potential to be high-impact and to address interesting questions in the adult stem cell field.
- The goal of this study is to examine if selective Wnt pathway activation through the Frizzled 7 (FZD7) receptor in human hair follicle stem cells (HFSCs) may be used to efficiently regenerate human skin following injury. If successful, the study will significantly advance stem cell science and regenerative medicine.
- The study will advance mechanistic understanding of human skin regeneration and might help to identify effective Wnt-based therapeutic approaches to promote healing of chronic wounds and severe burns.

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

- While it is well established that WNT is a key regulator in multiple biological contexts, focusing on detailed analysis of a single WNT ligand/Frizzled receptor pair (among a family of 10) in stem cell biology and, particularly, wound healing is significantly innovative.

- The focus on Wnt is not really new. This is a classic aspect of the stem cell field. The main issue is that Wnt controls proliferation. This effect can sometimes be difficult to distinguish from a direct effect on plasticity or cell fate choice. It could have been interesting for this proposal to explore potential interplay, as Wnt is unlikely to be the master regulator of stem cell potency.
- Aim 1 brings little novelty since it is based on conventional experiments in mouse models. However, Aims 2 and 3 are definitely more novel, as they will take advantage of human models.
- Technical innovation is strong. The use of a human Wnt mimetic is interesting and could help to address novel questions. The use of human models is also very relevant and will help to better understand species divergence.
- The investigators will apply the knowledge gained from the earlier mouse studies demonstrating a functional role of mHFSCs in skin regeneration to the human system.
- The investigators will use a humanized transgenic mouse model (HF7) expressing human FZD7, as well as a human-selective Wnt mimetic that specifically reacts with human but not mouse FZD7; both reagents have been previously developed in the Co-Investigator's lab. Overall, the proposed project is moderately innovative.

**Rationale: Evaluate the scientific rationale in the proposal**

- The rationale is well grounded in knowledge of skin biology, especially stem cell populations and wound healing. Models are well designed to use human or human-like modified receptors and ligands, and in some cases human cells in immune-deficient mice.
- Feasibility is strongly supported by previous work on development and repair, especially of the skin. The ability of hair follicle stem cells to give rise to "classic" epidermal stem cells is well established in high-impact publications from the PI's former group, in which the PI was a postdoc and with which she still collaborates.
- Similarly, prior work from the Co-Investigator, and his mentors and collaborators, on signaling via WNT/Frizzled ligand-receptor interactions provides a compelling rationale for the use of an engineered WNT ligand that binds selectively to a single receptor (FZD7) among a 10-member family.
- The rationale is sound, even if focusing only on Wnt might not generate the expected outcome. This might not be the right pathway to modulate stem cell potency, or it might not act in isolation. Thus, the core objective of this grant might be difficult to achieve.
- The proposal is well organized and rational. The preliminary data are convincing and support the lead hypothesis. The focus on Wnt is not new but remains of major interest in the adult stem cell field.
- The use of mouse models is well justified and strongly supported by human models in vitro and in xenografts.
- The rationale is based on the hypothesis that selective WNT/FZD7 signaling can broaden stem cell developmental plasticity by remodeling the epigenetic state and inducing an open chromatin state. The applicants propose that this chromatin remodeling will support efficient, scarless wound healing and skin regeneration mediated by hHFSCs.
- The rationale of the project is supported by promising preliminary data obtained in the humanized HF7 mouse and with the human Wnt mimetic F7L6.

**Plan & Design: Evaluate the project plan and design**

- The core focus on a single engineered WNT ligand signaling through one receptor target in the Frizzled family is a great strength in a project that aims to illuminate how WNT exerts its potent effects in stem cell biology, development, and cell differentiation. This should enable much clearer interpretation of consequent changes in gene expression and chromatin structure (epigenetics) and will illuminate the precise role of WNT in stem cell lineages, especially in the skin.
- The PI and Co-Investigator constitute an exceptional team. Both trained in outstanding laboratories and have also shown a high level of productivity and innovation as independent investigators. Both have contributed importantly to multiple high-impact publications. Their strengths are highly complementary.
- In addition, a senior, very highly regarded leader in stem cell biology and medicine brings a valuable dimension to the project team. His letter of support indicates a strong collaborative and mentoring relationship with the PI.
- Resources, budget, and timeline are appropriate.
- Use of a model with human skin grafted onto immunodeficient mice enables in vivo studies with human stem cells.
- The experimental plan is well organized, and the experiments are well justified.
- The backup plans are convincing.
- The focus on chromatin organization is understandable. However, this epigenetic aspect is incredibly dynamic and might only be the consequence of plasticity, not the cause. DNA methylation might be more informative in the context of potency and plasticity.
- Aim 3 is high-risk since the technology necessary for this part of the project will require some work. Nonetheless, this aim is also high-gain.
- The PI has a strong track record with landmark publications as first author. The PI obtained an independent position in 2022 and has shown strong leadership. The PI's work is well known in the skin field. The Co-Investigator brings complementary expertise in Wnt signaling. The collaborators will provide essential resources such as primary human tissues.
- The budget seems appropriate for such a complex and ambitious program.
- The design of Aim 1, solely relying on HF7 mice and FZD7 activation, raises concerns. The assumption behind this study is that the outcomes of skin regeneration in the humanized HF7/F7L6 system will fully reflect those in human skin. This might not necessarily be the case, given that the presence of the mouse niche is likely to affect the results.
- While Wnt signaling via FZD7 is likely to play an important role in the regeneration of injured human skin, this intricate process is almost certainly regulated by additional molecular and cellular human niche components interacting with the Wnt pathway that are not present in HF7 mice. Among such components, signaling through other human Frizzled receptors and the human immune system might be necessary.
- The human-to-mouse skin grafts in Aim 3 will be performed in immunodeficient NSG mice. The concern here is that NSG mice lacking an immune system might not be appropriate for studying human wound healing.

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

- The technology has potential value to a wide swath of the population. Where applicable, human skin samples will be obtained from a diverse population. A more complete assessment of any impact of

diversity, including skin pigmentation, on wound healing and other applications would come in later stages, when the project becomes more explicitly translational.

- The population impact section is interesting but could detail a bit more the importance of genetic background and population diversity in wound healing.
- Public engagement is strong.
- The proposed study addresses the mechanisms that orchestrate human skin repair and regeneration to improve the outcomes of wound healing and degenerative skin disease. The experimental design adequately accounts for genetic, environmental, and other factors that may influence the results.
- This work aims to develop and test advanced regenerative strategies that accelerate scarless healing in high-risk groups, such as military personnel and firefighters who often suffer skin injuries and burns, and to improve their recovery and long-term health outcomes. The proposed studies will include human samples representing biological sex as a variable, spanning young to elderly donors and diverse racial backgrounds.

| Application# | DISC5-19908 |
|---|---|
| **Title**<br>(as written by the applicant) | Generating biological age in a stem cell-based complex disease model of osteoarthritis (OA) |
| **Project Objective & Impact**<br>(as written by the applicant) | We will pioneer a rapid yet physiological aging system for stem cell derived tissues based on the ideas of heterochronic serum exchange and apply it to joint-on-a-chip systems that link adipocytes, chondrocytes, and osteoblasts. We will validate it against patient samples and secondly determine how disease phenotypes emerge from dysfunctional inter-tissue communication during the aging process. |
| **Specific Aims**<br>(as written by the applicant) | • Define aging phenotypes in a joint-on-a-chip complex stem cell model of OA<br>• Test how failure of inter-tissue communication drives OA with aging |
| **Statement of Benefit to California**<br>(as written by the applicant) | California's population is aging rapidly, and aging profoundly shapes the biology underlying chronic disease such as osteoarthritis (OA)—the leading cause of pain and disability with aging—yet age is rarely considered in experimental systems. To create foundational knowledge at the interface of aging and stem cell fields, we will develop controlled, human-relevant design-build-test-discover systems of adjustable biological age to dissect inter-organ crosstalk alterations driving tissue aging in OA. |
| **Funds Requested** | $2,499,999 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

**Final Score: 88**

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

| | |
|---|---|
| **Mean** | 88 |
| **Median** | 88 |
| **Standard Deviation** | 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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel’s discussion and scoring of the application, the members of the GWG were asked to

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

| **Key Strengths and Weaknesses** |
|---|
| • Use of aged serum in the context of osteoarthritis (OA) is excellent. Nice plan and wonderful preliminary data.<br>• This is an innovative, well-designed, and potentially high impact project. Enthusiasm is only slightly moderated by concerns that the heterochromic aging strategy might not be physiologically relevant.<br>• This is a well-designed application, it is likely to generate meaningful results. Strengths outweigh weaknesses. |
| **Significance: Evaluate the project's significance and potential for impact** |
| • There are no current drugs to treat osteoarthritis (OA), which is a huge issue.<br>• Interesting preliminary data are presented where the inclusion of human serum from young or old donors in a dish establish a defined biological age and age-related functional decline of cells/tissues.<br>• The project has the potential to advance the application of stem-cell derived models to identify the role of aging in disease. Most current models are immature and their relevance for aging is limited.<br>• The study has the potential to provide insight into the roles of adipocyte-secreted factors in OA. The principles identified here could extend to other age-related stem cell-derived models but it is unclear whether the serum effects are widely conserved.<br>• Mechanistic targets identified in Aim 2 could enable new strategies to prevent or treat OA.<br>• The lack of a strategy to identify mechanisms of aging induced by serum is a missed opportunity given the expertise of the team. This seems to be an important aspect of validating the model and extending it to other systems.<br>• The goal of the proposed study is to develop human iPSC-based multi-tissue microphysiological system (MPS) that will provide a reductionist in vitro model system to study aging and the mechanisms of OA.<br>• If successful, this study will provide a physiologically relevant system for defining factors that drive OA development and will significantly advance foundational knowledge of regenerative medicine. |
| **Innovation: Evaluate the project for innovation relative to the current state of research** |
| • Use of aged serum is novel (and essential for the proposed experiments).<br>• Serum from donors of different ages has been used to induce aging in in vitro models. The use in microphysiologic systems of joints in aging appears novel however.<br>• The combination of stem cell-derived MPS and multi-omics analysis is a powerful combination to study complex, multi-factorial diseases such as OA.<br>• The hypothesis that fat drives aging in the joints is innovative.<br>• Few models are currently available to study the mechanisms of age-related OA. Traditionally such work has been conducted in young preclinical animal models that do not reflect the complexities of human OA; |

moreover, most of the studies addressed questions related to advanced stage of the disease rather than OA development.

- The proposed study will employ a unique strategy that cuts across several disciplines - stem cell biology, microfluidics, organ on chips technologies, machine learning, and OA biology - to overcome these shortcomings.
- Overall, the project is highly innovative.

**Rationale: Evaluate the scientific rationale in the proposal**

- Extensive preliminary data is presented, most from the PI/team.
- The proposal has strong and compelling preliminary data. The data show serum from older people induces age-related phenotypes and gene expression in the stem cell-derived joint model. Benchmarking transcriptomics also supports the model.
- Preliminary data support the use of multi-omics to identify potential factors that mediate cellular crosstalk in models treated with young vs. old serum.
- The premise that fat cells mediate OA phenotypes and represent a potential therapeutic target is interesting, although is not very well-supported.
- The overall scientific rationale for the study is based on the hypothesis that in the OA joint, adipose tissue crosstalk to cartilage is driving increased age-associated inflammation and cartilage damage.
- This is a sound hypothesis backed by strong preliminary data. Moreover, the investigative team developed a multi-tissue MPS system - an aging joint model - that will allow them to directly address mechanistic questions of the adipose crosstalk with the other tissues. Availability of this model further strengthens the scientific rationale for conducting the study.

**Plan & Design: Evaluate the project plan and design**

- Pitfalls and alternative approaches are presented.
- Lots of preliminary data.
- The project has a very systematic experimental design with clear and deep evaluation of the effects of serum age on cell and tissue parameters related to aging and functionality.
- Benchmarking efforts to tissue samples and other models are a strength.
- The proposed efforts to discover adipocyte-derived factors that influence OA development are generally well-constructed. Strategies to focus on mechanistic experiments in the model could be strengthened since a large list of differential features and pathways is likely to be discovered. A path to validation of the model predictions in other systems is lacking.
- The distinction between maturation and aging phenotypes is not discussed or investigated. There are concerns that aged serum will lead to fetal-like, senescent cells.
- The specific aims are logical; the experimental plan is well-designed and thought-through and given the expertise of the team and promising preliminary data, there is a high degree of confidence that the project will yield meaningful results.

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

- Musculoskeletal (MSK) diseases are the leading causes of pain worldwide and affect over 20% Californians. There are a few therapies available. The results of the proposed experiments could be used to quickly develop new drugs/treatments.
- The experimental model accounts for age and sex, both factors in OA.
- Partnerships with osteoarthritis groups on dissemination and outreach are a strength of the proposal.
- The project considers multiple dimensions of external factors and human variability. First, is based on the important biological variable - human age. Second, it will account for biological sex by using hiPSC of both sexes paired with matched exposure to human serum form male and female donors. Third, multiple hiPSC lines will be used to account for genetic diversity of the California population.
- Considering OA and obesity through a diverse lens is important for the health of Californians since the state's population is diverse. This project will extend the applicability of discoveries to this diverse population by including in the study iPSCs obtained from both biological sexes, as both are affected by OA.

| Application# | DISC5-19839 |
|---|---|
| **Title**<br>(as written by the applicant) | Uncovering disease and cell-type-specific constraints on therapeutic delivery using a human CNS tissue discovery platform |
| **Project Objective & Impact**<br>(as written by the applicant) | Human CNS therapeutic delivery fails to translate from animal or in vitro models due to unknown, human-specific delivery biology. This project establishes a human tissue discovery platform to identify cellular pathways governing delivery, enabling rational design of safer, more effective gene and regenerative therapies. |
| **Specific Aims**<br>(as written by the applicant) | • Establish physiologic benchmarks for viability in ex vivo reperfused human CNS tissues<br>• Map cell-type specific molecular pathways governing therapeutic delivery in reperfused human tissues with high throughput lipid nanoparticle screening |
| **Statement of Benefit to California**<br>(as written by the applicant) | This project strengthens California's leadership in regenerative medicine by establishing a first-in-kind human tissue discovery platform. By de-risking human therapeutic development, it accelerates translation of safer gene and RNA therapies for Californians with neurologic and other genetic diseases, reduces costly clinical failures, and builds infrastructure that supports academic innovation and biotechnology statewide. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

**Final Score: 88**

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

| | |
|---|---|
| **Mean** | 87 |
| **Median** | 88 |
| **Standard Deviation** | 4 |
| **Highest** | 94 |
| **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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| **Key Strengths and Weaknesses** |
|---|
| • A highly innovative set of investigators combines unique expertise to address a key gap in the field of gene therapy.<br>• This is a highly compelling proposal, and if successful, it could have a tremendous, field-defining impact. The team is perfectly positioned for success.<br>• This is a high-risk, high-reward new drug screening platform. |
| **Significance: Evaluate the project's significance and potential for impact** |
| • New gene therapies will be advanced by discovering new delivery vectors.<br>• A successful outcome will establish, in academia, a whole-brain human system for basic and translational discovery. This has been established for other human organs or, in the case of the brain, only in an industry setting previously.<br>• The resulting knowledge and data will likely have broad impacts and implications in multiple areas of neuroscience and for multiple diseases, and will generate basic mechanistic understanding of brain function and therapeutic delivery.<br>• The objective is to establish a scalable ex vivo human platform to test by reperfusing post-mortem brain tissue; if successful, this would represent a transformative new paradigm for disease models and functional genomics. |
| **Innovation: Evaluate the project for innovation relative to the current state of research** |
| • The combination of perfusion technology with LNP gene therapy capabilities is highly novel.<br>• The project does not use stem cell models (iPSCs were only used in previously generated preliminary data) but rather seeks to extend post-mortem reperfusion to the human brain, which has only been done in an industry setting previously. In doing so, it brings novel and innovative approaches and frameworks to regenerative medicine.<br>• The project is high-risk, high-reward. |
| **Rationale: Evaluate the scientific rationale in the proposal** |
| • Human tissues have unique biology that these proposed studies probe with cutting-edge techniques.<br>• The fundamental rationale is sound and compelling, articulating the shortcomings of animal or human in vitro models for therapeutic discovery and for predicting the success of therapeutic delivery to the human brain in vivo. |

- The approach has already been established in a company setting and for other organs by the team's collaborators, supporting feasibility.
- This is likely to be a high-risk, high-reward approach. The main concern is the use of anesthetic and sodium-channel-blocking agents (pentobarbital, ketamine, lamotrigine) to ensure the absence of neuronal activity; this is necessary to comply with ethical guidelines but may confound the results. The broader ethical aspect is also an important, non-trivial consideration but perhaps beyond the scope of this review panel.
- Both aims rely on untested new technologies, with limited inclusion of optimization approaches and alternative strategies, as well as interdependence between the aims.

**Plan & Design: Evaluate the project plan and design**

- The approach is strong, given the extensive preliminary data for proof of concept.
- Some of the pitfalls could be better anticipated.
- The plan is relatively straightforward, builds upon robust preliminary data, and is likely to yield valuable information.
- The pitfalls and alternative approaches are well articulated, with the exception of discussing the possible confounding effects of anesthetics and sodium-channel blockers.
- The leadership, integration, resources, and staffing are appropriate.
- The team communication and management plan is effective.
- The plan to conduct a pooled screen simultaneously testing >100 lipid nanoparticle gene editors in reperfused human CNS tissues (brain and spinal cord) and peripheral organs (liver, kidney, spleen, and muscle; n=4 donor tissues each) is ambitious, if overly so (Aim 2).
- The success of Aim 2 is critically dependent on the success of Aim 1; Aim 2 might benefit from optimization in rodents prior to testing on a small number (n=4) of reperfused human brains.

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

- There are no concerns.
- The brain donors will represent the genetic diversity of the local population, and findings will be applicable to many affected populations.
- The outreach, partnership, and educational efforts are appropriate.
- This technology could be used for precision drug screening or gene editing in any recently deceased individual, with potential benefit to others sharing a similar disease etiology.

| Application# | DISC5-20195 |
|---|---|
| **Title**<br>(as written by the applicant) | Decoding Human Pancreatic Progenitor Biology to Enable Next-Generation Stem Cell-based Therapies for Type 1 Diabetes |
| **Project Objective & Impact**<br>(as written by the applicant) | Human stem cells are a promising source for replacement pancreatic beta cells for treating type 1 diabetes (T1D). Still, protocols to generate beta cells from stem cells suffer from low purity and fail to match adult beta cell function. Our proposal addresses these bottlenecks by decoding mechanisms underlying native human endocrine development to enable next-generation stem cell therapies for T1D |
| **Specific Aims**<br>(as written by the applicant) | • Define the spatial and temporal appearance of novel endocrine progenitor populations in the human pancreas across developmental time.<br>• Identify pathways underlying cell fate selection in human Pre-Beta endocrine progenitors.<br>• Define the impact of stem cell-derived enterochromaffin-like cells on SC-beta cell function in vitro and in vivo. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Type I Diabetes (T1D) is a significant burden in California, especially for children; according to estimates provided by the California Diabetes Program, ~2.3 out of every 1,000 children between the ages of 5-19 in California had diagnosed diabetes in 2008, with 83% having T1D. Research proposed here would represent a significant step towards the holy grail of T1D treatment: a therapy for patients without the need for the administration of insulin or frequent blood testing. |
| **Funds Requested** | $2,499,875 |
| **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.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

### Final Score: 88

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

| | |
|---|---|
| **Mean** | 86 |
| **Median** | 88 |
| **Standard Deviation** | 4 |
| **Highest** | 90 |
| **Lowest** | 75 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 11 |
| **(1-84): Not recommended for funding** | 4 |

# FINAL COMMENTS

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

**Key Strengths and Weaknesses**

- The proposal is strong and addresses an important question for the field of beta cell replacement therapy. The team has generated strong preliminary data and has the expertise to carry this project forward.
- The big concern is in Aim 2, the CRISPR KO experiments. The integrated lentiviral sgRNA library may be silenced after differentiation to stage 4 and stage 5.
- The project addresses a highly significant problem with innovative methods and a strong investigator team. It has excellent preliminary data and a strong rationale. Overall enthusiasm is high but somewhat tempered because, even if successful, the outcome would only take the field another step in a still lengthy process.

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

- The ultimate goal of the proposal is to improve the differentiation of SC-derived beta cells by leveraging information gathered from human developing pancreas datasets.
- The success of this project will improve the differentiation protocols currently used to generate beta cells from hPSC and allow the generation of a cell product enriched in beta cells.
- More generally, the idea that studying human developing organs can provide novel insights for the efficient, directed differentiation of hPSCs into specific cell types is being actively explored by several scientists across multiple tissues. The investigators are at the forefront of this effort, and the knowledge gained in this area will undoubtedly be beneficial to the broader scientific community.
- Moving forward, the large datasets being generated will likely be integrated with AI-based approaches to enable more unbiased analyses and the potential design of more targeted and efficient experiments.
- This proposal aims to address one major barrier in T1D cell therapy: current beta cell differentiation methods generate immature beta-like cells and the purity is low. This proposal tries to understand key pathways underlying endocrine progenitor cell fate decisions and uses that knowledge to improve hPSC to beta cell differentiation.
- Successful completion of this project could improve SC-beta cell differentiation efficiency, SC-beta cell function. All of these could advance beta cell replacement therapy for T1D.
- This project is at the discovery-stage and may produce new mechanistic knowledge that help us understand pancreatic development and thus help us design new methods for beta cell differentiation.
- Many barriers exist before SC-beta cell can be used for T1D therapies. including functional states of SC-beta cells; graft survival of beta cell after transplantation; long-term durability, etc. This project is trying to address the issue of making better SC-beta cells.
- Successful project outcome would advance understanding of pancreatic endocrine cell development, in particular insulin-secreting islet beta cells, helping to increase efficiency of beta cells derivation from hPSCs in terms of function (glucose-responsiveness) and yield.

- Successful outcome per preceding point would bring us closer to realizing the potential of hPSC-derived beta cells as a "cure" for type 1 diabetes (T1D), a major unmet need.
- Important to point out that there is at least one ongoing human phase 3 clinical trial of hPSC-derived beta cells in subjects with T1D and hypoglycemia unawareness. While earlier phase trials already demonstrated the ability of transplanted cells to reduce insulin requirements in such subjects, there is reason to believe that more efficient protocols for hPSC differentiation as proposed in this project could improve efficacy and reduce cost.
- More generally, successful outcome of the project would also inform more productive therapeutic approaches to functional cell replacement of other diseases for which hPSC-differentiation could be a source of relevant cells, e.g., dopamine-secreting neurons in Parkinson disease.

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

- The project primarily builds on established stem cell and regenerative medicine approaches (spatial transcriptomics, CRISPR screens, and reporter lines) rather than introducing new technologies. Nevertheless, it employs these methods in a purposeful manner that effectively supports the overall objectives.
- The collaborator brings expertise in pooled screening and will perform a CRISPR screen on hPSCs, testing for FEV+ EP expansion and beta or EC cell enhancers. Others have performed similar studies, but this approach is the first to systematically test the whole genome to identify genes crucial for lineage determination.
- This project plans to use human fetal pancreas biology and hPSC differentiation techniques to study beta cell differentiation.
- This project plans to use multiple techniques, such as CRISPR gene editing, reporter-based FACS, and in vivo transplantation. However, all of these technologies are established. The innovation lies more in the integration than in a fundamentally new technology.
- The project employs a unique synergy of technologies to achieve its objectives. Specifically, it uses both spatial transcriptomics (scRNA-seq) and in situ hybridization in the developing human fetal pancreas and then compares the results with those from comparable studies of hPSC differentiation into endocrine pancreatic cells.
- The project uses novel computational techniques to analyze data from the above-cited methods, yielding powerful insights into the development of insulin-secreting beta cells.
- Innovative methods are used to study the effects of knocking out key transcription factor genes on beta cell development. As well, novel approaches to studying cell-cell interactions in beta cell differentiation are employed.

**Rationale: Evaluate the scientific rationale in the proposal**

- The PI has pioneered this area of work by performing single-cell analysis of both mouse and human developing pancreas. Her work showed that the transcription factor FEV marks a population of endocrine progenitors that has the potential to develop into beta cells.
- The hypothesis that FEV-enriched endocrine progenitors would improve the efficiency of stem cell-derived beta cells from hPSCs is very intriguing. Importantly, it is backed by single-cell analysis/in situ hybridization and immunofluorescence in the human pancreas and in vitro studies using FEV KO hESC lines.

- However, it is conceivable that FEV marks not only beta cell progenitors but a broader population of islet-like progenitors. Overall, the rationale is sound, and the preliminary data support the central hypothesis, although alternative interpretations remain possible.

- Figure 4 showed that loss of FEV reduced beta cell differentiation efficiency. However, FEV did not fully abolish differentiation, indicating that FEV is not absolutely required for this differentiation.

- Data shown in Fig. 10 are not sufficient to demonstrate that the PI's group has successfully generated a FEV-GFP knock-in cell line. Sequencing results of the targeted loci are needed. Also, green fluorescent images of the sorted FEV+ cells should be shown. It is unclear why the PI did not show this in the Figure 10 image.

- The proposal assumes that insights from fetal development can be productively transferred to in vitro differentiation, which is plausible but not guaranteed.

- The scientific rationale for the proposed project is strong and supported by an impressive body of preliminary data. A critical piece of these preliminary data is the identification of the transcription factor FEV as a key marker of "pre-beta cell" development and its subsequent disappearance with mature beta cell development. Importantly, this was identified in both human fetal pancreas developmental studies and parallel studies of hPSC differentiation in vitro into beta cells.

- Studies of SC-derived "enterochromaffin" cells are well documented in the preliminary data, and the rationale for studying their interaction with SC-derived endocrine precursor cells is well justified in terms of potential effects on increasing yield and improving function of SC-derived beta cells.

- A mouse model is used for "rescue" of streptozotocin-induced diabetes by transplantation of SC-derived beta cells. This is a standard method in the field for in vivo evaluation of beta cell function.

**Plan & Design: Evaluate the project plan and design**

- The team is addressing an important and still unresolved question centered on our ability to understand the mechanisms driving endocrine lineage specification and how to control these processes in vitro to efficiently generate islet-like cells depleted of EC cells.

- The idea of testing whether sorting for FEV+ cells can improve the efficiency of beta cell differentiation, as well as whether knocking out FEV following endocrine progenitor induction may favor beta cell over EC cell specification, is compelling.

- The Co-Investigator brings valuable expertise in pooled screening approaches and will lead a CRISPR-based screen in hPSCs to identify regulators of FEV+ endocrine progenitor expansion and beta versus EC cell fate determination. While related studies have been performed previously, this approach is notable for systematically interrogating the entire genome to identify genes that are critical for lineage specification.

- Lastly, in Aim 3, the applicants propose to determine whether EC cells influence beta cell function both in vitro and in vivo, an important question that, as they correctly state, has not yet been formally addressed. As part of this aim, they plan to evaluate the feasibility of using specific surface markers to purify EC cells and subsequently reaggregate them with islet-like cells at varying frequencies.

- The applicants identify potential technical limitations and propose alternative strategies where appropriate. Overall, the team possesses the expertise and state-of-the-art technologies required to successfully carry out the proposed work.

- One suggestion would be to maintain an open mind regarding the developmental potential of FEV+ EP progenitor cells and to test for alpha, delta, and epsilon cell differentiation alongside beta cells.

- Additionally, the team should evaluate some of the more recently published differentiation protocols, as the current approach appears to generate a relatively large proportion of non-endocrine cells. This could

potentially impact the final CRISPR screen readout, as off-target cell populations may introduce noise and complicate the interpretation of the results.

- This team has the right expertise in pancreatic development, stem cell differentiation, and transplantation.
- Sub-Aim 2B has many problems. First, using a lentiviral gRNA library to infect hPSCs and then starting hPSC-to-beta cell differentiation will cause many integrated constructs to be silenced, which will bias the results. Second, many of the genes essential for endoderm formation may be discovered because this is the first step of differentiation, which is not important for understanding endocrine progenitor-to-beta cell differentiation.
- The project design, bolstered by the preliminary data provided in the application, is highly likely to yield meaningful insights that should provide a stronger basis for developing effective cell replacement therapy.
- The applicants identify a number of potential pitfalls and address steps they have taken to mitigate these.
- The budget and timeline appear to be appropriate.
- The project involves collaboration between the PI and Co-Investigator labs, both at the applicant institution, albeit on different campuses. Both investigators are leaders in their respective fields, and the resources available to them are superb. There is ample interdisciplinary integration: cell and developmental biology (PI) with state-of-the-art genomics (Co-Investigator). Superb core facilities add value.
- Team communication and management should be excellent. The PI and Co-Investigator labs have been collaborating for years, and an excellent plan for management and staffing is described in the application.

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

- The proposed improvements to the differentiation protocol could enhance efforts to develop more effective cell replacement therapies for diabetes and, as such, may ultimately benefit all individuals living with T1D. The team also indicates that they will use cells and tissues reflective of the population diversity of the state of California; however, limited details are provided in the main proposal regarding how this will be implemented, given the unpredictable nature of obtaining fetal samples.
- The proposal acknowledges disparities in T1D incidence and outcomes across racial, ethnic, sex, and socioeconomic groups.
- The plan to consider sex, race, and tissue-source diversity in human samples is appropriate.
- The applicants specifically address the diversity of the population they will study (reflected in tissue samples to be obtained) and potentially target for cell replacement therapy (allogeneic).
- While the main target population is those affected by T1D, the applicants point out that beta cell replacement therapy could eventually prove useful in subjects with T2D, which is far more prevalent and affects a more diverse population.
- The application describes well the several outreach and educational efforts to be employed, including CIRM Bridges, SEP, SRTP, and PROPEL programs.

| Application# | DISC5-20158 |
|---|---|
| **Title**<br>(as written by the applicant) | Engineering Nephron Connectivity in Human Kidney Tissue Using Synthetic Signaling Centers |
| **Project Objective & Impact**<br>(as written by the applicant) | Human kidney tissues grown from stem cells lack reliable internal connections, limiting their usefulness for studying disease and developing therapies. This project addresses this bottleneck by defining how nephron connections can be intentionally guided rather than left to chance. Success would enable more accurate human kidney models for regenerative medicine and drug discovery. |
| **Specific Aims**<br>(as written by the applicant) | • Define rules for distal nephron-collecting duct connectivity using synthetic signaling centers to achieve reproducible epithelial connections.<br>• Determine how proximal nephron-vascular connectivity forms, distinguishing permissive versus instructed modes of endothelial engagement.<br>• Engineer proximal fate–directing signaling modules to control nephron identity and morphogenetic alignment.<br>• Establish timing and geometric rules for endothelial integration with patterned human nephrons.<br>• Identify molecular signatures of successful nephron connectivity at epithelial and vascular interfaces.<br>• Integrate distal and proximal connectivity modules to build human kidney tissue with directional, dual-end connections. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Kidney disease affects millions of Californians and places a major burden on patients and the healthcare system. By enabling more accurate human kidney tissue models, this research will accelerate discovery of regenerative therapies and safer drug testing. The project strengthens California's leadership in stem cell innovation and supports future biomedical workforce development. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

**Final Score: 87**

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

| | |
|---|---|
| **Mean** | 86 |
| **Median** | 87 |
| **Standard Deviation** | 2 |

| | |
|---|---|
| **Highest** | 88 |
| **Lowest** | 81 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 13 |
| **(1-84): Not recommended for funding** | 2 |

## FINAL COMMENTS

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

**Key Strengths and Weaknesses**

- The significance of the project's aims within the context of CIRM's mission; the excellent preliminary data, innovative approaches and excellence of the team make me enthusiastic. The high risk nature of the project is a plus but the clear possibility that the aims will not be accomplished, especially within the allotted timeline, temper my enthusiasm.
- This is a well-designed innovative project, and it is likely to yield important information for predictable engineering of nephron-on-a-chip. A weakness of the application is that it lacks functional analysis of the engineered nephrons. Overall, the application's strengths outweigh weaknesses.

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

- This proposal aims to improve the functionality of kidney organoids generated in vitro from hPSCs. For that, the applicants want to use synthetic morphogenic organizers which can control signalling pathways known to control kidney development.
- Kidney diseases are a major health care challenge and the absence of in vitro models limit the development of new therapies.
- The kidney is a difficult organ to model in vitro due to the complex connection between kidney cells and vasculature. Major progress has been achieved during the past few years especially with models generated from hiPSCs. However, improvements are still needed. Thus this proposal seems to be timely.
- The main focus of the proposal is developmental biology but the knowledge generated could have a translational impact. In this aspect, it would have been really interesting to include some functional test to demonstrate that the cellular connections created with their approach has an impact beyond marker expression.
- A successful project outcome would have broad implications beyond the proposed ability to engineer hPSCs into proximal and distal functional nephron connectivity. For example the methods proposed could be applied to engineering functional organoids of other spatially complex organs such as liver and heart.
- Chronic kidney disease is a major unmet medical challenge. By transforming self-organization into programmable architecture, successful completion of the this project will enable next-generation platforms for kidney disease modeling, drug discovery, and regenerative medicine, while laying a foundation for future translational development aligned with CIRM's mission.

- The project has a potential to yield design principles for engineering of morphogen-based control of tissue organization and polarity – an important goal with broad implications for engineering different types of epithelial tissues.

- Since this project aims to yield nephrons recapitulating in vivo nephron architecture and polarity, it will generate a useful model of kidney function and is likely to inform development of therapeutics for a variety of kidney diseases.

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

- The concept of synthetic morphogenic organizers is intriguing. The applicants want to use this concept to induce self-patterning and self-organization of nephron cells and vasculature.

- This proposal focuses on developmental biology but uses hPSCs as relevant model for human developmental biology. The approach based on human in vivo data is also interesting. Indeed, the applicants propose to infer in vivo analyses, spatial and single cells transcriptomics, to identity the pathways controlling kidney patterning and organization. They, they will use this information in vitro.

- Understanding how endothelial cells can instruct kidney organization would be definitely interesting and something that the developmental biology have tried to understand for a long time.

- The project is technologically innovative in its use of programmable signaling systems in combination with spatial transcriptomics to achieve spatial and functional control in hPSC-derived tissue. This goes beyond current approaches to differentiating stem cells into functional organoids that have no way of predictably leading to "functional connectivity."

- Engineering HEK cells to produce canonical WNT ligands (shown to be critical for inducing distal nephron connectivity by spatial transcriptomics of developing kidney) exemplifies the innovative approaches employed in the project.

- Instead of traditional passive chronic cell treatments with morphogens, the investigators will employ a programmable synthetic signaling system to control relative cell geometries, morphogen concentrations, and their timed local presentation to cells.

- This is a novel regenerative medicine framework. It will allow the investigators to identify and quantify how nephron axial patterning and connectivity respond to proximally and distally restricted WNT, BMP, and FGF ligands (known to control normal nephron patterning in development) in order to predictably pattern and connect nephrons to distal iCD (urine draining) and proximal endothelial iENDO (blood filtering) networks in their organoids.

- Overall, the project is innovative.

**Rationale: Evaluate the scientific rationale in the proposal**

- The proposal is based on the concept that controlled gradients of growth factors will induce self-organization of kidney organoids, especially interactions between nephrons and vasculature. This concept is sound, since it has been used for most protocols. The technical approach based on synthetic organizers is more novel, as is the use of primary human tissues.

- The application contains a lot of primary data. Some figures are extremely small and difficult to read. The rationale for these data is also sometimes difficult to follow. Nonetheless, the data presented seem convincing.

- While definitely interesting from a developmental biology point of view, it is not clear how the knowledge generated will be applicable to generating improved kidney organoids.

- There is a strong scientific rationale for the proposed research. This is bolstered by a significant body of preliminary data well presented in the application. As noted in the application, the goals for Aim 1.1 of the project (distal connectivity) are feasible based on substantial preliminary data. This is not the case for the Aim 1.2 goals (proximal connectivity), nor for the more ambitious Aim 2 of achieving simultaneous proximal and distal connectivity in a functional nephron.

- The scientific rationale of the project is based on the hypothesis that nephron connectivity can be engineered by coupling spatial patterning between appropriate cellular counterparts using defined combinations of morphogens—BMP, WNT, and FGF—to generate epithelial (CD epithelia) and vascular interfaces (endothelium). This hypothesis is solidly based on knowledge of normal kidney development and thus provides a sound scientific rationale for the project.

- The rationale and feasibility of the project are supported by promising preliminary data that collectively show that nephron connectivity and higher-order structure can be driven by the local application of single and dual synthetic organizers that control morphogen fields and generate multiple cell fates in a spatially predictable pattern.

**Plan & Design: Evaluate the project plan and design**

- The proposal is very complex and difficult to follow. It lacks focus and clarity. The technical complexity is extremely high and risky. It might be difficult to generate meaningful results. The applicant will have to simplify their approach. Aim 1 is very detailed, while Aim 2 is less structured.

- The use of HEK cells for the synthetic organizers could be problematic, as these cells are unlikely to secrete physiological quantities of growth factors. Thus, the patterning is likely to be abnormal. Similarly, morphogen gradients work between cells in relatively close proximity, and it is not clear that such aggregates of cells could model this aspect.

- The use of iCD (hiPSCs) is definitely more interesting in this context. However, transgene induction is likely to be extremely heterogeneous due to silencing, and very few cells might express the necessary WNT factors. The grazoprevir (GZV)-responsive promoter system is interesting but will strongly increase the complexity of the system. Overall, this approach is high-risk.

- The lead applicant has a solid track record and brings the core expertise in signaling pathways. However, the kidney expertise comes from the Co-Investigator, who has solid knowledge of this organ. The Co-Investigator also has experience with project management and could have been the lead PI. Nonetheless, their expertise is synergistic, if not complementary. There is no doubt that this team will deliver interesting results.

- The budget seems appropriate for such a complex project.

- The project has a high likelihood of yielding meaningful insights. While it is a high-risk, high-reward project with certainly no guarantee of achieving Aim 2 with fully functional, proximally and distally connected nephrons generated, the important data generated by the project will prove critical for eventual success.

- Potential pitfalls for both aims are clearly delineated. Alternative approaches are presented, but to some extent these seem to be a bit like “more of the same” and hoping for a better outcome.

- The budget seems appropriate, but it is hard to say whether even three years is adequate for successful completion given the high-risk nature of the project.

- The project brings together two outstanding investigators: a leader in synthetic biology and a leader in the study of kidney development. This is a synergistic combination that makes the proposed project attractive.

- The resources at the applicant institution and in the respective laboratories are excellent, as is the proposed staffing.

- Plans for communication and management of the project are well described and appear excellent.
- By the end of the project, the investigators expect to establish a programmable framework for controlling nephron polarity and connectivity in human kidney organoids. They argue that by defining quantitative design rules for distal and proximal nephron connectivity, this work will move the field beyond stochastic self-organization toward reproducible, engineerable kidney tissue architecture.
- This is indeed a desirable goal, and the team is well equipped to achieve these goals. However, a weakness of the project is that it lacks any functional analysis of patterned kidney organoids. To learn if these organized structures would function better than the disorganized cell assemblies, and thus represent a superior model of kidney function, it is necessary to carry out comparative functional assays of patterned and unpatterned organoids.
- To this end, the project would benefit from the addition of a section where such assays could be carried out. For example, they could include tubular transport assays and glomerular filtration assays; both have been described in the literature for functional testing of kidney organoids.

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

- The information provided does not address genetic diversity or the impact of kidney diseases on different populations. There is also no information on how this research will benefit affected populations and communities.
- Information about public outreach is limited.
- The project's design, which includes benchmarking against human developmental atlases and focusing on conserved signaling logic and architectural constraints, increases the applicability of its findings to diverse affected populations beyond the diversity of the hPSCs employed.
- Close alignment with a CIRM center and core laboratory at the applicant institution assures broad dissemination of results across relevant research groups in California. The applicants have a good track record of engaging with kidney disease patient communities.
- The experimental design of this project will allow the systematic definition of design rules that govern patterning of nephron organoids, as well as their spatial orientation and epithelial and vascular connectivity, across multiple independent iPSC lines and experimental replicates, thus accounting for genetic and other external factors that may influence research findings.
- The team will engage with kidney research and patient-focused communities through scientific meetings, workshops, and collaborative networks to inform ongoing project development and future translational directions. Open data sharing, deposition of engineered tools, and dissemination of validated design rules will ensure that discoveries from this project can be adopted by a broad community, thus amplifying their impact and enhancing the population relevance of these discoveries.

| Application# | DISC5-19638 |
|---|---|
| **Title**<br>(as written by the applicant) | Systematic cell-type-resolved single-base resolution functional characterization of neurodevelopmental risk genes |
| **Project Objective & Impact**<br>(as written by the applicant) | We will close the gap between the clinical consequences of neurodevelopmental disorder mutations and the underlying cellular mechanisms by systematically reconstructing disease-causing mutations in human neural and glial cells using a combination of innovative technologies supporting the discovery of therapeutics that modify these effects, and ultimately generalizing across diseases. |
| **Specific Aims**<br>(as written by the applicant) | • Use scPRIME to test 100 variants in neurodevelopmental and psychiatric disorder risk genes in iPSC-derived neurons, linking genotype to gene expression, chromatin state, and neuronal function.<br>• Characterize 100 variants in iPSC-derived microglia to reveal cell type-specific effects by comparing their transcriptional, epigenomic, and cellular impact with those in excitatory neurons.<br>• Determine the cell type-specific consequences of variants during human cortical neurogenesis and bridge the gap between cellular and patient phenotypes with ARX variants as a test case. |
| **Statement of Benefit to California**<br>(as written by the applicant) | This research will accelerate the functional annotation of variants in genes that cause neurodevelopmental and psychiatric disease, thereby improving genetic diagnosis and precision medicine for Californians. By characterizing functions at the variant level across various cell types and diverse genetic backgrounds, our research will help reduce diagnostic uncertainty for families, inform the development of novel therapeutics, and strengthen California's leadership in biomedical innovation. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 86

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

| | |
|---|---|
| **Mean** | 86 |
| **Median** | 86 |
| **Standard Deviation** | 1 |
| **Highest** | 88 |
| **Lowest** | 85 |
| **Count** | 14 |

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

## FINAL COMMENTS

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

**Key Strengths and Weaknesses**

- This is a strong and technically innovative proposal supported by compelling preliminary data and a highly capable team, although concerns regarding mechanistic depth somewhat temper enthusiasm for overall impact.
- This is a strong proposal that builds on strong preliminary data, and this approach will be valuable to functionally annotate individual single-nucleotide variants (SNVs). There are a few technical concerns that were not addressed, but not enough to dampen the enthusiasm.
- Key strengths: using novel technology to address an important question, experiments are logically presented and potential pitfalls are well discussed. Minor weakness: aim 3 is relatively distant from other aims.

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

- This project addresses a major gap in stem cell biology: linking patient-derived genetic variants to functional outcomes in human cells. A successful outcome would advance understanding of how single-nucleotide variants affect gene regulation and cell function across neural lineages, using relevant iPSC-derived and primary stem cell models. The impact is strong within neurodevelopmental biology, though somewhat limited in scope beyond neural systems.
- Proposal has moderate potential to generate broadly informative insights by systematically examining variant-level and cell-type-specific effects. This could refine current gene-centric models toward more context-dependent interpretations of genetic risk. However, the underlying concepts are not entirely new, so the work is more likely to extend existing paradigms rather than establish fundamentally new ones.
- The successful completion of this project would aid in the interpretation of the functional consequences of individual genetic variation linked to NPDs. Examining the effect of specific genetic variants, as opposed to knock out or knock down screens, will more closely recapitulate the perturbation of gene function present in individuals with NPDs and is likely to yield valuable mechanistic insight.
- Systematically characterizing NPD-associated variants in iPSC-derived cortical neurons, microglia and primary neural stem cells. defining the functional impact of disease-linked variants in NPD risk genes may provide clues of how shared genetic risk gives rise to divergent disease phenotypes.
- The integration of single-cell transcriptomics, chromatin analysis, and cellular phenotyping provides a solid framework for generating mechanistic insights, particularly for prioritized variants. These findings could inform future therapeutic targeting and biomarker development. However, given the scale of the study, mechanistic depth may be limited to a subset of variants, and translational implications remain somewhat indirect.

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

- The proposal demonstrates strong integration of genome editing, single-cell transcriptomics, and stem cell-based disease modeling. The combination of precise variant engineering with simultaneous genotype–phenotype readouts at single-cell resolution is a strength and reflects cross-disciplinary synergy. The inclusion of multiple human cell systems further enhances this integration.
- The project applies a relatively novel framework by shifting from gene-level perturbation to variant-level functional interrogation in human stem cell-derived systems. This represents a conceptual advance, particularly in the context of interpreting human genetic variation. However, the broader framework—linking genetic variation to transcriptional and cellular phenotypes—is already emerging in the field, so the innovation lies in systematic implementation and not new conceptual model.
- The use of scPRIME is a key innovation, enabling precise introduction of single-nucleotide variants with single-cell readouts. This overcomes limitations of CRISPR loss-of-function approaches and represents a meaningful technical advance. While innovative in its application across cell types, it builds on existing technologies, and its impact will depend on robustness and scalability.
- This project proposes to extend the applications of scPRIME, an approach coupling pooled prime editing and scRNAseq, to iPSC-derived and primary neural cells. While pooled approaches using prime or base editing have been demonstrated in human cells before, this has not been done at scale in iPSCs. Establishing and benchmarking the method in iPSCs specifically is thus valuable for the field.
- The combination of screening assays in both iPSCs and human primary cells is innovative.
- Technical innovation of using single cell prime editing (scPRIME) for high throughput screening of NPD associated risk variants. The use of three human cell models - human primary neural cell cultures and human iPSC derived neuronal and glial cell cultures.

**Rationale: Evaluate the scientific rationale in the proposal**

- The scientific rationale is strong and well grounded. The proposal addresses a gap between genetic variant discovery and functional interpretation, particularly for single-nucleotide variants that cannot be modeled by conventional loss-of-function approaches. The hypothesis that variant effects are context- and cell-type-dependent is well supported and provides a solid foundation for the proposed work.
- The rationale is supported by strong preliminary data, including validation of the scPRIME platform and demonstration of genotype–expression coupling at single-cell resolution. These support feasibility, though some technical risks (e.g., editing efficiency, scalability) remain. Approach appears workable and justified given the potential knowledge gain.
- The experimental systems and approaches are appropriate and well justified. The use of human iPSC-derived neurons, microglia, and primary neural stem cells enables relevant, cell-type-specific analysis. The integration of single-cell transcriptomics, chromatin profiling, and functional assays is well suited to capture variant effects, with isogenic validation strengthening the design. However, the broad scope may limit depth of mechanistic follow-up.
- The rationale is sound and robust, and the need to interrogate single-nucleotide variants (SNVs) in human neural cells is well justified.
- The proposal builds on compelling and extensive preliminary data, and the experiments are well designed and feasible.
- The selected models are well suited.
- Linking genetic risk variants to function and disease phenotypes remains challenging. Strong preliminary data support the feasibility. The rationale for the six chosen genes could be better described and justified.

**Plan & Design: Evaluate the project plan and design**

- The proposal identifies several relevant technical risks and provides reasonable alternative strategies, such as modifying timing of editing or leveraging improved editing systems. These are appropriate and demonstrate awareness of key challenges, although some contingencies remain incremental rather than transformative if core approaches underperform.
- The investigative team appears well qualified, with complementary expertise in genome engineering, stem cell biology, and single-cell genomics. Access to relevant models, technologies, and institutional resources is a clear strength, supporting feasibility and interdisciplinary integration.
- Well designed to generate meaningful insights, with a clear progression from high-throughput variant screening to targeted mechanistic follow-up. The integration of single-cell, genomic, and functional assays is appropriate and should enable multi-level characterization of variant effects. However, the breadth of variants, cellular systems, and downstream assays introduces substantial risk that outputs may be broad but mechanistically shallow across many variants.
- The leadership, staffing and resources are appropriate.
- The communication plan is appropriate.
- scPRIME will be used to simultaneously detect targeting gene variants and the gene expression at single cell level. One hundred disease associated variants of six genes will be screened for their function at transcription level.
- Cellular and omics assays will be performed with hits from the initial screen in both NGN2-induced cortical excitatory neurons and microglia and primary neural cell cultures. The proposed study on ARX in neurodevelopment and its association with clinical severity appears to be more independent and outside of the scope.
- The overall scope is ambitious relative to a 3-year DISC5 timeline, particularly given the number of variants, cell types, and downstream assays. While feasible in principle, successful execution will require strong prioritization. The budget appears broadly appropriate for the scale and technical demands of the project.
- The possibility of barcode swapping, which is often a concern in pooled screens with complex pegRNA constructs is not discussed or addressed.
- There is no power calculation or other justification for the number of cells per perturbation.
- The project is well designed overall, but there is no plan to analyze and integrate data across the 3 aims.

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

- The proposal demonstrates thoughtful consideration of population-level variability by incorporating multiple donor-derived iPSC lines, primary cells from diverse genetic backgrounds, and balanced representation of sex. The use of genotyped samples and alignment with population reference datasets strengthens the ability to account for genetic diversity. Environmental factors are less directly addressed but are reasonably considered within the constraints of in vitro systems.
- The inclusion of diverse genetic backgrounds and primary human samples supports the potential for findings to be more broadly applicable across populations. The effort to examine how genetic background modulates variant effects is a notable strength and may enhance generalizability. However, the extent to which findings will translate beyond the specific models and selected variants remains uncertain.

- The team demonstrates a strong commitment to diversity, outreach, and training, including involvement in programs that support underrepresented groups and access to diverse patient-derived samples. These efforts are appropriate and may enhance the broader impact of the work. However, the connection between outreach activities and direct influence on study design or interpretation is somewhat limited.

- The variants were prioritized based on a genetically diverse population. Two iPSC lines will be used in the screens (one male, one female). The primary cells in Aim 3 are derived from individuals of diverse ancestries, mirroring the genetic diversity of the population in California.

- The outreach and education plan is acceptable.

- The proposed study will examine multiple risk genes associated with NPD. The team's proposed outreach, partnership, and educational efforts are acceptable.

| Application# | DISC5-19747 |
|---|---|
| **Title**<br>(as written by the applicant) | Leveraging lessons from normal development to overcome barriers to efficient transplantation of stem cell derived cortical neurons. |
| **Project Objective & Impact**<br>(as written by the applicant) | Our project seeks to improve the fidelity, safety and robustness of future cell therapies. We will develop protocols for deriving cortical neurons from human pluripotent stem cells and for enhancing integration of these cells into the adult brain environment. This project may lead to novel therapeutic candidates and new methods for enhancing cell and circuit replacement therapies. |
| **Specific Aims**<br>(as written by the applicant) | • Developing methods to derive transcriptomically-defined neuronal subtypes from human PSCs.<br>• Identifying strategies to promote regeneration of axons and synapses from PSC-derived PNs in the adult brain.<br>• Establishing how activity and experience enhance maturation and circuit integration of human PSC-derived neurons. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Californians asked CIRM to support stem cell research towards treatments for brain disorders by earmarking 25% funds for CNS projects. Cell therapy applications in the nervous system have the potential to address CNS disorders, including Epilepsy, Stroke, and Neurodegenerative Disorders. However, neural stem cell transplantation is inefficient and poorly understood in human brain tissue. Our project directly addresses these issues using a combination of genetic and cellular approaches. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

### Final Score: 86

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

| | |
|---|---|
| **Mean** | 86 |
| **Median** | 86 |
| **Standard Deviation** | 2 |
| **Highest** | 90 |
| **Lowest** | 80 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 13 |
| **(1-84): Not recommended for funding** | 2 |

# FINAL COMMENTS

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

**Key Strengths and Weaknesses**

- The overall goal of advancing a cell replacement therapy is significant and with years of work on identifying suitable cells. Cell replacement in humans needs to move to the next step to become a viable option for future investments. This proposal has the potential to significantly advance the field.
- The work proposed would help to leverage previous work and identify robust strategies for human cell replacement approaches.
- This proposal adequately addresses issues of pathfinding, cell purity and integration. However, how scaling and availability of the large cell numbers that would be needed for a clinical approach (and are according to the applicants an important bottleneck) are not addressed.
- The approaches described do not include any kind of injury or disease paradigm in which the cells would need to function. This makes it unclear whether the normal brain environment is the appropriate way to test the efficacy of future cell therapies.
- The critical "period mismatch" problem where transplanted neurons might be too immature relative to the host circuit or might be unable to compete with established synapses is not well addressed.
- The project is innovative, but there are important limitations that have not been properly addressed.

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

- The unmet need is real and clearly framed: long-range corticofugal pathway loss has no restorative therapy, and projection neuron (PN) replacement is the only credible strategy for reconnection.
- The applicant will identify cortical projection neurons in transplanted organoids, define their integration into the adult brain and identify means for enhancing function that are required for future cell based replacement therapies.
- No suitable therapeutic approaches have been identified to make long term improvements in diseases like Parkinson and ALS. If the proposal is successful, the impact and significance could be very high.
- A successful outcome would enhance knowledge of genes and mechanisms that contribute to successful transplantation of a subset of pyramidal neurons in the brain.
- The choice of corticostriatal (IT) and corticorubral (ET) PNs as exemplar nodes is well justified because they span the basal ganglia and brainstem motor systems respectively, providing credible cross-disorder reach.
- Mechanistic insights into experience-dependent integration would generalize to most cell-replacement therapies, for which circuit integration in the adult CNS is needed if validated.
- The significance argument leans on potential motor-disorder breadth without quantifying which patient population would benefit first or by how much.

- It is possible (but unclear) that similar genes and mechanisms would also be relevant to additional cell types and diseases.
- The specific scientific milestone that the work enables (e.g., the cell-product specification for a future preclinical program) is not articulated, weakening the impact case.
- Claims of broader implication for autism and OCD via corticostriatal PNs are speculative and do not strengthen the central argument.
- Some proof of concept exists that cell transplantation therapy approaches have value (i.e. interneurons), but the translation to the clinic for using PN is lacking and requires highly purified and large numbers of these cells as well as a proof of concept that such neurons can effectively integrate into the adult brain. This proposal claims to address these unique challenges that thus far make the transplantation of long-range PN problematic.
- This may, in the long run, increase the likelihood of successful integration of transplanted human neurons in the human brain.

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

- The approaches are overall innovative, in particular for linking the neural activity to functional integration.
- The integration of systems-neuroscience experience-dependent plasticity with stem-cell biology is innovative.
- Rather than applying novel frameworks, the project extends the application of novel approaches (including transplantation) to new cell types.
- If successful, a new set of markers would be available to optimize cell growth, purity and integration.
- The applicant uses highly sophisticated tools to define and characterize the specific population and to test integration into the brain.
- This project employs multiple cutting-edge models and approaches ranging from organoid cultures, to mouse models, resected human specimens, and CRISPR and optogenetic approaches.
- A major novel aspect is to use adult brain slices to test integration and to incorporate activity dependent firing into the study. The applicants developed surgically resected human brain tissue ex vivo cultures as a novel platform for studying integration into the adult brain. This is exciting.
- CRISPRi screening with targeted barcodes is a genuinely novel platform and a logical extension of the team's prior CIRM DISC0 work.
- The proposed imaging with optogenetic inhibition is a first-of-its-kind paradigm for testing cell-therapy timing.
- Aim 1B is conceptually similar to recent interneuron enrichment work and is incremental rather than novel.
- Re-expression of developmentally appropriate guidance cues to enhance adult-brain axon outgrowth has been published; the contribution here is the screen output, not the strategy itself.

**Rationale: Evaluate the scientific rationale in the proposal**

- The applicants have already shown that the desired neuronal phenotype emerges in human organoids. The next logical step is to define and identify the integration of organoid transplanted neurons into the brain network.
- Preliminary data are convincing: an established organoid-to-xenograft pipeline, retrograde labeling of cells, selective cell enrichment, working CRISPRi iPSC lines, dual-color imaging, and prior demonstration of replay-like co-firing.
- The use of human surgical explants is relevant but quality, access and disease injury may vary.
- Power analyses are provided per aim with effect-size estimates derived from preliminary data.
- The Aim 1B premise that surface-protein markers exist that cleanly separate IT from ET PNs is unproven; the TF-overexpression contingency would be more significantly complex.
- The axon-outgrowth screen scope (library size, guide composition) is not explicitly stated, making the feasibility of in vivo validation hard to judge precisely.
- The rationale for selecting the specific organoid differentiation protocol is unclear. As well articulated in the application, the generation of the IT and ET PNs in sufficient numbers is the main challenge or bottleneck, and their purification from the organoid cultures appears to be cumbersome. It is unclear why this specific approach was chosen, as opposed to other approaches to generate possibly more pure populations of these neurons.
- The rationale for the CRISPRi screen to enhance axonogenesis in human brain explants (Aim 2), and the investigation of the link between neural activity and functional integration (Aim 3) is compelling, and the experiments are feasible.
- The models (organoids, human brain explants and mice) are appropriate.

**Plan & Design: Evaluate the project plan and design**

- The project is likely to increase the understanding of the mechanisms that underlie successful integration of PN subtypes after transplantation.
- Potential pitfalls are overall discussed, and alternative approaches presented, and compelling for Aims 2 and 3.
- The three aims are logically sequenced; each can yield meaningful findings if downstream aims fall short. Caveats and alternative strategies are stated for every aim with concrete fallbacks.
- Team composition is genuinely complementary — the PI's organoid and genomics expertise with co-I's miniscope and behavior platform — and preliminary data already came from this collaboration, which de-risks team functioning.
- In Aim 1A human iPSC derived cortical organoids will be transplanted into the juvenile mouse brain. Retrograde labeling and scRNA will be used to identify PNs. For Aim 1b cell type-specific surface protein markers will be identified, isolated by FACS and molecular identities confirmed via scRNAseq.

In Aim 1C these enriched PNs will be transplanted back into juvenile cortex of mice and after 12 weeks cells analyzed as before. This seems a reasonable and feasible.

- Enrichment relies on cell surface protein selection which could be risky. The applicants offer direct differentiation of PNs using forced overexpression of transcription factors but this is not tested and might be a new bottleneck.
- Aim 2 addresses the bottleneck of enhancing integration and outgrowth in an adult environment. The applicants received CIRM funding to developed methodologies to increase migration and axiogenesis in adult brain tissue explants. They now propose CRISPRi screens with axon-targeted barcodes to identify genes that can drive axon outgrowth of transplanted PNs in the adult cortex.
- Preliminary data show that transplanted excitatory PNs intermingle with organotypic human brain slice culture -whether this represents integration is not clear and might be a premature conclusion.
- The approach in Aim 2 is ambitious where the applicant will infect target cells with a library of over 120 genes to find the signature that would overcome the inhibition of axon extension. Considering that a network of many factors may need to act in concert at different times and potentially different concentration, it is difficult to envision whether this approach could really identify a robust genemap that works in every environment under both healthy as well as disease conditions.
- Long axons of projection neurons require myelination by oligodendrocytes. This is not specifically addressed.
- In Aim 3 they will use the data generated from aim 1 and aim 2 to test the hypothesis that temporally structured patterns of host neural activity drive the functional integration of PSC-derived PNs into adult circuits, and identify when host–transplant co-activation is critical.
- This is a exciting aim that tests integration in addition to activity. If successful this could really add a new dimension to cell therapies that is definitely needed. The applicant will capture activity from transplanted cells in mice during motor behavior and during rest and sleep which has the advantage of "within session" comparisons.
- Survival of the transplant in immune competent or even slightly immune suppressed environment is also not addressed nor is it clear whether the approaches would be valid in a deceased environment. Theses limitation are not well discussed.
- Integration might be successful but to what extent PN neuron misfire, potentially causing negative side effects is not addressed.
- The leadership, integration, resources and staffing are appropriate.
- The team communication and management plan are effective.
- The communication plan (monthly PI meetings, aim-specific subgroup calls, weekly science reviews) is concrete.
- Institutional resource base is strong and well-leveraged.
- No explicit Gantt-style timeline is visible; Aim 3 (closed-loop optogenetics during sleep) is backloaded and at risk of not being completed within 3 years if Aim 1 is slower than expected.

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

- Sex of iPSC lines and recipient mice is addressed explicitly in study design — both XY and XX iPSC lines and male and female recipients.
- Two genetically and karyotypically distinct iPS cell lines (XY and XX karyotypes) will be tested.

- The clinician-investigator on the team is positioned to keep the research questions tied to real patient populations through ongoing engagement with the institution's stroke center, movement disorders clinic, and ALS center, which represents a credible link between discovery work and the populations it ultimately needs to serve.
- The project utilizes primary human brain tissue derived from adult surgical resections. Samples are derived from diverse patient population and will include provide coverage across all dimensions of diversity (sample availability is not limited).
- The experiments in Aim 2 account for genetic factors by examining brain tissue samples from a diverse set of donors.
- The iPSCs used in the proposal are only from two donors, and the results may thus be vulnerable to genetic variation. However, given the complexity of the proposed experiment, a larger number of cell lines would be difficult to accommodate.
- Genetic ancestry is not addressed in iPSC line selection or in the design of the validation cohort, which is a clear gap given that motor-injury epidemiology has documented disparities by ancestry.
- The proposal does not consider how axon-regeneration biology might vary across age, sex, or genetic background, even though the adult-brain environment is the central biological variable in Aim 2.
- The outreach and educational efforts plans are appropriate.
- The outreach commitments are conventional (society meetings, advocacy presentations) and do not extend the project's relevance or applicability to specific underserved populations in a quantifiable way.
- The applicant argues that the completion of these goals would not be limited to the specific context of PN neurons involved in disease of motor dysfunction. Examples given are not compelling.

| Application# | DISC5-19949 |
|---|---|
| **Title**<br>(as written by the applicant) | Neurovascular control of follicle growth using stem-cell derived ovary assembloids. |
| **Project Objective & Impact**<br>(as written by the applicant) | This project will substantially advance knowledge of neurovascular coupling - a concept well-studied in heart and brain but entirely unexplored in reproductive organs. Our efforts will yield the first neurovascular-ovarian follicle assembloids, paving the way to identifying therapeutic strategies for ovarian aging and PCOS and advancing in vitro gametogenesis for infertility treatment. |
| **Specific Aims**<br>(as written by the applicant) | • Aim 1: Map neurovascular–ovarian cellular networks in health, aging, and PCOS.<br>• Aim 2: Interrogate the interdependence of sympathetic nerves and endothelia in the ovary in vivo.<br>• Aim 3: Model ovarian neurovascular coupling with stem cell-derived follicle assembloids. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Ovarian aging affects all California women, with menopause increasing risks of cardiovascular disease, osteoporosis, and cognitive decline. PCOS impacts 10% of reproductive-age women, disproportionately affecting Latino and South Asian populations. This project will identify therapeutic targets to extend ovarian function, develop platforms for screening rejuvenation strategies, and advance in vitro gametogenesis for cancer survivors. Resulting public datasets will accelerate statewide research. |
| **Funds Requested** | $2,499,997 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

### Final Score: 85

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

| | |
|---|---|
| **Mean** | 86 |
| **Median** | 85 |
| **Standard Deviation** | 2 |
| **Highest** | 90 |
| **Lowest** | 80 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 13 |
| **(1-84): Not recommended for funding** | 2 |

# FINAL COMMENTS

Proposals were evaluated and scored based on the review criteria shown below, which are also described in the PA/RFA. Following panel discussion and scoring, reviewers entered 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 for clarity by CIRM.

**Key Strengths and Weaknesses**

- This work is relevant to over half the global population and if successful could garner insights that impact longevity, cardiovascular health, cognitive well being and fertility in women. Also it could provide new insights into in vitro fertilization options. This proposal was among the better ones in the pool with significant benefits that could extend beyond just women's health.
- This is an interesting proposal to look at neurovascular coupling during ovary development and aging. Innervation and vascularization may be under-appreciated contributors of follicle health, and this grant may shed light on the significance of this biology. However, Aim 3 is risky, which detracts from the overall significance.
- The shortcomings of the proposal are limited to the technical complexity of the assembloids proposed in Aim 3 and may all be addressed with pilot experiments to identify the appropriate matrix for assembloid culture and address other technical challenges. Overall, the proposal is exciting, highly significant to the field, and well thought out.
- Good team, assembloid might not work.

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

- Creating ovary "assembloids" comprised of 3 ovary cell types then combined with ovarian neurovascular organoids to improve ovary organoid maturation allows asking reciprocal questions about tissue vascular interdependence and homeostasis.
- Ovarian aging potentially impacts health both of women and their offspring. Understanding its genesis and the role of a vascular or neurovascular component is a next logical step in the field.
- The applicant hypothesizes that ovary aging is impacted by altered sympathetic neuron (SN) organization, which has been shown to be altered in aged and post-menopausal ovaries. The applicant calls these changes "neurovascular coupling" as the innervation is also associated with ovarian vasculature. At present, this is a correlation only, and does not exclude the importance of other factors in ovarian aging, or directly prove that ovarian failure is neuronally based.
- This group may succeed in making ovarian organoids from stem cells, though only the mouse genetic experiments described in aim 2 can really test is neurovascular coupling is critical to ovarian function.
- Overall, this work might shed light on whether (or not) neurovascular function is important for the survival and function of follicles in ovaries.
- This work could also help refine the understanding of polycystic ovary disease.
- Improved approaches for in vitro gametogenesis are a pressing need in assisted reproduction and reproductive biology, and neurovascular coupling in the ovary is a much-needed area of investigation—the successful completion of these aims may be truly transformative for the field, and to date no one appears to have explored these questions.

- The multifaceted approach to neurovascular coupling in the ovary would yield both mechanistic insights and therapeutic targets for reproductive medicine, and the proposed approach is uniquely positioned to bring about these results.
- The incorporation of polycystic ovary syndrome (PCOS) phenotypes throughout the proposal will be highly valued by the field, as this is an understudied area and access to tissues and appropriate models is limited.

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

- Vascular aging contributes to ovarian decline beginning at about age 35. This study is the first application of neurovascular coupling to the ovary organoids to create novel multilineage human constructs for analyses and comparison to mice in vivo data. Prior atlases of mouse and human ovaries have been constructed but lack neurovascular data. Applicant's goal is to add neurovascular components across age and PCOS in unique tissue assemblies (n=3 ea).
- Given the known role of vascular aging in pregnancy duration and the impact of neurovascular aging in other systems, it is reasonable to evaluate the neurovascular components of ovarian aging.
- The use of iPSC-derived organoids alongside follicles is new and if successful will open the door to more in depth understanding of cell interactions ex vivo with implications for IVF and infertility.
- The use of mice to assess ovarian neuronal function is appropriate because innervation and connection with the brain and/or celiac ganglia is critical to see if innervation affects follicle function and survival. It is not possible to model this only with stem cell in vitro models.
- The proposal brings strong technical and mechanistic innovation to the fields of regenerative and reproductive medicine. The multifaceted approach to investigating neurovascular coupling is thorough in tackling this question from many angles; the proposed assembloid work if successful would revolutionize follicle culture and assisted reproduction, but this aim seems to have limited feasibility compared to other parts of the proposal.
- The use of iPSC-derived organoids alongside follicles is exciting and new to the field. While the feasibility is questionable in this preliminary review, the introduction of iPSC support into the in vitro follicle culture community is compelling and will likely enable future, more broad research in this area.

**Rationale: Evaluate the scientific rationale in the proposal**

- Ovarian aging occurs in every woman beginning around age 35 and impacts not only fertility but cardiovascular risk and general health.
- The central hypothesis is that neurovascular coupling is critical to ovarian function and survival of follicles. However, there is concern since the mouse genetic data shows that when SNs are perturbed in mice, that considerable follicles remain, though they are decreased. This suggests that neurovascular coupling may be only part of the story.
- Aim 3 proposes to make ovarian organoids (this is good on its own). However, there is also a plan to include sympathetic neurons in these organoids and even making neuronal organoids to be made into "assembloids". Vascular organoids will also be included in assembloids. However, there is considerable risk that these will not behave similarly to in vivo follicles that are both vascularized and innervated.
- There is some mouse model work proposed, which is warranted as the grant explores the role of vascular and neuronal control of the ovary, which cannot be modeled in organoid systems effectively.

- The applicants have provided solid justification for the specific investigation of SNs from the celiac ganglia using prior datasets; however there is limited discussion of other neuronal subtypes in the background information or alternative approaches section.

- The proposal investigates the SN and vascular density aspects of neurovascular coupling, but none of the blood flow aspects of neurovascular coupling. This may be beyond the scope of the proposal, but investigation of blood flow, volume, and oxygenation are central to understanding the functional importance of neurovascular coupling for ovarian function and follicles.

**Plan & Design: Evaluate the project plan and design**

- Project plan is designed appropriately to undertake in vivo analyses in mice and test comparability in human tissue constructs.

- Aim 1: The approach is to study neurovascular structure in more detail in normal ovaries, aged ovaries, and in polycystic ovarian syndrome. Aim 1 is purely descriptive in nature, and may not yield "a comprehensive foundation for understanding neurovascular networks" (as stated as their aim1 goal) since the proposed experiments are descriptive only. However, this concern is addressed in other aims.

- Aim 2 is strong experimentally, as they propose to ablate SNs and Schwann cells in mice using cre-lox approaches. Similar approaches will ablate angiogenesis in the ovary. The hypothesis is that these genetic disruptions will perturb ovarian function, but if this is not the case, the central hypothesis is likely not valid.

- Aim 3 consists of an attempt to make follicle organoids from stem cells (mouse and human) and to see if neurovascular coupling is affected. A concern here is that there is no brain or hormonal control of SNs in such a system.

- The preliminary data in Fig. 3 shows deficient follicles in mouse ovaries made deficient for SN. However the magnitude of follicle deficiencies is only modest. This helps to support the neurovascular coupling idea, but also suggests that other factors must contribute to follicle growth and function, especially in the growing follicle stage. Fig. 4 shows that there is a good start in making ovarian organoids.

- The proposed aims comprehensively tackle the overarching scientific question from many angles, with both mechanism and therapeutic targets in mind. The technical approach is generally well balanced between innovation and feasibility, and most concerns are with Aim 3.

- Aim 3 seeks to use neural and vascular iPSC-derived organoids along with human follicles to model neurovascular-follicle coupling in vitro. The combination of two organoid types alongside early stage human follicles seems far-fetched especially with regard to the number of exogenous factors needed to sustain each component of the system.

- Aim 1 is a logical start to the proposed work and leverages prior datasets alongside new SN data from the celiac ganglia.

- The targeted genetic knockouts at the center of Aim 2 are elegant and well-explained. Intrabursal delivery seems feasible and preliminary work in this area is promising. Stimulation with gonadotropin is mentioned but it is unclear how exogenous manipulation of folliculogenesis may alter the terminal analyses of follicle development and oocyte competence.

- The applicants also propose to use an inert agarose matrix, or if necessary a fibrin-alginate matrix, to support this assembloid system, but this fails to carefully consider how each component of the assembloid may need to remodel or lay down ECM like what happens in vivo. The problem of a proper matrix for the assembloid is concerning, and the system seems too complex to become widely usable in the field.

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

- Applicants state human ovarian tissue will be sourced from donors (n=3 each at 25-35 and 35-50) representing CA's diverse population with age ethnicity and demographics used to stratify. PCOS occurs at a higher frequency in "Hispanic and South Asian populations"; including these women is an attempt to address population.

- The proposal shows comprehensive consideration of genetic background and population diversity for human samples and well-established relationships for acquiring the tissues needed.

- Age is a central variable in this proposal and is considered throughout the proposed aims.

- Understanding PCOS mechanisms and identifying therapeutic targets are pressing needs, but it is unclear how PCOS status will be scored for patients. It is also unclear whether only severe PCOS will be included and whether the applicants will have support from a collaborator with expertise in PCOS.

- The population impact is not strong, consisting mostly of a pledge to recruit a diverse research team. The project does not consider diverse human ovaries or the use of diverse stem cell sources.

| Application# | DISC5-19810 |
|---|---|
| **Title**<br>(as written by the applicant) | Impact of Sex-Differences on Intestinal Stem Cell Function in Pediatric Inflammatory Bowel Disease. |
| **Project Objective & Impact**<br>(as written by the applicant) | The intestine is a sexually dimorphic organ in which renewal, barrier integrity, and immune function are influenced by estrogenic and androgenic pathways. While sex hormones are known to modulate growth, differentiation, and inflammation in multiple tissues, how these differences shape intestinal cell states during development and contribute to diseases like pediatric IBD is poorly understood. |
| **Specific Aims**<br>(as written by the applicant) | • Define the sex- and age-dependent intestinal intrinsic and microenvironmental gene regulatory mechanisms in children and adolescents with and without IBD.<br>• Aim 2. Evaluate the impact of sex hormone signaling on intestinal stem cell (ISC) differentiation and function using patient-derived organoids |
| **Statement of Benefit to California**<br>(as written by the applicant) | Crohn disease, a form of IBD, shifts from male predominance in childhood to female predominance after puberty. In contrast, risk for Ulcerative Colitis, a second form of IBD, decreases in females after puberty. Pubertal onset coincides with profound endocrine changes, marking a critical period for growth, development, and therapeutic choices. Deciphering the sex-specific regulation of intestinal stem cells and gut healing may unlock new diagnostic, prognostic, and therapeutic options for IBD. |
| **Funds Requested** | $2,499,999 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

### Final Score: 85

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

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

# FINAL COMMENTS

Proposals were evaluated and scored based on the review criteria shown below, which are also described in the PA/RFA. Following panel discussion and scoring, reviewers entered 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 for clarity by CIRM.

**Key Strengths and Weaknesses**

- This was my favorite proposal. It is clearly written, addresses pediatric IBD (Inflammatory Bowel Disease) by sex and will fill in a data set that today is missing: female IBD cohorts.
- This proposal is well-designed, the team has access to the human tissues required for the studies and has the expertise to carry out the proposed work.
- The proposal is conceptually interesting, and there is a high likelihood that there are sex differences in stem cells that are not yet understood. Enthusiasm is dampened by a narrow focus on estrogen, limited preliminary data to support premise/feasibility, and limited depth in examination of stem cell biology/function.

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

- IBD is increasing/occurring at younger ages and shows significant sex differences pre/post puberty. This proposal will address gaps in knowledge both about IBD and about the cellular targets of the disease in pediatric patients. This is a clearly written proposal that articulates a step by step approach to evaluating pediatric IBD and the sex and age dependent changes in intestinal cells that may underlie it.
- This is a highly significant and exceptionally comprehensive proposal focused on understanding how sex and pubertal development impact inflammatory bowel disease.
- Despite clear clinical evidence that disease presentation and progression differ between males and females during puberty, the biological mechanisms underlying these differences remain poorly understood.
- The proposed studies directly address this important gap with a strong focus on how developmental age and sex reshape intestinal stem cell biology, epithelial repair mechanisms, and inflammatory responses.
- These studies are well positioned to identify sex-specific pathways, biomarkers, and therapeutic targets relevant to disease progression in pediatric IBD. More broadly, this work may establish a conceptual framework for understanding sexually dimorphic stem cell regulation in other tissues and disease contexts.
- Will examine how sex differences influence intestinal cells; relevant to Crohn's disease (CD) which increased in females post puberty (opposite for ulcerative colitis). And males being more likely to have CD in earlier childhood.

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

- A major innovation is simply studying female pediatric patients with IBD. That coupled with functional genomics assessments of the age and hormone-specific changes that underlie pediatric IBD make this novel and of benefit to the community.
- A paucity of data exist about pediatric females with IBD. Age and sex specific analyses of both the disease onset and progression coupled with functional genomics assessments of intestinal cells could provide new insights into how to treat disease in children and perhaps how to mitigate disease via regenerative strategies.

- A key innovation is the integration of single cell multiome and spatial transcriptomics to address how puberty and sex hormones influence intestinal epithelial biology and IBD progression.
- Another important strength is the focus on pediatric patient-derived samples across pre- and post-pubertal developmental stages, which represents a uniquely valuable and understudied biological context.
- Employs single-cell genomics, spatial transcriptomics, and functional organoid studies.
- There has been work examining estrogen effects on intestinal epithelial organoids, which dampens novelty.

**Rationale: Evaluate the scientific rationale in the proposal**

- The current therapies for IBD involve lifelong immunosuppression in pediatric patients, which pose significant development and health risks over their lifetime.
- Given that IBD differs clinically by sex and age, the lack of mechanistic understanding in this area represents a major gap that the proposed work should fill.
- The paucity of data regarding age and sex specific analyses of both the disease onset and progression coupled with functional genomics assessments of intestinal cells could provide new insights into how to treat disease in children and perhaps how to mitigate disease via regenerative strategies.
- The scientific rationale for this proposal is exceptionally strong and well supported by both clinical observations and emerging biological evidence. The investigators build a compelling case that puberty-associated hormonal changes may fundamentally reshape intestinal epithelial and inflammatory programs, thereby contributing to divergent disease phenotypes between males and females with IBD.
- Given the recognized clinical importance of puberty and sex in IBD management, the lack of mechanistic understanding in this area represents a major gap that strongly justifies the proposed work.
- The proposed approaches are scientifically sound and highly appropriate for the questions being addressed. The use of patient-derived biopsies spanning sex and pubertal stages, combined with single-cell multiomics and spatial transcriptomics, is well justified for defining cell-intrinsic and microenvironmental regulatory programs.
- The proposal is ambitious, but the extensive preliminary rationale, strong conceptual framework, and complementary expertise of the investigative team support overall feasibility.

Even where aspects remain exploratory, the potential knowledge gains and translational implications clearly justify the proposed investment.

- The proposal is lacking preliminary data supporting the overarching premise, although it is plausible that hormones will affect stem cells based on published studies.

**Plan & Design: Evaluate the project plan and design**

- The straightforward functional genomics assessment of patient samples across sex and age is clearly articulated and supported.
- The project is exceptionally well designed and logically organized to generate meaningful mechanistic and translational insights. The aims are highly complementary, progressing from unbiased molecular characterization of patient tissues to spatial analysis of tissue organization and finally to functional testing in organoid systems.

- Aim 1 is particularly strong in its integration of single-cell multiomics and spatial transcriptomics to define sex, age, and disease dependent regulatory programs across epithelial, stromal, and immune compartments. The proposed analyses are sophisticated and well aligned with the central hypotheses.
- Aim 2 appropriately extends these findings into functional studies using donor-derived organoids to test the direct effects of estrogen signaling on intestinal stem cell renewal, epithelial integrity, and inflammatory injury responses. This translational progression from patient tissue profiling to mechanistic validation is a major strength of the proposal.
- Potential challenges, including patient variability and tissue heterogeneity, are acknowledged and partially mitigated through the large and diverse pediatric patient population available through host institution's hospital.
- The team is highly appropriate, bringing together investigators with complementary expertise in intestinal biology and functional genomics. The institutional environment, patient access, and technical resources appear outstanding and well suited to support successful completion of the work.
- Aim 1 will use a single-cell multiomic approach for epithelial, stromal, and immune compartments and then track sex-associated changes in gene expression patterns and transcription factor activity. While this approach will identify correlative differences, there are other confounding variables that need to be thoroughly ruled out.
- Aim 2 proposes to evaluate the impact of sex hormones on stem cell differentiation. This is an important aim, however it is limited in scope to estrogen and female organoids.
- Proposal integrates experienced intestinal epithelial expertise and functional genomics researchers to globally identify associations in expression and chromatin profiles.
- The proposal includes immunofluorescence and RNAscope in situ mRNA hybridization on mucosal biopsies combined with cell-specific markers for androgen receptor (AR) and estrogen receptor (ER) to determine cell expression patterns across all donor categories.

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

- The major weakness is that population impact is not clearly articulated. Mention is made of recruiting pre/post pubertal males and females across race, ethnicity and home zip code suggesting that diverse genetic groups will be captured.
- Utilizing demographic information such as zip code suggests the investigators will also begin to be able to address social determinants of health in this population.
- The proposal demonstrates an excellent commitment to understanding disease mechanisms across biologically and demographically diverse populations. A major strength is the deliberate inclusion of pre and post pubertal male and female donors, which directly addresses an important but historically understudied source of biological variability in IBD research.
- Applicant will enroll pre/post-pubertal male and female donors on an ongoing basis and anticipate capturing diversity across genetics, environment, and age.

| Application# | DISC5-19686 |
|---|---|
| **Title**<br>(as written by the applicant) | Allelic Expression States as Determinants of Neurodevelopmental Disorder Risk |
| **Project Objective & Impact**<br>(as written by the applicant) | A major barrier in stem cell–based disease modeling is understanding how cell-type–specific allelic expression states arise during human neurodevelopment and create selective disease vulnerability. Defining mechanistic control of allele-biased transcription will improve the interpretation of genetic risk and functional outcomes in human stem cell–derived neural systems and future therapies. |
| **Specific Aims**<br>(as written by the applicant) | • Define developmental maps of allelic expression in stem cell–derived neural cells.<br>• Identify chromatin and TF mechanisms controlling allelic bias.<br>• Test stability and reversibility of allelic states in human neural models. |
| **Statement of Benefit to California**<br>(as written by the applicant) | This project strengthens California stem cell leadership by improving the biological fidelity of human neurodevelopmental disease models and improving the outcomes of gene and cell therapies for neurological conditions. By defining allele-specific regulatory mechanisms, the project enhances the interpretation of genetic risk in disorders affecting Californians. Open datasets, methods, and training in allele-aware stem cell genomics expand statewide research capacity and collaborative innovation. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

### Final Score: 85

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

| | |
|---|---|
| **Mean** | 85 |
| **Median** | 85 |
| **Standard Deviation** | 2 |
| **Highest** | 90 |
| **Lowest** | 80 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 13 |
| **(1-84): Not recommended for funding** | 2 |

# FINAL COMMENTS

Proposals were evaluated and scored based on the review criteria shown below, which are also described in the PA/RFA. Following panel discussion and scoring, reviewers entered 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 for clarity by CIRM.

**Key Strengths and Weaknesses**

- This was a very strong application with very few weaknesses. There is excellent preliminary work, a detailed experimental plan, and a reasonable budget and timeline.
- This is a strong and fundable proposal with impressive technical sophistication and strong preliminary data, although the ambitious scope and complexity somewhat reduce confidence in the depth and completeness of mechanistic insight across all aims.
- The project will generate valuable datasets, and is overall meritorious. There are a few concerns about the lack of clarity of the genome-wide versus locus specific focus analysis, and a lack of preliminary data of Aim 2.

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

- This project addresses a fundamental gap by investigating how allelic expression states are dynamically regulated during human neurodevelopment. By moving beyond gene dosage to allele-specific regulation in human stem cell–derived systems, it has strong potential to advance foundational understanding of gene control and disease vulnerability.
- The proposal introduces a potentially impactful framework in which allelic expression is treated as a dynamic, regulatable state rather than a fixed genetic outcome. This could refine current models of disease risk, though the broader generalizability and biological impact of these allelic states are not clear.
- The outcome of the work will most certainly advance stem cell biology knowledge as it relates to human cortical development.
- The successful completion of this project will advance fundamental knowledge of allelic expression states, and how they are regulated, across neuronal development. This foundational knowledge will likely have broad implications for our understanding for regulation of gene expression during healthy brain development, and disease. This is, in turn, likely to have implication on downstream therapeutic development.
- The biological insights will be informative broadly for neurodevelopment and neurodevelopmental disorders.
- The study results will inform how, when and where to intervene for some neurodevelopmental disorders.
- The integration of allele-resolved genomics and causal perturbations is well suited to generate mechanistic insights, particularly on reversibility of allelic states. However, translational implications are largely indirect.
- One concern is the lack of clarity, from an analytical perspective, on genome-wide analysis versus a focus on the proposed locus, which could affect the generalizability of the findings, and how wide-ranging they may be.

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

- The project is innovative and the employs innovative stem cell research to understand under appreciated issues in neurodevelopmental biology.
- A key innovation is the proposal of allelic expression states as dynamic, regulatable regulatory configurations, rather than fixed genetic outcomes. This represents a potentially important conceptual advance.
- The project is largely innovative, since allelic expression states are not systematically studied in neurodevelopmental disorders.
- Proposal demonstrates strong integration of stem cell biology, allele-resolved genomics, chromatin architecture, and transcription factor network analysis. The combination of single-cell multi-omics, 3D genome mapping, and organoid-based human models demonstrates cross-disciplinary approach.
- The use of allele-resolved, single-cell multi-omic profiling combined with targeted perturbations in human cortical organoids is innovative and well suited to the problem.
- The proposal uses cutting edge genetic and epigenetic approaches.

**Rationale: Evaluate the scientific rationale in the proposal**

- The scientific rationale is strong. The proposal addresses a key gap in understanding how allelic expression contributes to selective vulnerability in NDDs, challenging conventional gene-dosage models.
- The rationale is supported by compelling preliminary data showing dynamic monoallelic and biallelic expression at the proposed locus, including evidence of reversibility. These findings support feasibility and justify the exploratory aspects of the project, although the extent to which findings generalize to other loci is not clear.
- The experimental strategy is well justified and logically aligned with the central hypothesis. The use of human iPSC-derived cortical organoids is appropriate, with non-human models used appropriately for validation. The integration of allele-resolved multi-omics and targeted perturbations is appropriate for moving from observation to mechanism.
- The scientific rationale is sound and compelling.
- The studies are rationally designed and with the team in place they are feasible but somewhat ambitious.
- The preliminary data largely support the feasibility of the proposed experiments, with the exception of Aim 2 where there is no preliminary data for allele resolved snm3C, and this analysis and interpretation is likely challenging as the team acknowledges.

**Plan & Design: Evaluate the project plan and design**

- This project introduces an underappreciated idea – that allelic expression states at autosomal transcription factor loci embedded within gene-poor, regulatory-dense genomic regions are dynamic.
- By integrating transcriptomics with chromatin and transcription factor–centric analyses in fully human stem cell–derived neural models, the team will define the extent of allelic expression occurring and whether these impart selective vulnerability in the context of mutations causing neurodevelopmental disease.
- Well structured, with a clear progression from descriptive mapping of allelic states, to mechanistic dissection and causal testing. This design is logically aligned with the central hypothesis and is likely to

generate meaningful mechanistic insights, particularly through integration of allele-resolved multi-omics and perturbation strategies.

- The leadership team is strong, with complementary expertise in stem cell biology, genomics, and computational analysis. The plan for team coordination is appropriate.
- The leadership, integration, resources and staffing are appropriate.
- The team communication and management plan are effective.
- The budget and timeline are appropriate.
- Identifies relevant technical and biological risks and provides thoughtful and appropriate mitigation strategies, including use of multiple donor lines, complementary assays, and alternative model systems. Proposed assays rely on subtle allele-specific signals that may be difficult to resolve robustly across heterogeneous organoid systems.
- Aim 2 in particular lacks sufficient detail: bulk assays are proposed for validation in “defined neural populations”. It is unclear which neuronal populations are referred to, and which protocols? Will this be performed on all cell lines, or a subset?
- The project is overall likely to yield informative insights, although in most cases, the focus on the specified gene vs genome-wide allelic states in unclear.
- No preliminary data is presented for allele resolved snm3C.
- Focus on discovery and causality is a strength, although scope is highly ambitious and may limit mechanistic depth and interpretability across all loci and developmental contexts.
- The project is ambitious for a 3-year DISC5 timeframe, given the complexity of multi-omic profiling and perturbation studies. While feasible in principle, the breadth of multi-omic profiling, perturbational studies, and cross-line comparisons introduces substantial execution risk within the proposed timeframe.

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

- The team will employ hiPSC lines derived from donors representing diverse genetic ancestries and mixed backgrounds to determine if the findings are conserved or how they are divergent. This will be meaningful to the field.
- The proposal thoughtfully incorporates genetic diversity and sex as key variables, using iPSC lines from donors with diverse ancestries and including isogenic controls to separate allele-specific effects from background variation. Developmental stage and cellular context are also explicitly considered.
- By examining whether allelic regulatory principles are conserved or vary across genetic backgrounds, the project has good potential to inform the generalizability of findings. The team demonstrates strong commitment to community engagement and science communication.
- The experimental design adequately captures genetic diversity by investigating several lines from diverse backgrounds.
- The resulting framework may be extending to investigate variants prevalent in specific communities.
- Prior and proposed outreach are appropriate.

| Application# | DISC5-20061 |
|---|---|
| **Title**<br>(as written by the applicant) | Characterizing how neurons from human stem cell transplants integrate into spinal neural circuits following injury |
| **Project Objective & Impact**<br>(as written by the applicant) | Human neural stem cell grafts into sites of spinal cord injury can restore some motor function. However, how the neural circuit formation and firing patterns relate to motor recovery are unknown. We will study human neural stem cell circuit formation in high detail which could serve as foundational principles of neural stem cell treatment strategies for all neurological disorders and injuries. |
| **Specific Aims**<br>(as written by the applicant) | • Analysis of emergent cell types and connections from transplanted human neural stem cells.<br>• Define the neural activity within human neural stem cell grafts during locomotion. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Hundreds of thousands of California residents have experienced some degree of neurological injury or disorder such as stroke, spinal cord injury, traumatic brain injury or Parkinson's disease. We will uncover fundamental regenerative neuroscience principles that could help enhance treatment and maximize quality of life for Californians afflicted with these diseases. |
| **Funds Requested** | $2,499,999 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

### Final Score: 85

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

| | |
|---|---|
| **Mean** | 85 |
| **Median** | 85 |
| **Standard Deviation** | 1 |
| **Highest** | 86 |
| **Lowest** | 83 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 13 |
| **(1-84): Not recommended for funding** | 2 |

# FINAL COMMENTS

Proposals were evaluated and scored based on the review criteria shown below, which are also described in the PA/RFA. Following panel discussion and scoring, reviewers entered 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 for clarity by CIRM.

**Key Strengths and Weaknesses**

- This application could easily also go into the non-funding category. The problems in the experimental plan are significant ones (a single cell line, lack of strategies on decision points and quantification strategies for good versus less-good results, lack of attention to glial cell contribution to neuronal function, and other problems as addressed earlier).
- There is importance in trying to better understand what happens to transplanted cells, and the applicant team is well qualified to try and address this problem. The concern is that the way the applicant proposes to do this will make sub-optimal use of the tissue in providing information to alter future strategies.

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

- The proposal addresses a central mechanistic gap in regenerative neuroscience — whether NSC grafts function primarily as permissive, gain-modulating relays or as instructive neural circuits — a question that directly limits the rational optimization of graft-based therapies for SCI and potentially other CNS injuries, such as stroke and TBI.
- Proposed outputs, including cell-type-specific Cre lines, V2a-silencing functional datasets, and a spatial transcriptomic atlas of graft-derived circuitry, would constitute valuable community resources for investigators developing neural graft therapies. Generation of multiple genetically engineered H9 Cre lines for spinal interneuron subclasses provides a potentially durable platform resource extending beyond the immediate aims of the proposal.
- Technical integration of neural identity, neurophysiology, and locomotor behavior at a cell resolution level would be a step forward for the transplant field.
- The group's prior demonstration of substantial recovery of skilled hand function following focal cervical SCI and similar transplantation in non-human primates provides a strong biological rationale for pursuing mechanistic dissection of graft function.
- Significance is closely tied to one specific cell product platform; broader applicability to alternative NSC products or manufacturing strategies is asserted rather than experimentally addressed. It's also not clear how the exogenous trophic factor component of grafts will be accounted for.
- The proposal does not define quantitative decision thresholds that would alter future graft-engineering strategies (e.g., the magnitude of V2a contribution required to justify subtype enrichment), leaving translational implications somewhat qualitative.
- Although the proposal references progress toward IND-enabling studies, clinical translation activities themselves are outside DISC5 scope.
- Proposal addresses a critical gap in regenerative neuroscience: understanding how transplanted neural stem cells functionally integrate into host neural circuits after injury. While prior work demonstrates structural integration and partial recovery, the cellular identities, connectivity patterns, and activity dynamics of graft-derived neurons remain poorly defined. This project has strong potential to advance foundational knowledge in neural repair.

- The proposal has moderate-to-high potential to shift the field by reframing NSC grafts as active, functionally organized neural circuits rather than passive relays. However, the conceptual advance is refinement of an emerging idea rather than entirely new paradigm.
- The study is well positioned to generate mechanistic insights by combining spatial transcriptomics, synaptic mapping, and electrophysiology during behavior. These data could inform optimization of cell types, circuit design, and stimulation strategies for future therapies. However, direct paths to biomarkers or near-term therapeutic translation are largely indirect.
- Transplanting neuroepithelial stem cells into injured spinal cord has provided promising improvement in function in large animal models, but does not constitute full repair. It would be helpful to have more information on what is happening to transplanted cells in terms of their differentiation and function.

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

- Chronic in vivo electrophysiological recording from NSC grafts in awake behaving animals using ultraflexible (1 μm) NET probes represents a highly innovative and potentially field-advancing application of spinal recording technology.
- The planned custom human 500-gene MERFISH panel for graft characterization is innovative, and feasibility is strengthened by the group's prior experience developing analogous mouse spinal cord panels.
- The investigators have already generated a CRISPR-engineered V2a-Cre H9 line and demonstrated Cre-dependent inhibitory DREADD silencing in vitro, enabling subtype-specific causal perturbation studies.
- Planned generation of additional V1, V3, and MSE Cre knock-in lines would substantially expand the toolkit available for future mechanistic studies of graft-derived circuitry in addition to the emphasis on V2a interneurons.
- Individual components of the experimental strategy (MERFISH, Syn-Tom tracing, DREADDs, ultraflexible probes) are established technologies; innovation derives primarily from their integration within the graft-repair context rather than from the development of fundamentally new methodologies.
- Causal perturbation experiments are limited to the V2a subclass within the current proposal period; broader cell-type-specific functional dissection is deferred to future studies.
- The proposal frames graft function largely as a binary passive-versus-instructive distinction, whereas actual graft physiology will likely reflect heterogeneous and partially overlapping network mechanisms. The framing is almost certainly an oversimplification, and interpretation is likely to be complex.
- Proposal is somewhat innovative.
- The proposal demonstrates strong integration of stem cell biology, systems neuroscience, spatial transcriptomics, and in vivo electrophysiology. The combination of human NSC transplantation with real-time neural recordings in behaving animals is a good step - combining individual technologies that are already established.
- A key conceptual element is the distinction between passive versus instructive roles of graft-derived circuits, framing NSC transplants as active neural networks whose function can be interrogated and optimized. While this represents a useful and important shift, it builds on emerging ideas rather than introducing a fundamentally new framework.
- The use of flexible nanoelectronic thread electrodes to record activity from graft-derived neurons in awake animals is a significant technical innovation. This is complemented by cell-type–specific tracing, spatial transcriptomics (MERFISH), and chemogenetic silencing (DREADDs), enabling causal and mechanistic interrogation. The generation of genetically defined NSC lines further strengthens the approach.

- The innovation lies in the application of technology but is otherwise limited. The transplants have been studied for a long time, and the goal here is to apply currently available advanced techniques to better characterize what is happening with the transplanted cells.

**Rationale: Evaluate the scientific rationale in the proposal**

- Preliminary support is strong across multiple domains: stable NET-based spinal recordings have been demonstrated in behaving animals; the V2a-Cre H9 line has been generated and shown to be DREADD-responsive in vitro; MERFISH workflows are already operational in the applicant's laboratory; and prior studies demonstrate robust NSC-mediated functional recovery in large animal models.
- The central passive-versus-instructive circuit hypothesis is conceptually clear and experimentally testable using the proposed convergent physiology, transcriptomic, and perturbation approaches. However, little is mentioned about the complexity of entering supraspinal inputs and how they will be resolved.
- Major technical approaches are already established within the participating laboratories, minimizing dependence on external collaborators for core feasibility, and allowing for a reasonable ramp-up to the experiments.
- Statistical planning for Aim 2.2 is reasonably concrete and includes effect-size assumptions and power estimates.
- Sample sizes for Aim 1 (≥12 sections from 4 animals) are very modest relative to the likely heterogeneity of graft-derived cell populations and spatial organization. These experiments are underpowered.
- Potential technical challenges specific to human graft MERFISH analysis in mouse tissue — including segmentation ambiguity, species-specific transcript assignment, and spectral complexity — are not discussed in detail.
- Although stable recordings have been demonstrated in intact spinal cord, direct preliminary evidence for chronic single-unit recording specifically within grafted lesion tissue is limited, and may be significantly more challenging in injured, partly repaired tissue.
- The proposal relies substantially on convergent inference to assign physiological activity to defined neuronal subclasses; direct in vivo subtype identification of recorded units is not yet fully demonstrated.
- The rationale is well established.
- Rationale is strong and addresses a major unresolved question in regenerative neuroscience: how transplanted NSCs integrate into host circuitry after SCI. Although prior studies show that NSC grafts can survive, form synapses, and improve recovery, the activity dynamics of graft-derived circuits remain poorly understood. The distinction between passive relay versus instructive circuit models provides a mechanistic framework for studying stem cell–mediated repair.
- The rationale and feasibility are well supported by strong preliminary data demonstrating survival, connectivity, and functional effects of H9-derived spinal NSCs in rodent and large animal SCI models. Pilot data support the feasibility of chronic spinal recordings using NET probes.
- The experimental approaches are well aligned with the hypotheses. Rodent SCI models are appropriate and supported by prior translational work. A key challenge will be interpreting graft activity patterns and distinguishing passive versus instructive circuit dynamics.
- The central rationale of the proposal is that more information about what is happening functionally in the transplanted NSCs may provide information about how to improve their performance.

**Plan & Design: Evaluate the project plan and design**

- The proposal is logically organized, with appropriately staged aims progressing from molecular/circuit characterization to functional perturbation studies. Aim 2 is well phased into population recording (2.1), V2a perturbation (2.2), and future-enabling line generation (2.3).
- Potential pitfalls and alternative strategies are addressed thoughtfully, including fallback histological methods, higher-density probes, and future optotagging approaches. The data-sharing and computational plans are appropriate for the scale and complexity of the proposed MERFISH and electrophysiological datasets.
- Geographic proximity and complementary expertise of the applicant teams provide a strong collaborative framework for execution. Inclusion of an experienced data project manager strengthens feasibility for handling large multimodal datasets.
- The scope of longitudinal surgeries, behavioral analyses, chronic recordings, transcriptomics, and chemogenetic experiments may be ambitious for a three-year DISC5 mechanism.
- The communication and conflict-resolution plans are relatively minimal compared with the overall technical sophistication of the proposal.
- The proposal does not clearly describe how mechanistic findings would be prioritized or incorporated into downstream translational optimization strategies. The relationship between Aim 2 findings and future graft-engineering decision-making remains somewhat underdeveloped.
- Well structured, progressing from mapping cell types and connectivity to functional circuit analysis. Design is well aligned with the goal of linking molecular identity to function and should yield meaningful insights. The use of complementary approaches is a strength, though the ambitious scope may limit depth.
- The proposal identifies key technical risks (e.g., recording stability, variability in graft activity) and provides practical and generally appropriate alternatives, such as higher-density probes, multiple probe insertions, and computational modeling approaches.
- The project is ambitious for a 3-year timeframe, given the complexity of in vivo recordings, multi-modal analyses, and generation of engineered cell lines. While feasible in principle, success will depend on prioritization and efficient execution.
- The leadership team is a major strength, combining world-leading expertise in spinal cord circuitry and translational stem cell biology. Collaboration is highly complementary and well justified, with access to advanced technologies and infrastructure that support feasibility.
- The collaborative framework benefits from co-located teams and clearly complementary roles, which should facilitate coordination. However, explicit details on project management, decision-making, and data integration workflows are quite limited given the complexity of the effort.
- One major shortcoming is that the proposal is entirely neurocentric in its formulation, even though the transplanted cells also differentiate into glial cells (which are vital for neuronal function). Thus, if decreased function is due to issues with the glial cells then this proposal will not be helpful.
- If not all types of relevant neurons are generated by the transplants, it is unclear which ones need to be added, in what ratio, how to control their relative survival and function, and whether they integrate with other transplant cells or with host tissue. Questions of this nature are not addressed.
- Experimental strategies are tied to one NSC product, despite information from other laboratories that there is great variability in the effective of different NSC populations that are putatively the same as each other.
- The proposal lacks information about how decisions about favorable or unfavorable results will be determined.

- Interpretations seem greatly oversimplified considering the complexity of these cell populations and their interactions.
- Direct evidence for success of the proposed electrophysiological recording system in the lesion environment is lacking.
- There is no information on how data outcomes will inform next stage decisions.

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

- Planned engagement with patient advocacy and neurological disorder foundations is appropriate for a preclinical regenerative neuroscience project. The proposal appropriately emphasizes broad potential applicability of mechanistic insights across CNS injury contexts. However, outreach activities are primarily educational and foundation-facing; the proposal does not articulate project-specific strategies to improve relevance or accessibility for underserved patient populations.
- Both male and female recipient animals are included explicitly in the study design. However, assertions that graft-derived circuitry is expected to function similarly across sex, genetic background, and populations are not substantially supported by experimental evidence or literature discussion.
- The proposal does not meaningfully address potential biological variability related to sex, immune compatibility, or broader diversity considerations relevant to eventual clinical translation. Use of a single XX-derived H9 ES cell line without discussion of HLA diversity, alternative donor strategies, or future manufacturability considerations limits the depth of the population-impact framework.
- The proposal gives limited attention to population variability. It assumes that neural circuit organization and graft behavior are broadly conserved across sex, genetic background, and populations, with some inclusion of both male and female animals.
- The work is positioned as broadly applicable to conditions such as SCI, stroke, and TBI. However, this generalizability is largely assumed rather than empirically tested.
- The team demonstrates strong commitment to outreach and engagement with patient advocacy organizations.
- Spinal cord injury is a problem for all ethnic groups.
- The use of a single cell line provides no insight regarding variations in cells from different individuals.
- Other aspects of population concerns are standard and are in the acceptable range.

| Application# | DISC5-19811 |
|---|---|
| **Title**<br>(as written by the applicant) | Mapping Human Retinal Niches to Improve Stem-Cell Therapy for Geographic Atrophy |
| **Project Objective & Impact**<br>(as written by the applicant) | Geographic atrophy causes irreversible vision loss, yet stem-cell therapies show unpredictable survival near disease lesions. This project addresses the lack of human-based understanding of why transplanted cells succeed or fail in specific retinal regions. Success will define biological barriers and opportunities for improving regenerative strategies. |
| **Specific Aims**<br>(as written by the applicant) | • Define the cellular, molecular, and structural features of distinct human retinal regions in geographic atrophy, with emphasis on the transition zone where vision loss progresses.<br>• Test how human disease microenvironments influence stem-cell–derived retinal graft behavior using engineered retinal models and living human retinal tissue.<br>• Determine whether stressors that impair graft survival represent fixed disease constraints or biologically modifiable barriers by selectively probing human retinal systems. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Geographic atrophy affects a growing aging population in California and leads to permanent vision loss and loss of independence. By advancing human-based understanding of why regenerative therapies fail or succeed, this research supports development of more effective future treatments, reduces long-term healthcare burden, and strengthens California's leadership in regenerative medicine research. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

**Final Score: 85**

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

| | |
|---|---|
| **Mean** | 85 |
| **Median** | 85 |
| **Standard Deviation** | 5 |
| **Highest** | 90 |
| **Lowest** | 75 |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 10 |
| **(1-84): Not recommended for funding** | 4 |

# FINAL COMMENTS

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

**Key Strengths and Weaknesses**

- The models have limitations that may or may not be important. Working with human tissue is important in studies of GA.
- Strength: The significance is obvious, as successful completion has the potential to advance a clinical intervention for thousands of patients who are going blind.
- The hypothesis pursued in this application is based on the observation that the GA transition is biologically distinct, but its specific molecular and cellular features remain unknown. It is, however, not clear whether such differences are functionally relevant to graft rejection. The proposal will shed light on this question and thus address an important knowledge gap.
- Weakness: The very exciting Aim 3 is heavily dependent on the outcomes of Aims 1 and 2 and will address whether graft failure at the GA border is an intrinsic property of the diseased human niche or, in contrast, is manipulable by changing a limited number of factors. This is an important question but depends on the success of the other aims. A better discussion of alternative approaches would have been helpful.
- Bioinformatic expertise is not explicitly described.
- The scientific rationale for conducting the proposed research is fundamentally very strong.

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

- Targeting the transition zone in GA is a very active area of research. The proposal has the potential for enormous impact on a very important disease.
- The proposal addresses a significant knowledge gap and, if successful, would have a major impact for millions of individuals worldwide who experience irreversible vision loss.
- Defining the microenvironmental niche that supports regenerative cell survival in the geographic region that harbors surviving cells is a critical step toward a therapeutic intervention.
- There is a poor understanding of the retinal niche microenvironment in the regions where RPE and photoreceptors are viable, dying, dead, or atrophic.
- Successful completion of the project would reveal new paradigms and improve our understanding of the transition zone microenvironment, as well as the human retinal factors that influence cell survival and death.
- A successful outcome would generate important mechanistic knowledge that could inform the development of future therapeutics and/or biomarkers in regenerative medicine for a leading blinding disease worldwide for which no therapies are currently available.
- Of note, there are no animal models for dry AMD because of the complex, age-related, multifactorial pathology of the disease. A major strength of this proposal is its use of tissue from diverse human donors

to understand the underlying microenvironment that could influence iPSC-derived RPE cells as therapeutic products.

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

- Using retinal explants from GA and in vitro models is highly innovative.
- A novel aspect of this proposal is the use of human donor retina rather than tissue derived from animal models or indirect approaches.
- While microdissection itself is not novel, the use of a high-precision robotic system to isolate a very small, specific region that cannot be dissected manually is innovative.
- The integration of a 3D-printed, layered human retina-like construct as a manipulable cellular platform for drug development is exciting.
- This project cuts across traditional silos and employs a unique synergy of technologies, including stem cells, living human retinal organoid tissue, iPSC-derived cells, microrobotic surgery, and engineering, to achieve its objectives.
- This proposal applies novel frameworks to stem cell biology and regenerative medicine by utilizing retinal organoid tissue that is, in fact, affected by dry AMD/GA. By understanding the biomarkers, microenvironment, and factors that change during this pathologic state in diseased human tissue, the investigators will gain insight into factors that could impact stem cell transplant viability.
- The approach is extremely innovative and creative, utilizing human retinal organoids and transplanting iPSC-derived cells into tissue from diverse human donors.

**Rationale: Evaluate the scientific rationale in the proposal**

- The transition zone is important in GA. However, the remainder of the relatively normal macula and the central atrophic area are also important with respect to the biochemistry of GA.
- It is already known that the most relevant target cells for regenerative intervention reside in a microscale satellite niche defined by molecular heterogeneity. The applicants will use a microscale robotic system to harvest transition cells and identify niche features that are unique to this region.
- The use of GA-related stressors in a human three-layer retina-like engineered platform established by the investigators to define graft vulnerabilities is a reasonable approach.
- The applicants will determine whether graft failure at the GA border can be overcome. This is a critical step in defining a therapeutic strategy, and the rationale for the approach is solid.
- It is possible that the composition of the GA region is not responsible for graft survival but instead is a consequence of the cell atrophy that occurs in this region. A discussion of alternative hypotheses would have been helpful.
- For the FDA and other regulatory bodies, New Approach Methodologies (NAMs) are innovative, non-animal testing methods, including human cell-based models and computational tools. Driven by the FDA Modernization Act 2.0 and the FDA's Scientific Roadmap, NAMs aim to improve human relevance, reduce animal use, and streamline Investigational New Drug (IND) submissions for biologics and chemical entities.
- The rationale and feasibility are supported by the available data. GA has a substantial unmet need for innovative stem cell therapies. No animal models exist, and transplanted stem cells face challenges in

surviving. Although the preliminary data are limited, the knowledge to be gained justifies the investment of resources.

**Plan & Design: Evaluate the project plan and design**

- Some aspects of GA are not being modeled here, such as the role of inflammation in disease progression. When interpreting the experimental results, it would be important to compare findings from the transition zone with those from the relatively normal macula and the central atrophic area, as toxic factors in these regions may be as important as, or more important than, those in the transition zone.
- Preliminary data suggest that regenerative cell survival is microenvironment-dependent, and it is therefore likely that the specific condition of the GA microenvironment contributes to graft failure. The applicants demonstrate the feasibility of identifying the GA border and transition zone, which is a critical capability for this application.
- Aim 1 is straightforward and will yield important information about the molecular and cellular composition of the GA transition zone. The use of human tissue is a clear strength. The fact that each donor eye contains regions of preserved retina, a transition zone, and an atrophic core enables within-subject analyses.
- Whether a consistent pattern specific to the GA transition zone emerges across diverse samples remains to be seen and is appropriately discussed as a potential pitfall.
- Aim 2 builds on the data generated in Aim 1 to perform functional studies focused on the mechanistic question of how stem cell-derived RPE grafts respond to stress associated with the GA transition zone. This is an exciting aim.
- In Aim 2b, the applicants propose to transplant freshly derived iPSC-RPCs into deceased human tissue, specifically at the anatomically narrow GA transition zone. This is another exciting aim, assuming the transplants survive long enough to permit analysis of region-specific signatures. The proposed mitigation strategy—focusing on early endpoints that are most predictive of engraftment success—is reasonable.
- The project is well designed to generate meaningful insights, with each aim building logically on the previous one. The proposed budget and timeline are appropriate. The leadership, interdisciplinary integration, resources, and staffing are well supported, and the PI has the expertise and infrastructure needed to conduct the work. The plan for team communication and management of the collaborative effort is well documented and appears readily implementable.

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

- The proposal has the potential for a huge impact.
- The proposal does not discuss the etiology of GA, and therefore it is not clear whether external factors contributing to the disease should also be considered.
- If successful, the proposal could lead to therapeutic approaches that would benefit many people across California.
- The proposal will generate a large amount of omics data that will require integration, including potentially complex normalization across variable tissue samples. It is not clear that sufficient bioinformatics expertise is available to manage these analyses.
- The proposal and experimental design account for genetic, environmental, and other external factors that may influence the findings. The use of human retinal organoids from donors with diverse backgrounds enhances the potential for translatability across populations. However, it is unclear how

many donors will be included, which genetic backgrounds will be represented, and what other potential confounding factors will be considered in establishing the models and evaluating the results.

| Application# | DISC5-19893 |
|---|---|
| **Title**<br>(as written by the applicant) | Revealing the Mechanistic Architecture Governing CAR-T Cell Potency and Failure at Single-Cell Resolution |
| **Project Objective & Impact**<br>(as written by the applicant) | A bottleneck in human disease research is the inability to explain why engineered immune cells succeed or fail at a single-cell level, despite similar manufacturing & potency metrics. This project will define how immune state and engineering design shape cell–cell interactions, generating a reusable atlas that enables mechanistic insight, biomarker discovery, & rational design of regenerative therapies. |
| **Specific Aims**<br>(as written by the applicant) | • Design and test CAR regulatory elements that control the level, timing, and stability of CAR expression, and determine how these features shape T-cell behavior.<br>• Generate patient-derived CAR T cells from cancer and autoimmune diseases using non-viral engineering and profile their phenotypic and functional states before and after antigen exposure.<br>• Analyze direct CAR T–target cell interactions at single-cell resolution to capture functional outputs and transcriptional changes during real cell engagement.<br>• Integrate and analyze all datasets using machine learning to build a single-cell based atlas that links CAR design, patient biology, cell state, and interaction behavior to function. |
| **Statement of Benefit to California**<br>(as written by the applicant) | California is home to hundreds of thousands of patients with cancer and autoimmune diseases, many of whom do not respond to existing therapies. CAR T treatments can be life-saving, but failure rates remain high and poorly understood. By studying diverse patient samples from California to uncover why CAR T cells succeed or fail, this project will help investigators design more effective therapies, improve response rates, and strengthen California's leadership in cell therapy research and care. |
| **Funds Requested** | $2,499,738 |
| **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."<br><br>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** | 4 |
| **Highest** | 85 |

| | |
|---|---|
| **Lowest** | 75 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 13 |
| **(1-84): Not recommended for funding** | 2 |

## FINAL COMMENTS

Proposals were evaluated and scored based on the review criteria shown below, which are also described in the PA/RFA. Following panel discussion and scoring, reviewers entered 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 for clarity by CIRM.

**Key Strengths and Weaknesses**

- If successful, the project will generate a rigorously curated CAR T cell atlas that enables novel mechanistic insight into single-cell CAR T behavior and serves as a reusable discovery resource for the broader cell therapy field, supporting improved treatment design and more effective next-generation therapies.
- Aims are well-developed; investigators have appropriate expertise to complete project; will generate significant amount of data though better definition of how this atlas will be used is warranted.
- This is a strong grant addressing an important knowledge gap about CAR T cell potency and failure, leveraging innovative single-cell approaches and assessing cells from both patients with cancer and patients with autoimmunity. Enthusiasm is dampened by a lack of clarity about how the large datasets that will be generated will enable a mechanistic understanding of CAR T cell functional responses in vivo in patients. In vitro effector capacity is not directly predictive of in vivo potency.

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

- The project addresses why CAR T products with similar bulk properties can vary in function, persistence, and clinical performance. The application's central hypothesis is that productive versus non-productive CAR T responses are shaped by CAR expression dynamics and the starting immune state of patient-derived T cells.
- This proposal aims to identify controllable, mechanistic determinants at the single-cell level that differentiate productive from non-productive CAR T cell responses in malignant and autoimmune diseases. This is a significant and potentially impactful area of investigation.
- The approaches used are cutting-edge and will provide new a new atlas of CAR T cell states induced in vitro.
- If successful, the project will generate a rigorously curated CAR T cell atlas that enables novel mechanistic insight into single-cell CAR T behavior and serves as a reusable discovery resource for the broader cell therapy field, supporting improved treatment design and more effective next-generation therapies.
- The project will benefit the CAR T field as a whole as it remains unclear how transcriptional control of CAR expression mediated by enhancer–promoter configurations interacts with starting immune states within patients to drive early activation, dysfunction, or exhaustion.
- The project will be using a framework that incorporates patient-derived products from both malignant and autoimmune sources.

- The project studies generalizable variables: regulatory cassette design, donor immune state, antigen exposure history, and single-cell encounter outcome. However, the proposal remains focused on CD19-directed systems, and the extent to which the findings will generalize to other antigens, CAR architectures, solid tumors, or non-T cell platforms is not directly tested.
- Potential outputs are an atlas that could help identify pre-manufacturing or post-manufacturing immune-state features associated with a higher probability of productive CAR T–target interactions. It could also identify CAR expression programs that reduce tonic signaling while preserving effector function.
- The main limitation is that the proposal does not fully explain how single-cell signatures from heterogeneous products would be translated into actionable manufacturing or selection strategies, especially when the most productive cells cannot obviously be prospectively isolated or expanded.
- It is unclear how well the chosen in vitro readouts of CAR T cell function replicate the complex in vivo environment, and discussion of how findings from the in vitro assays will be translated to ensure in vivo relevance is inadequate. There are known T cell states that demonstrate robust effector responses in vitro but perform poorly in vivo because of homing and survival defects.

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

- Several technologies are integrated in this proposal, including EXTRA-seq enhancer-promoter screening, non-viral targeted CAR insertion, a 36-panel high-dimensional T cell characterization assay, Cell-Cell-seq, CITE-seq, and machine-learning.
- Aim 1 proposes to screen approximately 7,500 enhancer–promoter combinations in Jurkat cells, then down-select four and ultimately two regulatory cassettes for primary-cell studies based on basal activity, inducibility, timing, amplitude, and reversibility of CAR expression.
- Applying single-cell approaches to CAR T cells from both malignant and autoimmune contexts and comparing different CAR designs and responses to short and prolonged stimulation adds novelty. Approaches such as EXTRA-seq and CELL-CELL-seq add novelty.
- Project leverages technically novel single-cell phenotyping, functional secretion profiling, interaction-resolved transcriptional analysis, and quantitative CAR regulatory screening technologies developed and integrated across the applicant and partner institutions.

**Rationale: Evaluate the scientific rationale in the proposal**

- The rationale is sound. There is plenty of evidence to support importance of exploring individual CAR T–target encounters and the contribution of CAR design/expression dynamics, immune status and quality of patient T cells, and attempting to better understand transcriptional and functional aspects.
- The proposal is based on the idea that CAR T cell failure emerges from early antigen-counter decisions that are influenced by CAR expression dynamics and baseline T cell state.
- Aim 1 is supported by major works in the field showing that constitutive CAR expression can lead to tonic signaling and T cell dysfunction/exhaustion. Identifying other expression cassettes may be able to improve CAR T cell function.
- Aim 2 will use non-viral CAR integration into specified loci for controlled expression. This eliminates confounding variables such as the effect of viral transduction or semi-random integration.
- Aim 3 focuses on encounter-level interactions and repeated stimulations then performs transcriptomics on T cells and target cells to identify productive and non-productive interactions. This data can be

mechanistically important but it is not clear how the data will be actionable to build better therapeutics unless specific cell populations can be enriched.

- Comparing effects of starting with cells from patients with cancer versus autoimmunity is very interesting, as are the EXTRA-seq and CELL-CELL-seq approaches.
- The proposal focuses on all in vitro testing of CAR T cells and targets in isolation of other immune cells and microenvironmental factors. It is unclear whether productive cytotoxic effects in the absence of TAMs, MDSCs, and Tregs would translate into productive in vivo responses.
- The objective of learning causal drivers or CAR T cell failure is a good one with broad implications. However, this is a complex and multifactorial problem, with high likelihood of individual variation from one patient to the next, and our ability to predict in vivo functionality based on in vitro cell-based assays is poor.
- Limited preliminary data was included to support feasibility.

**Plan & Design: Evaluate the project plan and design**

- The four aims are logically sequenced as identify regulatory cassettes, manufacture CAR T products, characterize encounter-level function, and integrate the data into an atlas.
- 60 engineered products will be developed and characterized at baseline and after antigen encounter through a 36-panel high dimensional flow assay that provides excellent T cell characterization.
- With single-cell-cell interactions, it is not clear what if the proposal is statistically powered to make associations with the multiple variables tested.
- Jurkat cells are used in Aim 1 despite other groups demonstrating high feasibility of library screening in primary human T cells.
- The proposal would be strengthened by a clearer explanation of actionability. Aim 3 may identify individual CAR T cells or interaction states associated with productive killing, but because the final CAR T products are heterogeneous, it is unclear how these findings would be used to isolate, enrich, manufacture, or release better products.
- The PI has relevant expertise in CAR T engineering, manufacturing, and translational immunotherapy; One institution provides CAR T engineering, spectral flow, quality systems, and computing; another institution provides nanovial and Cell-Cell-seq expertise. A third institution provides access to clinically relevant donor material. The communication plan is adequately addressed and appropriate for a multi-institutional project.
- While a large amount of informative data will be generated using cutting-edge approaches, it is not clear how the data will result in mechanistic insights for in vivo CAR T functionality. Discussion of how the findings will lead to in vivo testing is important.
- Aims are well-developed; investigators have appropriate expertise to complete project; will generate significant amount of data though better definition of how this atlas will be used is warranted.

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

- Patient-derived leukapheresis material from malignancy and autoimmune CAR T cell settings will be utilized from [redacted institution], suggesting that the products may be clinically diverse.

- Proposal does not include enough detail on how demographic, genetic, environmental, or treatment-history variables will be powered and analyzed; difficult to assess whether robust conclusions about subgroups can be drawn.

- Appropriate.

- The applicants discuss study designs that incorporates biological diversity at the discovery stage and collaboration with [redacted institution] and CIRM-funded institutions to ensure broad population relevance.

| Application# | DISC5-19870 |
|---|---|
| **Title**<br>(as written by the applicant) | Foundational Mapping of Splicing-Derived Neoantigen Recognition in Human Sarcoma Using Stem Cell-Enabled Immune Platforms |
| **Project Objective & Impact**<br>(as written by the applicant) | This project addresses a key bottleneck in human disease research: the lack of scalable human-relevant systems to mechanistically link tumor antigen presentation and endogenous T cell recognition, which cannot be achieved with costly xenograft animal models. It will deliver reproducible stem cell-enabled platforms to define immune recognition mechanisms and advance regenerative medicine discovery. |
| **Specific Aims**<br>(as written by the applicant) | • Aim 1: Foundational discovery of public-like splicing neoantigens in human sarcoma using junction-centric transcriptomics integrated with immunopeptidomics.<br>• Aim 2: Robotic, discovery-scale quantification of T cell recognition using Single Chain Trimer-enforced antigen presentation and renewable universal iPSC-derived immune platforms.<br>• Aim 2A: Robotic pooled Single Chain Trimer library screening in universal iPSC-derived dendritic cells to enrich rare neoantigen-reactive tumor-infiltrating T cells at scale.<br>• Aim 2B: Automation-enabled high-throughput T-cell receptor recovery and antigen-specific deconvolution using reporter to establish a platform for quantifying cohort-level recognition frequencies.<br>• Aim 3: Mechanistic mapping of immune-tumor interactions in mass-Bioprinted human sarcoma microenvironments using AI-assisted automated spatial image analysis. |
| **Statement of Benefit to California**<br>(as written by the applicant) | This project will generate foundational discovery tools, datasets, and stem cell-enabled immune platforms that strengthen California's leadership in regenerative medicine and cancer research. By leveraging CIRM-supported infrastructure and sharing scalable methods and data, the work benefits California researchers, trainees, and patients while supporting equitable, statewide scientific advancement. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>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** | 4 |
| **Highest** | 93 |
| **Lowest** | 75 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 8 |
| **(1-84): Not recommended for funding** | 7 |

## FINAL COMMENTS

Proposals were evaluated and scored based on the review criteria shown below, which are also described in the PA/RFA. Following panel discussion and scoring, reviewers entered 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 for clarity by CIRM.

**Key Strengths and Weaknesses**

- The overall project is an exciting approach that may identify novel neoantigens with broad translational potential for clinical practice. Aims 1 and 2 are outstanding and may yield important outcomes, whereas Aim 3 is overly aspirational and does not fully account for the antigen-presentation capacity of dendritic cells (DCs).
- Discovery of new splice variants is a sound approach, but it is unclear how this strategy is innovative relative to other methods for uncovering these neoepitopes. iPSC-derived dendritic cells require validation against primary human dendritic cells, and there is little focus on MHC-II. It is also unclear whether the tumoroids used in Aim 3 adequately recapitulate the tumor microenvironment; validation data demonstrating this would strengthen the approach.
- Aim 3 seems misplaced.
- Good team.
- The project addresses a crucial need for an underserved population, particularly patients with aggressive sarcomas, including younger patients. It has clear potential to generate targets for precision immunotherapy and will be carried out by an excellent team.

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

- The proposed stem cell-enabled automated system for immune-tumor mapping in 3D sarcoma microenvironments (AIM 3) has broad potential applications in immunotherapy for solid tumors, and also in tissue engineering and regenerative medicine.
- The proposed work will uncover new tumor-specific RNA splicing events that lead to the generation of neoantigens, which will be examined for their ability to activate T cells. The significance is high, as neoantigens represent an important therapeutic target and splice variants may be shared across tumors of the same type in different patients.
- The significance is tempered by the focus on a single HLA haplotype and by the fact that the experimental approach does not test antigen processing of the nominated antigens by the PSC-derived dendritic cells.
- The project is significant and could address an important unmet need for patients with refractory sarcomas through the identification of new therapeutic targets.

- Significant impact.
- Immunotherapy for many cancers is limited by the paucity of neoantigens, especially in tumors with low tumor mutational burden (TMB). Fusion protein junctions represent a potentially important class of accessible neoantigens that can be exploited for targeted immune therapies. The proposal offers a stem cell-enabled platform for deep, systematic discovery of tumor-specific RNA splicing events that generate neoantigens, which are common and potentially exploitable in sarcomas and other cancers.
- The proposed stem cell-enabled automated system for immune-tumor mapping in 3D sarcoma microenvironments (Aim 3) has broad potential applications in immunotherapy for solid tumors, as well as in tissue engineering and regenerative medicine.

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

- Although the notion that tumor-associated splice variants can generate neoantigens is not novel, aspects of the proposed approach are innovative.
- New splice variants identified through this work could help address an unmet need, but it is not clear how this approach differs from other efforts aimed at uncovering therapeutic targets.
- Very innovative.
- The concept of "dark matter" in cancer immunology refers to a growing field focused on factors beyond straightforward protein-coding sequence mutations and gene fusions that can contribute to tumor rejection. High-throughput sequencing and peptidomics are uncovering unexpected neoantigens that rank among the previously underappreciated factors potentially contributing to the host response to tumors. The applicants propose an efficient, high-throughput platform to discover such neoantigens.
- Thus, the strong focus on fusion-derived and aberrant splicing-derived neoantigens, rather than amino acid substitutions arising from random mutations, is innovative. This approach has potential significance for cancer immunotherapy, especially in low-TMB cancers, including many rare cancers that primarily affect children, adolescents, and young adults, an underserved population.
- All three specific aims are technologically innovative. Together, they link: (1) discovery of splicing-derived neoantigens using RNA sequencing and immunopeptidome analysis; (2) functional studies of neoantigen recognition using circular RNA-encoded single-chain trimers (antigenic peptide/beta-2 microglobulin/MHC Class I or Class II heavy chain) and antigen presentation using iPSC-derived dendritic cells; and (3) automated bioprinting of a reproducible 3D tumor immune microenvironment.

**Rationale: Evaluate the scientific rationale in the proposal**

- The rationale for examining tumor-specific RNA splice variants is clearly outlined and supported by previous publications. The proposed work will adapt this approach to sarcomas.
- The scientific rationale for conducting the proposed research is sound.
- The rationale is sound.
- The rationale for discovering and evaluating splicing-derived neoantigens for immunotherapy is strong, particularly in a family of cancers with low tumor mutational burden (TMB). There is already a clear example of a fusion-generated protein being successfully targeted for immunotherapy via a peptide vaccine in fibrolamellar carcinoma (Nature Medicine, December 2025). Systematic discovery of splicing-mediated neoantigens in sarcoma offers an opportunity to extend this approach to a larger and clinically important class of cancers.

- The proposed discovery platform appears well thought out, technologically sound, efficient, and high throughput. A notable strength is that the project is based entirely on fresh human tumor samples rather than cell lines or mouse models. The combination of a clinical center that treats a large number of sarcoma patients and a center laboratory employing cutting-edge technologies is a strength.

**Plan & Design: Evaluate the project plan and design**

- The plan is carefully structured and clearly laid out, including clinical collaborators and bioinformatics experts who will assist with sample acquisition and curation of RNA splice variants with significant potential to encode neoantigens. Aim 1 will obtain sarcoma tissues, perform HLA typing, and isolate HLA-bound peptides for LC-MS/MS analysis using a custom peptide database, which is a key aspect of the approach.
- Although Aim 1 will isolate HLA-bound peptides, and thus peptides that are functionally processed by the tumor-cell proteasome, there is no confirmation of this key antigen-presentation step when dendritic cells are used to present antigen, as this will be performed using a peptide-HLA construct. It would be important to determine whether the dendritic-cell immunoproteasome is also able to process these neoantigens into peptides.
- Aims 2 and 3 are, by design, entirely dependent on Aim 1, which is the discovery-focused aim. In some cases, this dependence may be viewed as a potential weakness.
- Aim 2 will employ single-chain trimer (SCT) libraries to activate T cells and identify immunologically relevant peptide complexes that activate reporter T cells or tumor-infiltrating lymphocytes (TILs). SCTs will then be expressed by PSC-derived dendritic cells, followed by TCR cloning. This aim would be highly labor intensive, but if the proposed automated approach performs as suggested, it may be achievable.
- Aim 3 represents a departure from the neoantigen-discovery and TCR-identification approaches used in the earlier aims. Here, the applicants will use tumor organoids to test the ability of SCT-expressing dendritic cells to activate predefined TCR-bearing T cells and induce attack of the organoid. This is a cumbersome approach for testing T-cell function and lacks a key control involving dendritic cells that do not express SCTs.
- The project is well designed to achieve meaningful results, but it is unclear whether the tumoroids described in Aim 3 will faithfully recapitulate the heterogeneity and immunosuppression observed in vivo.
- The project is exceptionally well designed. Experiments addressing each aim utilize cutting-edge, high-throughput, automated technologies. The flow and integration among the aims are logical and clearly described in both the text and the schematic diagram. The statistical plan is strong.
- Aim 3—the construction of a robust, reproducible, automated system for 3D immune-competent tumor models—is ambitious but appears feasible. The absence of preliminary data on the bioprinted microenvironment is noted. The team should be encouraged to validate this system in parallel with activities under Aims 1 and 2.
- The PI has an impressive track record in iPSC research dating back to the early days of the field, as well as in the management of a Stem Cell Engineering Center. The team also appears to have a good track record.

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

- Population impact is carefully considered and described.
- The experimental design accounts for genetic, environmental and other external factors that may influence research findings

- The experimental design depends entirely on accession of tumors from surgical procedures carried out at an active clinic at the PI's institution. As such, it should accurately reflect the cancer patient population of a large California metropolitan area.

| Application# | DISC5-19807 |
|---|---|
| **Title**<br>(as written by the applicant) | A precision organoid platform to investigate APOE4 dependent cerebrovascular impairments |
| **Project Objective & Impact**<br>(as written by the applicant) | Human-specific mechanisms governing neurovascular unit assembly, blood–brain barrier function, and vascular–neural crosstalk remain poorly defined due to inadequate models. This project will elucidate how stem cell–derived neural, glial, and vascular lineages self-organize and interact, transforming understanding of cerebrovascular biology in human disease. |
| **Specific Aims**<br>(as written by the applicant) | • Create and characterize functional vascularized brain organoids.<br>• Evaluate cerebrovascular dysfunctions in APOE4-mutant functional vascularized brain organoids.<br>• Establish functional vascularized brain organoids.-based functional readouts as a field-enabling platform. |
| **Statement of Benefit to California**<br>(as written by the applicant) | This project will benefit California by advancing human stem cell–based models to study cerebrovascular dysfunction underlying prevalent neurological diseases affecting Californians, including Alzheimer's disease and related dementias, stroke, and brain injury. By leveraging diverse California-derived iPSC resources and multidisciplinary expertise, this work strengthens California's leadership in regenerative medicine and supports equitable, population-relevant biomedical innovation. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>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** | 6 |
| **Highest** | 90 |
| **Lowest** | 75 |
| **Count** | 13 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 7 |
| **(1-84): Not recommended for funding** | 6 |

# FINAL COMMENTS

Proposals were evaluated and scored based on the review criteria shown below, which are also described in the PA/RFA. Following panel discussion and scoring, reviewers entered 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 for clarity by CIRM.

**Key Strengths and Weaknesses**

- The proposal addresses an important unmet need with a strong team and a well-designed APOE3/APOE4 comparison. However, the central feasibility step—integration of bioprinted vessels into organoid slices—is not supported by preliminary data, and weaknesses in the rationale and experimental design modestly outweigh the strengths.
- Establishing a human organoid model of the vascularized brain and the blood-brain barrier is likely to significantly advance drug discovery and development for diseases of the nervous system.
- The investigative team is outstanding.
- The proposed work in Aim 2, focused on Alzheimer's disease mechanisms and the role of APOE, should proceed only if experts in this challenging field are recruited to the project.

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

- One of the main limitations of organoids is their limited viability. A vascular system could substantially improve this by helping to prevent necrosis deep within the organoid. In addition, modeling how the vasculature integrates with neurons would represent a parallel technological advance relevant to diseases involving this interface.
- Whether this approach will dramatically improve viability or accurately model the neurovascular interface remains to be determined.
- Vascularized human brain organoids address a major limitation of the field. Brain organoids develop necrotic cores beyond approximately 3 mm in the absence of blood vessels, and there is currently no human-relevant blood-brain barrier (BBB) model available for study.
- APOE4 is a high-priority unsolved problem, and modeling its cerebrovascular contributions in human tissue addresses an important biological question.
- Blood-brain barrier (BBB) function is essential for normal brain physiology, and BBB dysfunction contributes to many neurological diseases. BBB permeability is also a critical consideration in the development of therapeutics for brain disorders. Currently, BBB function is typically assessed in vivo using animal models. The availability of a human in vitro model to assess BBB function would greatly facilitate studies of BBB physiology and pathology and has the potential to make a major impact on drug development.
- Establishing an in vitro organoid system to study BBB function is likely to advance stem cell biology more broadly. In addition, it could significantly change drug discovery and development by reducing reliance on expensive and time-consuming animal studies.
- Establishment of an in vitro organoid BBB model may be viewed positively by the FDA and could ultimately help inform future regulatory guidelines.

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

- The innovation really comes from the integration of different technologies and the comprehensiveness of the experimental plan to establish a viable, bioprinted, lumenized, endothelial-pericyte-containing brain organoid.
- There are no concerns with any particular step in this proposal, but in terms of the broader field, this is going to be a useful intermediate step rather than a game changer. In short, it reads like a slam-dunk R01 with important advances, but not one that is potentially revolutionary, as are some of the other applications (a high bar).
- Both halves of the technology already exist independently: pericyte-containing organoids and bioprinted vessels have been published. The novelty lies in combining them via a sandwich configuration in which 500-µm organoid slices are placed around a printed vessel.
- Other groups have extensively pursued organoid vascularization through co-culture with endothelial progenitors, transplantation into rodent hosts, and microfluidic chips. The proposed sandwich approach is novel but not obviously superior to these alternatives, and no preliminary data demonstrate that it is.
- The applicants are highly experienced in the field of organoid generation and bioprinting. They represent the cutting edge in this area of research. Extending their published work from non-neural systems to neural systems represents a logical step in their effort to generate functional in vitro models of human organs.
- The proposal integrates input from many areas of biological and neurobiological research, including molecular biology, cell biology, stem cell biology, and bioengineering.
- The project is highly innovative, particularly the aspects related to bioprinting. The applicants are pioneers in this field.

**Rationale: Evaluate the scientific rationale in the proposal**

- 3D-printing vessels: To invest in neurovascular model systems, barring some huge breakthrough, this is probably the approach to fund.
- The biological inspiration is unclear for the particular shape of the printed vessels, nor is this a variable that is studied. In natural systems, neurons and vessels undergo guided, parallel growth, and the lack of such organization in this system is a source of concern.
- The sandwich configuration is essentially an unproven step. There are few preliminary data demonstrating that EPVs actually integrate into brain organoids, and the geometry itself does not reflect how cerebrovascular systems are organized in vivo.
- The available preliminary data rely on HUVECs and other non-brain endothelial cells, which lack true BBB properties and limit the translational relevance of the proof of concept.
- Aim 2 is completely dependent on the success of Aim 1. Aim 2.3 lacks a clear therapeutic hypothesis; VEGFA, already used in earlier aims as a barrier disruptor, reappears as the proposed perturbation.
- The scientific rationale is based on earlier publications by the applicants and other investigators in the field. This prior work suggests that it is possible to generate vascularized tissue organoids using bioprinting methods. Expanding this approach to the nervous system by generating FVBOs (functional vascularized brain organoids) is a logical next step.
- Aim 1, the establishment of the FVBOs, while ambitious, follows a logical and scientifically convincing path. Some of the technical details are poorly described in the application. However, the applicants answered follow-up questions succinctly and convincingly. It seems likely that the goals can be achieved, and the applicants are optimally positioned to pursue them.

- There are concerns regarding Aim 2, in which the FVBOs will be used to study APOE functions related to Alzheimer's disease. In principle, this approach makes sense once the FVBOs are fully established and the methodologies to monitor BBB function are well developed. At this point, however, the research proposed in Aim 2 seems overambitious. In addition, there are technical concerns (see below).

**Plan & Design: Evaluate the project plan and design**

- There are many components to this cell-diverse system, and the applicants have assembled experts in all of them.
- The neurovascular coupling assessment is reasonably multimodal.
- The experiments, despite being very detailed, are well described, logical, and clearly demonstrate the applicants' relevant expertise.
- Based on the experimental design, it is unclear whether differences in the APOE4 response are truly brain-like because there is no null experiment comparing APOE4 before and after a null printing process.
- The proposal could have included more comprehensive disease-related experiments, such as Aβ40/42 ratio, tau, cell death markers, synaptic markers, and markers of astrocyte activation. All of these would be expected to change if the construct is physiologically realistic.
- Many of the fallback approaches rely on less advanced, less innovative methods that are of less interest for funding.
- The proposal brings together a strong complementary team with excellent track records: the PI in organoids, a Co-I in bioprinting, a Co-I in genomics, and an expert advisor on BBB protocols. The APOE3/APOE4 isogenic comparison is appropriately designed.
- Significant concerns remain regarding the perfusion experiment. The revised data demonstrate perfusion only in bioprinted vessels alone, not in FVBOs. The flow rate is relatively high, the durability of the Cell-Tak seal over a month-long culture is uncertain, and tracer retention is meaningful only if the vessel is actually integrated.
- The shear stress aim is the least developed. The range of shear stress is not clearly defined, and treating the bulk mechanical properties of the entire construct as a proxy for endothelial mechanosensing is a stretch.
- The studies in Aim 1 are well designed and are likely to yield meaningful insights. The applicants are leaders in the field, understand the potential pitfalls, and adequately consider alternative approaches. The timeline for this part of the application is ambitious but reasonable, and the budget is adequate.
- Aim 2 goes beyond establishing the FVBO and addresses questions related to Alzheimer's disease pathology. APOE4 is a significant risk factor for Alzheimer's disease, and the applicants propose to study FVBOs generated from iPSCs derived from donors carrying the APOE2, APOE3, and APOE4 isoforms. However, there are no preliminary data suggesting that differences among these groups will be observed.
- Aim 2 would bring the applicants into the complex and controversial area of Aβ oligomer biochemistry. This field is littered with artifacts and irreproducible data. It is not possible to use oligomeric Aβ42 without appropriate, technically challenging analytical procedures. It is strongly recommended that the applicants establish collaborations with experts in this field before embarking on these studies.
- The applicants represent an outstanding team to undertake the proposed work. They are leaders in the fields of organoids and bioprinting. The PI is a well-trained and successful early-stage investigator, and this grant would significantly support the continued development of her independent research program.

- Team communication and management are highly adequate.

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

- Much of the justification is based on the fact that the PIs see patients in the Los Angeles area. While that is a strength, it is not obvious how it directly affects the proposed science.
- The application mentions that the iPSC lines will be diverse but provides almost no detail regarding the number of lines or their demographic breakdown, which would be expected if this were a priority.
- The overall plan for population impact is solid, with engagement of diverse populations, including Hispanic and African American participants, and the use of diverse stem cell repositories.
- Appropriate population impact enhancement strategies are proposed, including training sessions and integration of CIRM COMPASS and Bridges students.
- Successful completion of this program has the potential to positively affect all human populations across all stages of development, adulthood, and aging. The plans outlined by the applicants are adequate.

| Application# | DISC5-19957 |
|---|---|
| **Title**<br>(as written by the applicant) | Spatiotemporal Control of Human Primordial Germ Cell Fate by the Embryonic Niche |
| **Project Objective & Impact**<br>(as written by the applicant) | Human reproduction depends on primordial germ cells, the precursors of eggs and sperm. Using complementary stem cell–derived embryo models that generate early germ cells, we will map where and when these cells arise, identify the neighbouring tissues that guide them, and discover essential signals. This blueprint will reveal early origins of infertility and guide future therapies. |
| **Specific Aims**<br>(as written by the applicant) | • Define the composition, spatial organization, and dynamics of human PGC specification niches.<br>• Identify and mechanistically test niche-derived signals that control PGC specification and survival across platforms.<br>• Model infertility-related PGC–niche defects across platforms |
| **Statement of Benefit to California**<br>(as written by the applicant) | Infertility affects 1 in 6 people of reproductive age with most causes unexplained or suspected to have a genetic cause. By using stem cell–derived embryo models to define the signals and environment that establish germ cells, we will identify early mechanisms underlying infertility. We will generate a blueprint of human germline specification, strengthening California's long-standing leadership in stem cell research and informing future diagnosis and treatment. |
| **Funds Requested** | $2,425,796 |
| **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."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 85

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

| | |
|---|---|
| **Mean** | 83 |
| **Median** | 85 |
| **Standard Deviation** | 3 |
| **Highest** | 85 |
| **Lowest** | 75 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 9 |
| **(1-84): Not recommended for funding** | 6 |

# FINAL COMMENTS

Proposals were evaluated and scored based on the review criteria shown below, which are also described in the PA/RFA. Following panel discussion and scoring, reviewers entered 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 for clarity by CIRM.

**Key Strengths and Weaknesses**

- Strength: The project addresses a key question in human biology that remains unanswered. Only recently have highly advanced in vitro models become available that enable this type of study. Combined with high-resolution molecular and imaging approaches, the work could provide a valuable advance.
- Weakness: It is difficult to assess how closely the specified PGC-like cells resemble their in vivo counterparts. As a result, findings from the in vitro system may reflect, but not fully recapitulate, the in vivo biology.
- Great team.
- This is a strong grant that seeks to define and understand the niche components that harbor and support PGCs in a human in vitro system. The grant has no major weaknesses but is somewhat limited by the lack of a functional assay for developmental competence of the PGCs. Nevertheless, the work is likely to provide substantial insight into human PGCs (or PGC-like cells) and the signaling mechanisms within this niche.

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

- Human germ cell development is nearly impossible to study in vivo, and many questions regarding the initial signals and regulatory mechanisms therefore remain unanswered. This proposal, from two leading laboratories in the field, aims to shed light on these questions. The project appears highly feasible and is likely to generate novel insights into primordial germ cell (PGC) formation, some of which may ultimately help address human infertility.
- Large potential impact.
- The main goal of this application is to model primordial germ cell specification in humans using in vitro models of the gastrulating embryo. More specifically, the applicants intend to define the growth factors that control PGC production during early human development.
- If successful, this project will provide mechanistic insights into the early specification of PGCs. However, the topic is not entirely novel, and multiple groups are pursuing similar questions using human model systems. The niche factors under investigation appear to consist primarily of signaling pathways, which are also not entirely novel.
- The translational and stem cell aspects are limited.
- The knowledge generated by this project will be of interest to the developmental biology field and will address questions that could ultimately be useful for the production of gametes in vitro.
- This grant application aims to discover the origins and niche requirements of primordial germ cells. This is a very important and unresolved question in mammalian reproductive biology that has been difficult to address using in vivo approaches and is essentially intractable in humans.
- The use of human in vitro embryo models may, for the first time, allow determination of the origins of PGCs and their niche requirements.

- Insights into infertility associated with Turner syndrome may emerge as a medical outcome of this work.

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

- How and where PGCs are specified has been a longstanding question, but it has thus far been investigated primarily in model organisms. The main innovation is the ability to generate early embryo-like structures from inducible stem cells, complemented here by a well-characterized, simpler 2D culture model. The two-pronged approach used for imaging/spatial analyses, signaling studies, and disease investigation is noted as a key strategic advance.

- The use of human gastruloids is definitely interesting. However, several such models are currently available, and their relevance to natural development requires further validation. Architectural features are particularly important in this regard. Validation against existing in vivo datasets generated from animal models could be useful.

- The proposal is based on conventional technologies, including single-cell approaches and advanced microscopy.

- It is not clear what fundamentally new knowledge this application will provide. Signaling pathways controlling PGC specification have largely been identified in animal models and in human in vitro platforms. For example, BMP4 signaling has been known for more than a decade, and downstream molecular mechanisms have already been explored. It would have been interesting to use the hiEmbryoids platform to investigate the importance of mechanosensing mechanisms, extracellular matrix (ECM) interactions, and related factors.

- This research group has developed an early human embryoid model system that models early post-implantation development. This innovative model positions the team well to explore PGC establishment in a human cell-based system.

**Rationale: Evaluate the scientific rationale in the proposal**

- The proposal and its rationale are well described. The laboratories are well suited to the work based on their expertise, and the overall approach is sound. Whether the 2D and 3D systems are both necessary, or whether they will be equally informative, is not entirely clear. However, the synergy between the laboratories provides a compelling rationale for pursuing this approach.

- The rationale for the proposal is clear and is well supported, at least for Aims 1 and 2. This is, however, a relatively conventional approach. The identification of signaling pathways controlling PGC specification is a logical objective, and the project plan follows a classic strategy for understanding cell-fate decisions.

- However, the rationale for using two different platforms, which may be challenging to compare directly, is difficult to follow.

- A key issue is determining whether the generated PGCs are functional. The use of a small number of markers alone may not be sufficient. Additional approaches would strengthen this aspect of the work. For example, can the cells be differentiated further? What is their epigenetic state, including DNA methylation status?

- The spatial component is also unclear. The model is a 3D aggregate that only partially mimics the organization of the natural embryo. As a result, it is uncertain whether the spatial information obtained will be more informative than that provided by the Geltrex overlay model.

- Two independent cell-culture models will be used to study the PGC niche: a 2D Geltrex model and a 3D early embryo model. This is a sound approach, as findings that are shared between the two systems would strengthen interpretations regarding niche components and signaling factors.

- The use of single-cell and spatial transcriptomics will help identify cell types involved in niche formation and PGC development.
- There is a possibility that infertility associated with Turner syndrome is caused by sex chromosome deficiency (XO karyotype). The use of these cells in the proposed assays may help determine whether the infertility observed in Turner syndrome results from defects in PGCs, the PGC niche, or both.

**Plan & Design: Evaluate the project plan and design**

- Aim 1 will provide unprecedented insights into the spatial and temporal induction of PGCs. The combination of image-based analyses and single-cell multi-omics/ATAC-seq is well suited to addressing these questions.
- Aim 2 will provide an in-depth assessment of signaling pathways and receptor-ligand interactions, including BMP, WNT, TGF-β, NODAL, and FGF pathways. The combination of genetic and small-molecule perturbations is meaningful.
- Aim 3 will use the model systems to explore selected infertility-related conditions. Candidate genes have been selected and compared across the 2D and 3D platforms. Perturbation studies, molecular profiling, and patient-derived cell lines will facilitate investigation of causality and functional requirements.
- Taken together, the three aims will provide important insights into human germ cell biology.
- The rationale for using two different platforms to study signaling pathways controlling PGC specification is not entirely clear. An important initial step would be to compare PGCs generated using the two approaches to define their similarities and differences. The applicants may also find it difficult to use the same hPSC lines, as the protocols are likely to be line-specific. Furthermore, the synergy between the two laboratories is not fully apparent, and each team could potentially pursue this project independently.
- The system used in this proposal is interesting, but robustness remains a challenge. In addition, such models only approximate natural development. Therefore, data generated in vitro would still benefit from evaluation against in vivo datasets, such as those available from non-human primates or mouse models.
- Aims 1 and 2 are logical and follow a conventional progression for this type of project. The first step is to identify growth factors, and the second is to validate their function. However, Aim 3 appears disconnected from the earlier aims and may represent an effort to broaden the scope of the proposal. This aim could potentially constitute a separate project. The central hypothesis of Aim 3 would benefit from validation using patient datasets.
- The inducible knockout strategy proposed in Aim 2 may be challenging to implement. There are concerns that currently available systems may not function optimally after differentiation. In addition, the tetracycline-based system commonly used for such approaches is already incorporated into the gastruloid model. Temporal control may also prove difficult, as signaling pathways may need to be inhibited within narrow time windows, potentially less than 24 hours. Furthermore, the functions of individual pathways may change over time, and a constitutive knockout approach could obscure these dynamics.
- The use of small molecules may be the more practical option; however, some of the selected compounds may not be optimal. For example, Noggin may be preferable to LDN193189 because LDN193189 affects multiple pathways. Similarly, IWP2 is broad in its activity, and DKK1 could also be considered.
- The team is excellent but lacks complementarity. The reviewer questioned whether sufficient synergy exists, given that either group could potentially conduct the project independently.
- This is a large budget for a relatively conventional program.

- The two model systems will be combined with cells harboring two fluorescent reporters that mark PGC-like fate (SOX17 and TFAP2C), as well as reporters for niche function (ISL1 and TBX6), based on previous studies. These tools should facilitate investigation of niche biology.
- Aim 1 is primarily a cellular mapping effort designed to define and characterize the cells that form PGC niches using reporter genes and single-cell analyses.
- Aim 2 proposes mechanistic validation of PGCs and niche-derived signaling cues. Pharmacological inhibition of pathways such as BMP, WNT, and Nodal will support these studies.
- The proposal does not directly test the developmental competence of the PGCs. Although this is ethically difficult to assess in human systems, the lack of a functional validation assay is a concern. Follow-up studies in a non-human primate system may ultimately be needed to extend these findings.

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

- The choice of line may be relevant. Overall the results will be very foundational and hence be applicable across the population.
- This aspect could be reinforced. There is no plan to use diverse hiPSC lines or to look at different populations susceptibility for infertility syndromes especially in the context of Aim 3.
- The outreach activity is well described and convincing.
- Multiple stem cell lines will be used in both embryo model platforms. This helps to assess the impact of genetic diversity upon PGC specification and niche function.

| Application# | DISC5-19950 |
|---|---|
| **Title**<br>(as written by the applicant) | Temporal AI models for programming therapeutic cell state transitions |
| **Project Objective & Impact**<br>(as written by the applicant) | The proposed work seeks to develop time-aware artificial intelligence (AI) models to enable the prediction of effective interventions to program cells towards lasting therapeutic trajectories. We propose to apply these AI models to predict therapeutic targets for promoting resilience to disease-relevant stimuli in Alzheimer disease, addressing one of California's most significant medical needs. |
| **Specific Aims**<br>(as written by the applicant) | • Map the temporal perturbation landscape of neural cell types to predict factors to promote resilience to disease trajectories<br>• Develop a generative AI model (Cellformer-Perturb) for predicting perturbation responses across contexts<br>• Predict targets to program neural cell types towards therapeutic trajectories for AD |
| **Statement of Benefit to California**<br>(as written by the applicant) | Alzheimer disease (AD) is the most common neurodegenerative disease, affecting >720,000 Californians (12% of adults 65 years+) and rapidly increasing with the aging population, highlighting the urgent need to accelerate the discovery of targeted therapies for this devastating disease (Alzheimer Association 2025 Report). The proposed work to identify candidate therapeutic targets to promote AD resilience will benefit both the growing number of AD patients and their caregivers. |
| **Funds Requested** | $2,499,998 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

### Final Score: 85

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

| | |
|---|---|
| **Mean** | 82 |
| **Median** | 85 |
| **Standard Deviation** | 8 |
| **Highest** | 90 |
| **Lowest** | 60 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 8 |
| **(1-84): Not recommended for funding** | 7 |

# FINAL COMMENTS

Proposals were evaluated and scored based on the review criteria shown below, which are also described in the PA/RFA. Following panel discussion and scoring, reviewers entered 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 for clarity by CIRM.

**Key Strengths and Weaknesses**

- The proposed methods are high risk, but the studies could yield novel insights into Alzheimer's disease biology.
- This project represents a highly innovative approach to understanding Alzheimer's disease by modeling cell-state transitions from health to disease and identifying resilience mechanisms that may slow disease progression. The work has the potential to transform how we think about disease and its treatment more broadly.
- Strengths include great laboratories, an innovative approach, plans to generate high-value experimental data, and a good idea for a foundation model.
- The preliminary data are not strong. The experimental design is not well justified with respect to the strengths and potential biases of the foundation model approach. In addition, there are no published studies that have examined or benchmarked Cellformer-Time.

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

- The proposal targets a major unmet medical need. Alzheimer's disease affects more than 720,000 Californians and costs Medi-Cal approximately $5.7 billion annually.
- The platform (Cellformer-Time and Cellformer-Perturb) is intentionally generalizable beyond Alzheimer's disease. Success would generate reusable AI infrastructure applicable to many progressive diseases, substantially broadening the potential impact of the work.
- Establishing temporal and causal modeling would move the field beyond observational snapshot data. If critical inflection points can be resolved from these complex datasets, this would represent a paradigm-level advance rather than an incremental one.
- The team's prior model, [redacted model name], has been widely downloaded, demonstrating that the field is likely to adopt the group's new infrastructure.
- The link between AI-predicted targets and a CIRM-aligned regenerative therapy is indirect. The primary outputs are gene targets that would still require conventional drug development before reaching patients and would remain subject to the challenges inherent in that process. As such, the outputs are far upstream of clinical interventions.
- The "resilience trajectory" framework may be interpreted as a strategy to slow disease progression rather than to restore function.
- Effective therapeutics for Alzheimer's disease remain a major unmet need.
- The project would advance the ability of AI models to predict cell-state dynamics during transitions from healthy to disease states using transcriptomic data. This has the potential to improve understanding of these states and their stability, and to identify therapeutic targets that could maintain healthy states or reverse disease states.

- New models would be developed to predict the toxicity of drug perturbations in specific cell types, potentially accelerating drug development efforts.
- The project focuses on Alzheimer's disease and would be highly significant if successful.
- Alzheimer's disease is a highly complex, multifactorial disorder. There are concerns that analysis of a single pathway or cell type, even at the depth proposed here, may not be effective. Nevertheless, this is a relatively minor concern, as the study is likely to provide valuable insights into mechanisms contributing to the disease.
- The project could have a significant impact on understanding the biological mechanisms underlying Alzheimer's disease.
- Based on earlier results, there is reason to believe that organizing perturbed stem-cell assays into large, time-dependent foundation models could generate important new insights.
- The approach incorporates large-scale perturbational screens that will themselves constitute a valuable resource, independent of the modeling effort.

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

- Cellformer-Time is a first-in-class model that generates past, intermediate, and predicted future cell states across temporal trajectories. Cellformer-Perturb extends this capability to predict responses to perturbations in potentially novel, previously unseen contexts.
- The co-evolution of time-resolved CRISPRi Perturb-seq data generation and temporal transformer model training represents an innovative closed-loop design.
- In silico toxicity screening across cardiac, endothelial, and hepatic contexts adds a safety-prioritization layer that is absent from most target-discovery pipelines.
- The scale of the proposed data generation is impressive.
- It is unclear whether Alzheimer's disease is the optimal target for this work.
- The proposal features a strong combination of computational and experimental approaches.
- The concept of developing AI models to predict transitions from healthy to disease states using public databases is innovative.
- Extending the investigators' prior work to examine cell-state stability in response to drug perturbations is innovative.
- The concept of treating disease by promoting cellular resilience to perturbations is compelling and novel.
- Perturbation studies themselves are not innovative; however, applying a time-dependent foundation model to single-cell RNA-sequencing data is somewhat innovative.

**Rationale: Evaluate the scientific rationale in the proposal**

- Preliminary Cellformer-Time data showing accelerated aging in AD microglia and a validated rejuvenation target in the endothelium provide strong proof of concept.

- [Redacted model name]-predicted cardiac targets that experimentally improved iPSC microtissue contractility provide an external validation precedent for the in silico-to-wet-lab pipeline.
- The team's CRISPRi platforms in iPSC-derived microglia, astrocytes, and excitatory neurons are published and operational. This de-risks Aim 1.
- The benchmarking strategy (held-out sets, external embedding validation) is rigorous and well described.
- Primary screening uses a single male isogenic iPSC line. Sex- and ancestry-balanced replication is proposed but is limited in scope.
- Aim 3 is heavily dependent on the SEA-AD continuous pseudoprogression score. If the CPS is noisy or biased, disease-axis training could be compromised, and the proposal does not deeply address this dependency.
- Validation of the rejuvenation target via siRNA in primary endothelial cells is preliminary. The strongest in vivo-relevant validation precedent is in cardiac biology, not Alzheimer's disease.
- Well written.
- Leverages public databases well.
- The rationale is based on pioneering work by the PI using AI models to understand cell states and their transitions based on single-cell transcriptomics. The depth of the preliminary work provides significant confidence in the feasibility of the proposed study.
- The team has expertise in working with iPSC models of neural cells and using CRISPRi/a techniques that will be important for achieving the stated objectives.
- Integration of public AD datasets with iPSC-derived cell types provides complementary models for systematic variation in vitro and comparison with the in vivo disease state.
- There are concerns about the ability of iPSC-derived models to predict the complex etiology of Alzheimer's disease.
- The rationale is good, but feasibility is difficult to assess without additional evidence. Because much of the proposal depends on CRISPR perturbational screening as input data, it would be helpful to provide more detail regarding the experimental design of the CRISPR perturbational model. Additional discussion of how the foundation model is expected to perform in the presence of single-cell noise and bias in the time domain would strengthen the proposal. In particular, more detail on the relationship between the experimental design and the foundation model would be valuable.
- Cellformer-Time is very interesting, and the preliminary data are promising, but it is unclear whether the approach has been benchmarked sufficiently to demonstrate that a project of this scope is technically feasible under the stated assumptions and to clarify its strengths and weaknesses. Publications describing the temporal modeling approach, even in bioRxiv, would be useful for evaluating how the method handles noisy assay data. At present, the information provided on this topic is limited.

**Plan & Design: Evaluate the project plan and design**

- The three aims are tightly integrated: data generation, model training, in silico discovery, and wet-lab validation, with explicit alternatives at each step (e.g., pathway-level perturbations if single-gene predictions are too modest).
- The collaborative infrastructure is substantive, including adjacent locations, twice-monthly co-mentored student meetings, a biweekly perturbation-modeling group, and a dedicated Slack channel.

- The data management plan is excellent (GEO/SRA, CRISPRbrain, Hugging Face, Apache 2.0 licensing), and the team already maintains heavily used repositories, making compliance with FAIR principles credible.
- Both PIs explicitly serve as Data Project Managers, with a clear division of responsibilities between code/AI and experimental data.
- The project is very ambitious for a three-year period, involving development of two new foundation models, generation of the requisite Perturb-seq training data across multiple neural cell types, validation in iPSC AD models, and demonstration of cross-context toxicity prediction.
- Hepatocyte differentiation feasibility is acknowledged as a risk. The HepG2 fallback is reasonable but is not biologically equivalent to primary hepatocytes.
- The combinatorial in silico perturbation analysis (groups of 10 targets, 20 combinations, and multiple decades of life) generates a large search space, and its interpretability and statistical power are not defined as concretely as other aspects of the plan.
- No explicit Gantt-style timeline is presented in the narrative.
- The methodology has not yet been published.
- There are some concerns about benchmarking of the Cellformer-Time model.
- The aims are logically designed to advance AI models that predict different cell-type responses to perturbations.
- The experimental design is rigorous, with clear and detailed objectives and analyses. Strategies for processing the data, obtaining meaningful results, and evaluating outcomes are strengths of the project.
- It may be difficult to select a small number of targets for validation in each aim given the size and complexity of the data. This is a minor concern in light of the team's past record with similar analyses.
- The investigators have considered a broad range of cell types and perturbations relevant to Alzheimer's disease.
- Integration of multiple models to predict healthy-to-disease inflection points is a strength.
- The idea of a time-dependent foundation model is a good one because it allows the data itself to inform the model. However, this approach may also be susceptible to artifacts, such as learning batch, assay, technician, or culture biases, particularly if these factors are completely confounded with temporal sampling. The proposal should discuss how such issues will be addressed experimentally.
- The temporal-screening-versus-disease-modeling approach is sound, and foundation models should be capable of learning and capturing these patterns. However, additional benchmarking would be valuable to assess how the model handles time-dependent discontinuities, represents rare or sparse cell transitions of interest, and avoids inappropriate bridging between distinct transitions or discontinuities.

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

- The training dataset is balanced by sex and includes 3,805 donors representing a broad range of ancestries (European, African, Hispanic, East Asian, South Asian, Caribbean, Jewish Israeli, Middle Eastern, Pacific Islander, and Native American). This is a meaningful and scientifically appropriate use of population-level variability for a discovery-stage AI project.

- The team commits to drawing on public AD cohort data with attention to ancestral diversity reflective of California, which is appropriate for the proposed modeling work.
- Key wet-lab findings will be replicated in iPSCs from donors of different sexes and ancestries, representing a reasonable, feasibility-matched plan for a discovery-stage project.
- Open-source release of all models, code, and tutorials maximizes equitable access to the platform itself, extending the project's impact across populations of researchers and downstream users.
- Primary in vitro screening is performed in a single male isogenic iPSC line for cost reasons. Replication in a female line is appropriate but secondary, and replication across ancestries is mentioned only briefly.
- The proposal could more explicitly address how Alzheimer's disease progression differs across ancestries (e.g., APOE4 risk magnitude varies substantially by ancestry) and whether the model is expected to capture these differences.
- The work exploits population-level variation for discovery, for example by examining samples from AD-resilient individuals.
- The project will utilize existing datasets with exceptional diversity among Alzheimer's disease patient populations.
- Engagement of AD advocacy and outreach groups is a strength.
- The proposal discusses California's aging population. It would also be valuable to include perturbed iPSC cells from a more diverse range of ethnic and genetic backgrounds, if budget permits.

| Application# | DISC5-19900 |
|---|---|
| **Title**<br>(as written by the applicant) | Validating Safety and Efficacy of Extremeophile Stress Tolerance Transgenes in Stem Cells |
| **Project Objective & Impact**<br>(as written by the applicant) | Tardigrades (“water bears”) are microscopic animals that can survive in extreme conditions. We will engineer stem cells to express tardigrade genes, making them more resistant to damage. |
| **Specific Aims**<br>(as written by the applicant) | • Engineer stem cells with tardigrade stress tolerance genes.<br>• Determine if tardigrade genes protect stem cells from stresses similar to therapeutic use.<br>• Determine if tardigrade genes change how stem cells function<br>• Determine if tardigrade gene expressing cells are safe and effective to use |
| **Statement of Benefit to California**<br>(as written by the applicant) | Stem cell research and treatment is limited by damage to stem cells that are used in therapies. Some patients, such as those with diabetes, offer challenges for stem cell therapies due to tissue and organ damage. This research will increase cell stress tolerance, enabling function in difficult diseases, and expanding access to new diseases. Additionally, the research will be conducted at one of the CA’s most successful institutions for graduating underrepresented students. |
| **Funds Requested** | $2,499,999 |
| **GWG Recommendation** | **(85-100): Exceptional merit and warrants funding, if funds are available** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

### Final Score: 85

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

| | |
|---|---|
| **Mean** | 82 |
| **Median** | 85 |
| **Standard Deviation** | 9 |
| **Highest** | 95 |
| **Lowest** | 60 |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 7 |
| **(1-84): Not recommended for funding** | 7 |

# FINAL COMMENTS

Proposals were evaluated and scored based on the review criteria shown below, which are also described in the PA/RFA. Following panel discussion and scoring, reviewers entered 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 for clarity by CIRM.

**Key Strengths and Weaknesses**

- Strength: This is a highly innovative approach to render stem cells more robust to stress during injection and engraftment. Successful validation of TEAPs could establish them as the gold standard for the field. The significance to CIRM's mission is very high.
- Weakness: The choice of cells is not well aligned with those that would be used in clinical applications. A more detailed discussion of the rationale for cell selection would have been helpful. In addition, the application is limited to analyses of stem cells and does not include stem cell progeny, which are more therapeutically relevant.
- The potential negative effects associated with expression of foreign disordered proteins such as TEAPs are discussed insufficiently.
- An obvious application of TEAPs could be the protection of cells during cryopreservation. It is a missed opportunity that this is neither included in the proposal nor addressed in the discussion.
- The proposal presents an attractive starting concept but is substantially weakened by a lack of sophistication in stem cell biology, overgeneralization regarding the effects of "stress" in cell therapy, and only superficial consideration of potential problems and risks.
- A strength is the investigators' engagement with the community and with younger individuals interested in STEM. The PI has regularly partnered with the local Society of Hispanic Professional Engineers to support outreach through the annual Día de Ciencias (Day of Science) events.

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

- The significance of this proposal is potentially very broad and could affect many cell therapy approaches using a variety of cell types and disease paradigms.
- Limited survival of engrafted cells is a major bottleneck for cell therapies. This approach could provide a novel strategy to address that challenge.
- The project will test a fascinating idea: transducing various human stem cells with genes encoding a set of proteins from tardigrades ("water bears") that are responsible for these organisms' remarkable ability to withstand extreme environmental conditions. The goal is to overcome stresses that broadly limit the success of cell-based therapeutic products.
- The applicants claim that their proposed technology could revolutionize the entire field of cell therapy. However, the proposed broad survey of four cell types and five tardigrade extreme tolerance-associated proteins (TEAPs), including 10 pairwise combinations, does not provide a compelling path toward a specific practical application. The plan also does not adequately address the significant biological and regulatory hurdles to the safe and effective use of TEAPs in regenerative medicine.
- A weakness is the superficial treatment of the biology of the multiple stem cell populations the applicants propose to study. The applicants also take an overly generic view of factors that "stress" cells, with virtually no discussion of the specific functions of the proteins to be tested.

- The potential safety and immunological issues associated with introducing completely foreign proteins into therapeutic human cells are addressed only superficially.

- Proposal: Develop and validate a stem cell model using extremophile proteins to improve survival during grafting stress both in vitro and in vivo. There is clear significance in enabling stem cells to survive following grafting. Cells are exposed to stress during delivery and are often grafted into damaged or impaired tissues, and this proposal seeks to enable stem cells to better withstand these stresses. The potential impact is highly significant and broadly translatable across multiple diseases.

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

- The approach is unique and highly novel and could significantly change the field of cell therapy. The group is also one of the few studying these interesting proteins and could make key advances for the field in general.

- The approaches used are not novel but are appropriate.

- The concept that the proteins the applicants collectively designate as TEAPs might confer useful stress-resistance properties to mammalian cells used in medicine has been raised previously in the relatively sparse literature on tardigrades. The proposal is innovative in its ambitious breadth, attempting to build a platform applicable to multiple cell types used in regenerative medicine.

- However, this breadth comes at the expense of a more thorough consideration of the likely limitations to the safety and efficacy of the proposed technology. This is particularly problematic for a proposal ostensibly focused, as reflected in its title, on translating TEAPs into clinical applications rather than on gaining basic information about their function.

- This proposal is innovative because oxidative stress is a major contributor to many cellular and systemic diseases. Many extremophiles, such as haloarchaea, use thousands of small non-coding RNAs to manage free radicals and protect DNA and proteins from damage.

- The proposed outcome is to establish the feasibility and safety of TEAPs in relevant therapeutic stem cell models, providing the foundation for future studies directed toward determining disease-modifying activity.

- Aim 1: TEAPs will be expressed individually or in pairs using Sleeping Beauty vectors in ASCs, iPSCs, HSCs, and NSCs. The cells will be tested under representative stressors (shear, hypoxia, metabolic restriction, hyperglycemia, and chemotherapy) and assayed for biological changes.

**Rationale: Evaluate the scientific rationale in the proposal**

- The project is motivated by the general challenge of overcoming stress during engraftment, which results in low graft survival and limited therapeutic benefit. The applicants have leveraged the ability of tardigrades to survive extreme stress, and tardigrade-specific proteins have been shown to provide a unique set of protective benefits.

- Preliminary data show that expression of TEAPs increases stress tolerance in ASCs and significantly improves their survival following a number of stresses, including radiation and chemotherapeutic toxicity.

- Translating and validating the use of TEAPs in other therapeutically relevant stem cell models is a logical and highly rational extension of the discovery of this unique set of proteins.

- Aim 3 is a safety study involving transplantation into mice, which appears to be a reasonable approach.

- The applicants' stated value proposition is extraordinarily broad and nonspecific: "Increasing human stem cell stress tolerance is therefore an enabling technology for clinical use of stem cells." This lack of focus in the experimental plan substantially weakens the proposal.
- The preliminary data do not adequately support the rationale. The experiments were carried out in a commercial immortalized (TERT-expressing) line of "adipose stem cells," despite the general view that these are stromal cell populations with few, if any, true stem cells. One TEAP—a mitochondrial protein—significantly improved cell survival under nutrient deprivation (one-third-strength medium) and modestly improved survival following passage through a small-bore needle.
- The improved survival came at the expense of a substantial change in differentiation, with markedly increased osteogenic differentiation and decreased adipogenic differentiation under standard ASC conditions. The third ASC lineage, chondrogenic differentiation, was not reported.
- The notion that improved survival may result, in part, from reduced susceptibility to apoptosis is concerning. A major issue in cell therapy is the potential for transplanted cells to undergo malignant transformation. Resistance to programmed cell death is therefore a significant risk factor that the application fails to adequately acknowledge.
- The rationale is compelling: increasing human stem cell stress tolerance represents an enabling technology for the clinical use of stem cells. The approach has the potential to be translated across diseases, age groups, and therapeutic areas and could help advance the field of regenerative medicine.

**Plan & Design: Evaluate the project plan and design**

- The applicants will express single TEAPs and combinations of TEAPs in commonly used stem cell types and test them under relevant stressors using cell survival assays, integrated transcriptomics, molecular marker expression, and functional analyses. This is a straightforward approach that should set the stage for validation.
- Stem cell phenotyping using bulk RNA sequencing is an important step to determine whether TEAP expression affects stem cell differentiation and lineage commitment.
- Transfection controls and the definition of the protein expression levels required to optimize the effect are not well addressed.
- Aim 2 will determine whether these unique proteins are immune compatible, immunomodulatory, and immunogenic using both in vivo and in vitro systems. The proposed in vitro system is not appropriate and is unlikely to be informative.
- Interpretation of the results may not be as straightforward as anticipated. TEAPs may protect against some stressors but not others. The criteria for determining whether the approach is therapeutically viable are not clearly defined.
- The potential for tumorigenic outcomes is not well conceptualized or adequately controlled.
- The sheer amount of proposed work is daunting. Testing four distinct cell types against five different proteins, plus 10 possible pairwise protein combinations, generates 60 different conditions. This is further multiplied by the use of both male and female cells of ethnically diverse origin for each cell type. It is not clear that this broad survey can be accomplished within the proposed time and budget. There is too much breadth and very little depth.
- The experimental plan is not designed to test specific hypotheses regarding the individual cell types or transgenic proteins, nor does it define specific benchmarks for a successful cell type/TEAP combination. The lack of clearly defined deliverables is a major weakness, even for a Discovery-stage proposal.
- Potential immunogenicity of the tardigrade proteins is a significant concern and justifies testing mouse cells in mice. However, the proposed assays are rather generic. The studies would benefit from greater

emphasis on assays using cells expressing the proteins, together with appropriate positive and negative controls.

- The team appears strong in engineering and bioinformatics. However, neither the PI nor the Co-Investigator appears to have sufficient experience with human iPSCs, hematopoietic stem cells, or neural stem cells. These are not simple "off-the-shelf" systems. The team also appears to lack sufficient hands-on experience in stem cell-based therapies using differentiated cells. This may account for the proposal's lack of specific benchmarks for clinically relevant discoveries.
- The addition of an experienced leader in pluripotent stem cell research would be highly advisable, if not essential.
- The most challenging, but potentially most promising, application would be to improve the efficacy of cell therapies using differentiated products of iPSCs. Survival of the iPSCs themselves is primarily a manufacturing concern, as these cells are tumorigenic and are not candidates for direct therapeutic injection. The proposal only tests differentiation of TEAP-transduced iPSCs into primitive cells representing the three germ layers.
- The applicants do not acknowledge the obvious possibility that, if TEAP expression alters iPSC lineage commitment to the same extent that it altered ASC differentiation in the preliminary published study, it could substantially diminish the utility of the technology for cell therapies based on pluripotent stem cell derivatives.
- A strength is the evaluation of multiple stem cell types under a variety of relevant stressors. TEAPs will be expressed individually or in pairs using Sleeping Beauty vectors in ASCs, iPSCs, HSCs, and NSCs. Cells will be tested under representative stressors (shear, hypoxia, metabolic restriction, hyperglycemia, and chemotherapy) and assayed for survival using Live/Dead, EdU, MTT, and Annexin V assays.
- The team is strong, and the proposed budget and timeline are reasonable. However, the three investigators each commit only 10–15% effort, raising some concern that the work will rely heavily on junior staff who have yet to be identified.

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

- The team is ideally suited to conduct these studies and has dedicated considerable effort to the development and characterization of these interesting proteins.
- The impact is not limited to specific diseases but could affect many cell therapy approaches. The potential impact is therefore high.
- The proposal includes plans to test cells from both sexes and multiple ethnicities. Testing cells from both male and female donors is advisable.
- However, testing cells from multiple ethnic groups adds limited value at this stage of discovery research. A first-pass survey of the effects of intrinsically disordered proteins from an evolutionarily distant organism on human stem cells is unlikely to be substantially influenced by rare coding polymorphisms associated with diverse human populations. Such studies could reasonably await identification of specific gene and stem cell combinations that appear promising for therapeutic application.
- The proposal has the potential for a very large population impact across multiple diseases, populations, therapeutic areas, and age groups.
- Adipose stem cells are a foundational cell therapy with both immunomodulatory and regenerative properties. However, other clinically relevant cell types, including induced pluripotent stem cells (iPSCs), hematopoietic stem cells (HSCs), and neural stem cells (NSCs), are more sensitive to metabolic,

oxidative, and mechanical stress during engraftment. The proposed studies will include cells from donors of both sexes.

| Application# | DISC5-20142 |
|---|---|
| **Title**<br>(as written by the applicant) | APOE4 compromises BBB integrity through pericyte-microglia cross talk |
| **Project Objective & Impact**<br>(as written by the applicant) | A major unresolved gap in Alzheimer's research is how the APOE4 genotype drives early cerebrovascular dysfunction through cell-cell interactions at the blood-brain barrier (BBB). This work connects lipid metabolism, microglial state shifts, and immune-vascular interactions to BBB integrity, providing a unifying framework to understand vascular dysfunction seen in APOE4 driven Alzheimer's. |
| **Specific Aims**<br>(as written by the applicant) | • Investigate the cell autonomous impact of APOE haplotype in pericytes<br>• Define bidirectional microglia-vascular signaling that regulates microglia state and vasculature integrity<br>• Determine how APOE-dependent microglia-pericyte interactions drive vascular pathology in Alzheimer's disease |
| **Statement of Benefit to California**<br>(as written by the applicant) | APOE4 carriers are estimated to account for 432,000 cases of AD in California, more than in any other U.S. state, ranking 7th in terms of prevalence. California's Medicaid program spends an estimated $5.6 billion annually on AD care, covering medical services and long-term support. Current FDA approved drugs put APOE4 carriers at risk for adverse effects. Our proposed work would uncover how APOE4 impacts the brain vasculature and provide a model to discover new drugs that alleviate these effects. |
| **Funds Requested** | $2,499,999 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 84

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

| | |
|---|---|
| **Mean** | 84 |
| **Median** | 84 |
| **Standard Deviation** | 2 |
| **Highest** | 90 |
| **Lowest** | 81 |
| **Count** | 13 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 5* |
| **(1-84): Not recommended for funding** | 8 |

* See Minority Report below

# FINAL COMMENTS

Proposals were evaluated and scored based on the review criteria shown below, which are also described in the PA/RFA. Following panel discussion and scoring, reviewers entered 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 for clarity by CIRM.

**Key Strengths and Weaknesses**

- This proposal is addressing an important question in AD with novel models and novel hypothesis.
- Strengths:
    - Significance – APOE4 is the strongest genetic risk factor.
    - Innovation – the microglia-vasculature model and Osteopontin-vitronectin hypothesis.
    - Rationale – strong preliminary data.
    - Population impact – balanced by sex, plans for ethnic diversity.
- Weaknesses:
    - Very ambitious – three dense aims in three years.
    - PI has over 15 active grants (focus concerns).
    - Data Project Manager experience not well documented.
    - *[Redacted product name]* is not a credible therapeutic.
- PI overwhelmed with other commitments.

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

- This project addresses a major gap in Alzheimer's research: how APOE4 disrupts the blood-brain barrier via pericyte-microglia crosstalk. APOE4 is the strongest genetic risk factor for AD, and vascular dysfunction is an early, clinically relevant feature linked to treatment complications like ARIA-E.
- Large target population.
- Understanding of APOE4-specific vascular contributions to AD is limited and there is an unmet need. How different components within the BBB microenvironment are impacted by APOE and whether the interactions between them are the true drivers of vascular pathology in AD remain knowledge gaps.
- The proposal targets a real and under-explored gap: BBB breakdown precedes Aβ accumulation, ARIA-E complications limit anti-amyloid therapies in APOE4 carriers, and APOE4 is the strongest genetic AD risk factor. Using human iPSC-derived models, the work will generate mechanistic insights into lipid metabolism, immune-vascular signaling, and vessel integrity. While disease-focused, it also advances fundamental stem cell biology, aligning well with DISC5 objectives.

- Direct California population alignment: 8–10M Californians are APOE4 carriers, with 300K–450K APOE4 carriers among Californians living with AD.
- The iPSC-derived APOE isogenic system may provide a meaningful regenerative medicine platform for therapeutic testing.
- APOE4 is associated with altered lipid handling, mitochondrial dysfunction, and neuroinflammation — pathways strongly linked to PPARγ biology.
- Project significance is primarily mechanistic rather than translational; no direct path to an iPSC-derived therapeutic cell product is articulated.
- The therapeutic tested is exploratory and [redacted agonist] is not a credible therapeutic for development.

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

- The proposal is highly innovative. It uses APOE-isogenic human iPSCs to overcome species differences. The novel microglia-vasculature cord (MVC) model captures physical and functional interactions between microglia, pericytes, and endothelium.
- The self-organizing cord system is a novel model that captures bidirectional crosstalk in a way that 2D and standard organoid systems cannot.
- The hypothesis that [redacted gene] acts as “molecular glue” between microglia and pericytes, with pericyte-derived vitronectin competing for integrin binding, is of great interest and testable. Adding PPARG agonism and advanced lysosomal probes further distinguishes this work from conventional approaches.
- The hypothesis-driven integrated [redacted pathway] signaling is biologically novel and testable.
- Combination of lipidomics, proteomics, metabolite tracing, scRNA-seq, and spatial proteomics in iPSC-derived APOE isogenic vascular cells is methodologically integrative.
- The use of 4 isogenic APOE iPSC pairs with planned expansion across sex and ethnicity is a strength.
- The proposed axis is one of several plausible BBB-relevant pathways; the proposal commits heavily to this hypothesis without clearly described alternatives if it does not validate.
- PPARG agonism as a rescue is conventional pharmacology rather than an innovative therapeutic strategy.
- The use of a novel human iPSC-derived vasculature model. The use of multi-omics approach, including lipidomics, proteomics, and metabolomics. The proposal tests a novel hypothesis that BAM is a state of microglia.

**Rationale: Evaluate the scientific rationale in the proposal**

- The rationale is strong and well-supported by preliminary data. The team shows that APOE4 microglia and pericytes both have reduced APOE secretion, lipid accumulation, lysosomal dysfunction, and metabolic shifts.
- Core idea: Define how APOE4 disrupts microglia–pericyte coupling at the BBB through [redacted pathway]-mediated crosstalk and lipid metabolism, using a novel iPSC-derived microglia–vasculature cord (MVC) model with isogenic APOE pairs.

- APOE4 is the most significant genetic risk factor for late-onset AD; the impact of this isoform on BBB remains under studied. The role of microglia in AD and APOE4 linked BBB integrity is not known. Strong preliminary data support the hypothesis.
- APOE4 microglia also show reduced [redacted gene], while APOE4 pericytes increase [protein]. The MVC model preliminarily demonstrates that microglia enhance vascular complexity. These data logically justify each aim and the central hypothesis.
- Preliminary data are strong: APOE4 microglia show lipid droplet accumulation, APOE4 pericytes show mitochondrial dysfunction, lysosomal pH changes, and increased vitronectin, and APOE4 microglia have reduced SPP1 and impaired BAM-state formation across many patient lines.
- ROSMAP postmortem dataset integration validates findings against human in vivo biology — a meaningful externalization of the cellular work.
- The microglia-deficient AD model supports centrality of microglia in AD vascular pathology.
- Holding endothelial genotype constant is useful for control but limits insight into endothelial-APOE4 contributions, which may be a substantial part of the in vivo phenotype.
- The role of *[agonist redacted]* as decalcifier versus promotoer of mineralization may be context-dependent; the proposal treats decalcification as the operative function but this is not unambiguously established for the BBB and relies on data from peripheral vasculature. Other hypotheses are possible.
- Good preliminary data.

**Plan & Design: Evaluate the project plan and design**

- The plan is rigorous, with multi-omics, quantitative imaging, and functional rescue experiments. Aims flow logically from cell-autonomous effects to bidirectional signaling to APOE-specific pathology. Alternative strategies are provided.
- Concerns include high ambition across three dense aims within three years, the Data Project Manager's limited documented management experience, and the PI's many active grants, which raise questions about focus and feasibility.
- Three aims with a clear 1) cell-autonomous → 2) bidirectional → 3) APOE-dependent pathology progression. Aims are non-redundant, and logically dependent in the right direction.
- Resource base is exceptional: Applicant institutional cores, a co-I's multiple mass spec systems for lipidomics and MFA, and another co-investigator's lab's Celldiscoverer 7 for imaging.
- Pitfalls and alternatives are addressed: blocking-antibody and competitive-peptide strategies for ECM-integrin probing, time-lapse imaging if differences are subtle, with acknowledgment that PPARG rescue may be incomplete.
- The microglia-vasculature platform underpins Aims 2 and 3; if it does not robustly recapitulate the in vivo phenotype across APOE backgrounds, both aims are at risk.
- Sample sizes for the model perturbation conditions (4 APOE conditions × multiple perturbations × triplicates) are not consistently specified across aims.
- Complex three-aim study with multiple omics modalities may be challenging within the time frame.
- The PPARG rescue is a single-drug arm. Given how much rests on it, alternative lipid-pathway rescues would have strengthened the design.

- Multi-omics will be done to examine the impact of APOE in pericytes. Tests a new hypothesis using the microglia-vasculature platform and how APOE4 drives a pathological state shift.
- Experiments are proposed to test whether PPARG agonism can rescue microglia-pericyte interactions and vascular integrity in APOE4 contexts. One limitation is the lack of astrocytes in the microglia-vasculature platform.

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

- The application addresses population impact reasonably well. The applicant uses four isogenic iPSC pairs balanced for sex and plans to diversify lines further via collaborations. Validation in human postmortem datasets is included. Community engagement activities are documented.
- APOE4 is a strong risk gene for AD. Two pairs of female and two pairs of male isogenic lines with APOE33 and APOE44 genotypes will be used. The PI's lab has been actively engaged in outreach and education efforts. However, no description is provided for the co-I's activity.
- APOE4 risk is stratified quantitatively by ancestry (Caucasian ~12-fold, East Asian ~30-fold, Black/Hispanic ~2–5-fold but highest AD prevalence) and by sex (APOE4 females higher risk), and these factors directly drive study design.
- Active diversification of the iPSC bank through collaborations with two different institutions is a concrete, scientifically appropriate step.
- A core facility at the applicant institution is building APOE isogenic lines across sex and ethnicity, with the explicit intent to make these lines available to the community — extending project impact across populations.
- Study design includes 2 female plus 2 male APOE isogenic lines, which is a reasonable, feasibility-matched plan for discovery-stage work.
- The proposal could more explicitly address how BBB-pathology differences across populations (e.g., differential ARIA-E rates, calcification prevalence) might map onto the mechanistic findings.

## MINORITY REPORT

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

The minority of reviewers who supported funding considered this proposal to address an important and underexplored question in Alzheimer's disease (AD): how APOE4, the strongest genetic risk factor for AD, contributes to early blood-brain barrier (BBB) dysfunction through interactions between microglia and vascular cells. These reviewers noted the clinical relevance of this question, particularly given the association of APOE4 with vascular dysfunction and complications such as ARIA-E, as well as the large population of APOE4 carriers potentially affected by the proposed work.
Reviewers considered the proposal highly innovative, highlighting the use of APOE-isogenic human induced pluripotent stem cell (iPSC) models and the novel microglia-vasculature cord platform. They also viewed the proposed mechanistic hypothesis as novel and testable and noted the strength of integrating lipidomics, proteomics, metabolite tracing, single-cell RNA sequencing, and spatial proteomics.
The minority reviewers considered the scientific rationale to be strong and well supported by preliminary data demonstrating APOE4-associated changes in microglia and pericytes.
Finally, these reviewers viewed the experimental plan as rigorous and logically organized. They highlighted the strong institutional resources, complementary experimental approaches, consideration of alternative strategies, and inclusion of isogenic lines balanced by sex, with plans for further diversification. Overall, the minority reviewers

considered the project's significance, innovative human iPSC-based platform, strong preliminary data, and potential to generate important mechanistic insights into APOE4-associated vascular pathology sufficient to warrant funding.

| Application# | DISC5-19775 |
|---|---|
| **Title**<br>(as written by the applicant) | Mechanisms driving the tempo of life in human cells |
| **Project Objective & Impact**<br>(as written by the applicant) | Our goal is to understand how mechanisms underlying the tempo of life impact human stem cell and progeny, with the objective of boosting organ regeneration in aging and disease. Our hypothesis is that glycogen accumulation is critical for the regenerative potential of the human brain and that preventing abnormal glycogen accumulation is essential to counter aging and glycogen storage diseases. |
| **Specific Aims**<br>(as written by the applicant) | • Develop human organoid models to probe glycogen metabolism at different life stages<br>• Identify new functional glycogen regulators to boost human regeneration potential<br>• Generate human models of disease genes that impact glycogen storage |
| **Statement of Benefit to California**<br>(as written by the applicant) | This research will benefit California by advancing understanding of human brain regeneration and glycogen-related disorders. By developing human neural organoid models and identifying key metabolic regulators, it could lead to new therapies for age-related cognitive decline and glycogen storage diseases, improving health outcomes and quality of life for Californians. |
| **Funds Requested** | $2,494,573 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

**Final Score: 83**

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

| | |
|---|---|
| **Mean** | 83 |
| **Median** | 83 |
| **Standard Deviation** | 4 |
| **Highest** | 88 |
| **Lowest** | 75 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 7* |

| | |
|---|---|
| **(1-84): Not recommended for funding** | 8 |

* See Minority Report below

## FINAL COMMENTS

Proposals were evaluated and scored based on the review criteria shown below, which are also described in the PA/RFA. Following panel discussion and scoring, reviewers entered 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 for clarity by CIRM.

**Key Strengths and Weaknesses**

- The team is outstanding, and the preliminary data are convincing. There are some concerns regarding how well the organoid models will establish the aging axis.
- The proposal has the potential for high significance and the premise is supported by strong preliminary data and relevant models.
- This is a significant project centered on the hypothesis that glycogen metabolism affects the maturation and regeneration of the human brain, and that preventing abnormal glycogen aggregation is crucial for regeneration. The team has generated strong preliminary data and assembled an impressive set of tools.
- The main concern is that the project is largely descriptive, with relatively little effort devoted to defining the mechanistic basis of glycogen accumulation.

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

- Glycogen is a key metabolite, and the preliminary data support the accumulation of glycogen aggregates (Corpora Amylacea) in aging human brains. While the data presented are insufficient to determine how representative or common this phenomenon is, it is an intriguing observation that warrants further investigation. Aim 1 will develop and utilize in vitro models. Aim 2 will perform screens and validation studies to identify regulators of glycogen storage. Aim 3 will generate and characterize models from patients with glycogen storage disease.
- Strength: The work will provide novel insights into the regulation of glycogen storage and its connection to disease.
- Weakness: The models may not adequately reflect disease states, and the claims regarding drivers of the tempo of life, enhancement of regeneration, and therapeutic potential are less convincing.
- The proposal will examine how metabolism shapes stem cell function, regenerative capacity, and age-related changes in stem cell function. If successful, it could lead to new approaches to enhance regenerative capacity, which declines with age. This would have high significance.
- The idea that abnormal glycogen accumulation is linked to cellular dysfunction and aging is novel and could have a major impact on neurological diseases, as well as other organs, such as the liver, in which glycogen is processed.

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

- The methods are innovative, and the focus on aberrant glycogen storage is interesting and timely. The proposal incorporates powerful new readouts.

- The laboratory has developed in vivo screens for brain aging and an image-based CRISPR screen in human cells.
- The study is based on neural organoids and regeneration.
- The project is highly innovative, and the team has developed an impressive set of tools and protocols needed to carry out the proposed studies. In addition, they have access to all of the samples required to produce human brain organoids at various developmental stages.

**Rationale: Evaluate the scientific rationale in the proposal**

- The rationale is generally well supported. However, across all three aims, some of the high-level objectives do not align well with the experimental plan, or the proposed results do not directly address those objectives.
- This is an excellent model system—the best currently available.
- The preliminary data identify glycogen granules in astrocytes, which are cells that can influence regenerative potential.
- The premise is based on the logical rationale that understanding glycogen metabolism and storage defects will improve our understanding of neural stem cell potential.
- There is growing evidence that cellular metabolism, particularly glycogen metabolism, may underlie differences between short-lived and long-lived species. The investigators present preliminary data showing that glycogen granules are present in human neural progenitors within neural organoids and accumulate in human astrocytes both in vitro and in vivo. They also found that, as the human brain ages, glycogen forms abnormally large extracellular clusters known as Corpora Amylacea.

**Plan & Design: Evaluate the project plan and design**

- The three aims are well described, and the team is outstanding. The new approaches, including the antibody- and FACS/imaging-based analyses, are powerful when combined with the advanced culture models and screening modalities.
- Weakness: Inducing aging features in iPSC-derived organoids is somewhat artificial, and it is difficult to determine where glycogen granules fit into this process. Aging clocks perform well in vivo but can be readily influenced in vitro by perturbations that do not truly affect cellular aging. It is also unclear whether organoids form extracellular granules comparable to those observed in vivo. The in vitro images in Figure 3 are less convincing than the in vivo samples.
- Aging features are not the same as aging. Preliminary work shows that organoids undergo aging-related changes during years of culture but remain far from truly "old" tissue.
- In the summary of Aim 1, and throughout the application, the concepts of the "tempo of life" and glycogen storage are emphasized, but neither is directly investigated in the experimental plan. Similarly, a causal "driver" role is not tested. In Aim 2, the claim that glycogen accumulation is detrimental and that limiting it could be therapeutic by improving regeneration requires additional supporting evidence.
- The screen in Aim 2 appears robust and is likely to identify new regulators in U87 cells, although these cells may have unique metabolic requirements. In addition, while discussed in the alternatives section, it is unclear whether complete loss-of-function approaches will be informative, as they may have effects beyond glycogen granule formation. There is also no direct evidence that these interventions will enhance regeneration.

- The team includes expertise in human neural organoid models of brain development and the molecular and metabolic mechanisms of brain aging.
- The laboratories have developed organoid-derived astrocytes that accumulate glycogen granules during prolonged culture, which could provide an effective model for studying age-dependent changes.
- A potential concern is that measuring glycogen accumulation across all aims may not serve as an adequate readout for regenerative capacity.
- The main concern is that the project is largely descriptive and lacks studies linking changes in glycogen accumulation to mitochondrial function. Mitochondrial function is known to decline with age, resulting in reduced fatty acid oxidation and other metabolic changes.

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

- Aim 3 provides a direct translational link and will investigate multiple diseases using the established models.
- The proposed organoids will be generated to incorporate and track these factors. Clinical and community outreach activities are also proposed.
- The effort is insufficient. Using cell lines from only three diverse ethnic backgrounds does not adequately evaluate the effects of the findings across different populations.

## MINORITY REPORT

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

Supportive comments described the proposal as having convincing preliminary data, an "outstanding" team, an innovative approach, an "impressive set of tools," and a "well described" project plan. The project's significance was attributed to its potential to provide novel insights into the relationship between glycogen storage and disease, both in neural tissue and potentially in other organs. Notable strengths included the "intriguing" premise that abnormal glycogen aggregation limits regeneration in aging cells and the "excellent... best available" model system, namely organoid-derived astrocytes that accumulate glycogen granules during prolonged culture. Although all reviewers acknowledged potential limitations in the generalizability and physiological relevance of the models, supportive comments suggested that the combination of advanced model systems, innovative readouts, and screening results from Aim 2 outweighed these concerns.

| Application# | DISC5-19843 |
|---|---|
| **Title**<br>(as written by the applicant) | Causes and consequences of age-related myeloid bias |
| **Project Objective & Impact**<br>(as written by the applicant) | We will gain insights into the causes and consequences of a process called "myeloid bias" which can result when blood stem cells produce too much of one kind of immune cell. |
| **Specific Aims**<br>(as written by the applicant) | • To determine the genetic basis and disease associations of myeloid bias in human cohorts<br>• To assess the impact of HSC lineage bias on immune function and disease susceptibility<br>• To determine the impact of biological sex on human HSC myeloid bias |
| **Statement of Benefit to California**<br>(as written by the applicant) | As the population of California ages, we can expect an increased incidence of age-related diseases and poor immune response to infections to put a strain on our healthcare system. A better understanding of the causes of immune decline and increased risk of chronic inflammatory diseases in aging is needed. The work proposed here will comprehensively test the idea that myeloid-biased blood stem cells underlie some aspects of immune aging and might therefore represent a therapeutic opportunity. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

### Final Score: 83

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

| | |
|---|---|
| **Mean** | 82 |
| **Median** | 83 |
| **Standard Deviation** | 4 |
| **Highest** | 85 |
| **Lowest** | 70 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 6* |
| **(1-84): Not recommended for funding** | 9 |

* See Minority Report below

# FINAL COMMENTS

Proposals were evaluated and scored based on the review criteria shown below, which are also described in the PA/RFA. Following panel discussion and scoring, reviewers entered 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 for clarity by CIRM.

**Key Strengths and Weaknesses**

- This is a strong application with an exciting Aim 2 to test the impact of myeloid-biased HSCs and a nice combination of analyses of human cohorts and use of mouse models. However, the hypothesis is vague (seemingly all-inclusive), and the proposal does not adequately address how thymic involution may stand in the way of T-cell development, even with more balanced HSCs.
- The main goal of the proposal is important, as we do not fully understand how myeloid bias in HSCs affects health outcomes. However, experimental details are lacking, as is a clearly defined hypothesis to be tested.
- Human age-related myeloid bias is an established phenomenon with broad clinical relevance. It remains understudied, partly because of a lack of complementary tools to both determine whether this process results from alterations in HSCs and to assess its functional outcomes.
- The proposal features a strong combination of GWAS and patient data with models of human HSC bias, gene editing, and single-cell analysis.

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

- Strengths include a team of investigators with unique and synergistic expertise that will support a successful project outcome and inform potential future therapeutics.
- Mitigable weaknesses include the lack of clear data to support the hypothesis that myeloid bias with age is causal of, rather than merely associated with, negative health outcomes. This will be assessed in mice receiving either balanced or myeloid-biased HSCs, with a focus on lifespan. It is unclear what other negative health outcomes will be assessed. What if myeloid bias with age confers a fitness benefit? How will this be assessed?
- How will an involuted thymus confound efforts to boost lymphocytes with balanced HSCs?
- The main significance of the proposed work comes from the need to better understand the mechanisms underpinning age-associated changes in hematopoietic stem cell lineage potential and bias toward myeloid outcomes.
- Human age-related myeloid bias is an established phenomenon with broad clinical relevance. It remains understudied, partly because of a lack of complementary tools to both determine whether this process results from alterations in HSCs and to assess its functional outcomes.

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

- Innovative tools, including antibodies and mouse models, are utilized, and conceptually novel directions are pursued.
- The proposal builds on the applicants' previous findings characterizing distinct subsets of hematopoietic stem/progenitor cells that exhibit either myeloid-biased or more balanced differentiation outcomes.

- No new frameworks are being developed; rather, the proposal seeks to understand the genetic underpinnings of myeloid-biased HSCs versus balanced HSCs during the aging process, as well as whether sex influences these changes.
- The inclusion of human data from several databases, along with access to GWAS data, myeloid and lymphoid CBC data, genetic data, environmental exposure data, and disease outcome data, will allow the investigators to attempt to develop a risk score. The analysis does not account for social determinants of health (SDOH), but this is beyond the scope of the application.
- The proposal combines GWAS and patient data with models of human HSC bias, gene editing, and single-cell analysis.

**Rationale: Evaluate the scientific rationale in the proposal**

- The combination of human data, mechanistic mouse models, and human cell-based studies is a strength.
- Some figure legends provide insufficient information for clear interpretation of the preliminary data.
- The rationale for the work is clearly laid out in the proposal, but the experimental plan and design are less well developed and not as clearly outlined.
- However, for Aim 3, the stated hypothesis—"We hypothesize that these sex-dependent differences in myeloid bias originate at the HSC level through cell-intrinsic and/or cell-extrinsic mechanisms"—is not readily testable, as either, neither, or both mechanisms are possible. It is difficult to support a scientific endeavor without a hypothesis that can be falsified by the results.
- The proposal presents a strong scientific rationale and plan to investigate the function of different HSC subsets and to understand the physiological consequences of eliminating specific HSC populations prior to further study in humans.

**Plan & Design: Evaluate the project plan and design**

- There is insufficient discussion of thymic involution as a contributor to reduced T-lymphocyte production with age. Even if more balanced HSCs can be favored in elderly individuals, the issue of reduced thymic output would still need to be addressed.
- The proposal provides broad outlines of how each aim will be pursued but does not include sufficient detail to fully evaluate the proposed work. The focus on CD164 is interesting, however, and may lead to new insights.
- In Aim 2, it is not clear whether the investigators have the ability to deplete balanced HSCs, as proposed, while sparing myeloid-biased HSCs. While the reverse approach may be feasible, both strategies are discussed.
- In Aim 3b, it would be important to know how many mice will need to be engrafted to account for likely donor-to-donor variability. The investigators may also wish to consider using the same mice in a competitive engraftment approach, particularly when examining sex differences in donor cells.
- There is no Figure 6 in Aim 3a. In addition, this aim is only loosely outlined, beyond indicating that CB, BM, and MPB samples will be compared.
- Donor-to-donor variability may be a significant issue in Aim 3, as the authors acknowledge. Pilot experiments to assess variability and, if necessary, increased sample size are possible approaches to address this concern.

- No concerns were identified regarding data sharing. The investigators describe plans to deposit raw and cohort-level data and to make individual-level data available through the databases used for the study (e.g., risk score analyses). The proposal should also discuss compliance with HIPAA requirements governing the use and deposition of these data.

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

- This is handled appropriately.
- It is unclear whether the proposed work, as described, will significantly advance our understanding of the mechanisms regulating myeloid-biased HSCs versus balanced HSCs in elderly individuals, or whether these differences are sex-linked.
- The proposed analysis of large-scale biobank data (approximately 1 million individuals in total) will include cohorts with ethnic and socioeconomic diversity both within and beyond the United States. This will enable analysis of variables such as age, sex, and socioeconomic status, as well as the ability to infer genetic ancestry.

## MINORITY REPORT

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

Six of the fifteen scoring panelists give this application a score of '85'. A reviewer representing the supportive minority emphasized the synergistic investigator team, innovative tools, conceptually novel directions, and the combination of human data, mechanistic mouse models, and human cell-based work. There was excitement in particular for Aim 2, wherein the applicant proposes to assess the impact of HSC lineage bias on immune function and disease susceptibility. According to the same reviewer, a valid knowledge gap (how hematopoietic stem cells become progressively myeloid-biased with aging) has been identified by the applicant, and the proposed studies will help fill this gap.

| Application# | DISC5-19705 |
|---|---|
| **Title**<br>(as written by the applicant) | Leveraging natural and engineered hematopoietic surface receptor variation for non-toxic stem cell transplantation |
| **Project Objective & Impact**<br>(as written by the applicant) | A major bottleneck in our ability to use stem cell transplant for a wide range of diseases is the fact that highly toxic methods are currently needed to make room in the bone marrow for curative stem cells. The biology of how stem cells take up residence in the bone marrow has many key unanswered questions. Our study will answer these questions and lead us towards a way to do non-toxic transplants. |
| **Specific Aims**<br>(as written by the applicant) | • Quantify engraftment efficiency of CXCR4 variant-expressing donor HSCs<br>• Quantify engraftment efficiency of c-Kit variant-expressing donor HSCs<br>• Identify drug-resistant surface receptor variants for in vivo HSC selection |
| **Statement of Benefit to California**<br>(as written by the applicant) | Californians of all ages and backgrounds are affected by diseases cured with stem cell transplant, such as blood disorders, immunodeficiencies, metabolic disorders, and possibly Alzheimer's and autoimmunity. Stem cell transplant currently requires pre-treatment with chemotherapy or radiation, which can lead to death and major complications requiring lengthy hospital stays. Our study will eventually lead to non-toxic transplant methods that improve patient outcomes and decrease health care costs. |
| **Funds Requested** | $2,481,613 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

**Final Score: 82**

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

| | |
|---|---|
| **Mean** | 82 |
| **Median** | 82 |
| **Standard Deviation** | 4 |
| **Highest** | 88 |
| **Lowest** | 74 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 6* |
| **(1-84): Not recommended for funding** | 9 |

* See Minority Report below

# FINAL COMMENTS

Proposals were evaluated and scored based on the review criteria shown below, which are also described in the PA/RFA. Following panel discussion and scoring, reviewers entered 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 for clarity by CIRM.

**Key Strengths and Weaknesses**

- The project goals are creative, and this is a relatively novel approach. The experiments based on homing to the BM niche are not likely to be informative as designed.
- Aims 1 and 2 are essentially the same, and Aim 3, looking at resistance, is an add-on. It is not clear why OptiProt is required, as there are more than enough naturally occurring or resistant variants to test the overall hypothesis. No biology or mechanism is being studied.
- The problem of HSCT resistance and conditioning seems inflated. A reference or some data to support the notion that this will increase eligibility for HSCT, and to define the extent of the current problem, would be welcomed.
- Combining cutting-edge protein engineering expertise with high-throughput laboratory experimentation and machine learning to design hyperfunctional and drug-resistant CXCR4 and c-Kit receptors is an innovative approach.
- The applicants are leveraging known naturally occurring variants. This approach, if successful, may prove superior to selectively decreasing the activity of CXCR4 and c-Kit on endogenous HSCs, which has proved challenging.
- In some ways, it seems oversimplified to assume that expressing these variants on donor HSCs will completely solve the problem of donor cell, as opposed to endogenous/host cell, engraftment. It also does not account for the fact that, for many oncologic conditions, an additional purpose of cytotoxic conditioning is to augment MRD-negative remission.
- While this strategy may improve engraftment efficiency, the ability of the planned experiments to recapitulate the duration of engraftment and engraftment failure, especially using a doxycycline system here and planning for future mRNA-based delivery, is a challenge.

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

- The proposal aims to enable effective HSC engraftment without cytotoxic conditioning. If successful, the work could provide insight into whether donor HSCs can be engineered to outcompete endogenous HSCs by enhancing their ability to migrate to, occupy, and persist within the bone marrow niche.The project has potential impact because non-toxic or less-toxic HSC transplantation could expand the use of HSCT.
- The proposal focuses on CXCR4/CXCL12 and c-Kit/SCF interactions that are central to HSC homing, retention, survival, and proliferation.
- Optimization of HSCT is a worthy pursuit and has the potential to benefit many patients with a variety of diseases. The risks associated with conditioning are less concerning than indicated here, and clinical evidence to support these claims is required.
- The idea of engineering HSCs to increase their capacity is not unique, but the use of CXCR4 variants and c-Kit modulation holds promise in applications related to, or expanding upon, HSCT.

- Few mechanistic studies are proposed, but mechanistic insights could be inferred from the results generated.
- Chemotherapy and radiation conditioning cause significant morbidity and mortality, and hematopoietic-specific antibodies have yielded less-than-ideal results.
- Manipulation of transplanted HSCs to provide a selective advantage during engraftment and repopulation would have widespread benefits.

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

- The proposal will use naturally occurring hyperfunctional variants of CXCR4 and c-Kit as tools to probe and potentially enhance engraftment, as well as inhibitor-resistant variants. Both strategies are innovative.
- The OptiProt platform adds another layer of discovery by using mutations in CXCR4 and c-Kit generated through error-prone PCR to define new structure-function relationships for CXCR4 and c-Kit and potentially identify additional hyperfunctional variants.
- To date, no major efforts have focused on the study of CXCR4 or c-Kit variants, although this is highly specific to hematopoietic tissue and HSC biology.
- OptiProt is unique and has many potential applications.
- Combining cutting-edge protein engineering expertise with high-throughput laboratory experimentation and machine learning to design hyperfunctional and drug-resistant CXCR4 and c-Kit receptors is an innovative approach.

**Rationale: Evaluate the scientific rationale in the proposal**

- The scientific rationale is that CXCR4 and c-Kit are critical stem cell receptors for HSC engraftment, and that utilization of hyperfunctional variants in grafted HSCs may enable them to outcompete endogenous HSCs for bone marrow niche homing.
- The rationale for using a doxycycline-inducible lentiviral system to define temporal requirements is reasonable for investigating the timing necessary for HSC engraftment. However, there is some uncertainty regarding how well this system will translate to mRNA delivery. If durable or multi-week receptor expression is required, the translational path becomes less clear.
- The clinical bottleneck identified may be overstated. Fanconi anemia is unique because of its DNA repair defects and the conditioning required. There is no question that reducing or eliminating conditioning would benefit HSCT recipients (e.g., by reducing the inflammatory cascade), but it is unlikely to increase the number of patients eligible for transplantation.
- Age and the opportunity to titrate existing conditioning regimens prior to HSCT should be incorporated into the discussion, including reducing the use of Busulfan, Fludarabine, and Treosulfan, with or without radiation. Resistance, however, as correctly stated in the rationale for Aim 3, is very rare.
- Engineered HSCs and naïve donor HSCs are prepared and used very differently in transplantation, and the benefits of engineering HSCs would need to outweigh the effects of conditioning. This issue is not discussed or sufficiently acknowledged.
- The rationale is based on two well-established interactions between HSCs and the bone marrow: CXCR4 and c-Kit.

- The investigators are leveraging known naturally occurring variants. If successful, this approach may prove superior to selectively decreasing the activity of CXCR4 and c-Kit on endogenous HSCs, which has proven challenging.

**Plan & Design: Evaluate the project plan and design**

- Aims 1 and 2 test CXCR4 and c-Kit variants, respectively, using similar sub-aims. The receptors will be tested in chemotherapy- and radiation-free settings in humanized mouse models, receptor expression duration will be evaluated, and protein engineering will be used to discover improved variants.
- The main design concern is the temporal expression analysis. The proposed doxycycline induction periods of 1 day, 3 days, 1 week, 2 weeks, 4 weeks, and 20 weeks are broadly informative, but they may not be granular enough to identify the optimal window of expression (there is a large gap between 1 and 5 months). It is also unclear how a 2-month requirement for expression would translate into an mRNA delivery strategy.
- The preliminary data strongly support the feasibility of the proposal. PDX models, identified variants, and, most importantly, doxycycline-inducible lentiviral constructs are already in place. Execution of the proposed studies appears straightforward.
- The competitive in vivo assays, with and without conditioning, would have benefited from the inclusion of a pre-PDX in vitro assay to evaluate both naturally identified variants and those generated by OptiProt.
- Aims 1 and 2 are essentially the same, differing primarily in their focus on CXCR4 versus c-Kit. The use of Plerixafor and SCF/c-Kit antibody blockade is an interesting in vivo approach, but the rationale for using Plerixafor in the presence of c-Kit variants, and vice versa, is not explained. In addition, no biological or mechanistic studies are proposed, such as gene expression analyses, clonal engraftment studies, or other downstream biological assessments and appropriate controls to confirm that receptor expression subsides as expected.
- A 12-week PDX graft measures HSC capacity, whereas the application is focused on homing. Homing should instead be assessed 2–3 weeks after transplantation, with secondary transplantation performed as a functional assay more closely aligned with the goals of the proposal.
- The molecules being tested function differently in the mouse bone marrow niche than in the human niche, including their effects on homing. The limitations inherent to the human-to-mouse xenograft model should be acknowledged.
- The investigators demonstrate expertise in human hematopoietic xenotransplantation, provide proof of principle for OptiProt (although not specific to this work), and validate the doxycycline system, demonstrating that they can likely regulate gene expression using doxycycline.
- In some ways, the proposal seems oversimplified in assuming that expressing these variants on donor HSCs will fully overcome competition from endogenous host cells. It also does not account for the fact that, in many oncologic settings, an additional purpose of cytotoxic conditioning is to augment minimal residual disease (MRD)-negative remission.
- Although this strategy may improve engraftment efficiency, the planned experiments may not adequately recapitulate long-term engraftment or engraftment failure. In particular, the transition from the doxycycline system used here to a future mRNA-based delivery strategy presents a challenge.
- It also remains unclear whether manipulation of donor HSCs alone will be sufficient to overcome the lack of manipulation of endogenous HSCs.
- The proposal misses an opportunity to discuss the impact of conditioning on fertility and the potential advantages of donor HSC delivery, including gene-modified HSCs, for preserving fertility across transplantation indications.

| **Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations** |
| --- |
| • The applicant will source donor CD34+ cells from diverse HLA backgrounds, balanced for sex where possible.<br>• The use of diverse CD34+ HSC samples and the acknowledgment of potential sex differences that may affect the results are strengths of the proposal.<br>• Within the Population Impact Enhancement Strategies section, the response is vague and somewhat underdeveloped, as the National Marrow Donor Program is not the premier organization from which to obtain key stakeholder feedback in the HSCT field.<br>• The investigators describe an agnostic approach to patient genotypic diversity at the loci of interest. They will obtain CD34+ cells from an established cell repository and plan to HLA genotype small aliquots to ensure a diverse portfolio of HLA types. They also plan to include approximately equal numbers of male and female donors, seek broad representation across ages, and use an equal mix of male and female recipient mice. |

## MINORITY REPORT

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

| Minority reviewers viewed the proposal as highly innovative and potentially transformative, highlighting its strategy of engineering donor HSCs with hyperfunctional CXCR4 and c-Kit variants to enable engraftment without cytotoxic conditioning. The integration of protein engineering, machine learning, and established HSC biology, together with strong preliminary data and demonstrated expertise in hematopoietic xenotransplantation, supported confidence in the project's feasibility and potential impact. While the reviewers noted some uncertainty regarding the translational path from the doxycycline model to an mRNA-based therapeutic approach, these concerns were outweighed by the proposal's strong scientific rationale, innovative design, and potential to advance safer HSCT. |
| --- |

| Application# | DISC5-20120 |
|---|---|
| **Title**<br>(as written by the applicant) | Systematic Expansion and Decoding of the Cytokine Language for Predictable Stem Cell Fate Control |
| **Project Objective & Impact**<br>(as written by the applicant) | Stem cell differentiation protocols are largely developed by trial-and-error due to limited understanding of how combinations of extracellular signals drive fate across contexts. By expanding the signaling repertoire, generating combinatorial perturbation datasets, and building predictive models, this project will enable rational, predictable control of stem cell fate for regenerative therapies. |
| **Specific Aims**<br>(as written by the applicant) | • Expand the extracellular cell signaling repertoire for stem cell fate control by using AI-driven protein design to generate synthetic agonists, antagonists, and biased ligands.<br>• Build high-throughput combinatorial perturbation platforms to systematically map how signal identity, combinations, and timing drive stem cell fate transitions.<br>• Develop and apply predictive, context-aware cell fate control models to navigate stem cells toward therapeutic muscle regeneration. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Stem cell therapies remain limited by slow, trial-and-error control of cell fate, delaying treatments for many diseases affecting Californians. This project will replace empirical approaches with a predictive platform that expands and decodes cell signaling to enable rational programming of human cell fates. By accelerating development of regenerative therapies, it will improve patient outcomes, reduce healthcare costs, and strengthen California's leadership and workforce in the life sciences. |
| **Funds Requested** | $2,423,140 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 80

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

| | |
|---|---|
| **Mean** | 82 |
| **Median** | 80 |
| **Standard Deviation** | 5 |
| **Highest** | 90 |
| **Lowest** | 75 |
| **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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

**Key Strengths and Weaknesses**

- The proposed work is extensive and aspirational, and even if only a fraction of what is being proposed is achieved, it would still provide a tremendous impact on the field.
- The team is good. The grant is well written.
- The proposal is very much in the "high-risk, high-reward" category. The well-organized overall plan and the exceptional team give hope that the project will generate significant results. A major weakness is the lack of specificity in considering mechanisms of signaling by potential engineered cytokines.
- Overall, the proposal is well written, and the ideas and concepts are detailed and explained well. The work and grant are interesting.
- At its core, this grant suggests that "natural cytokines provide a sparse and evolutionarily constrained set of inputs, fixing receptor identity, geometry, and pathway engagement into rigid molecular architectures." The introduction is very repetitive, with the same comments and justification regarding the ability to produce novel cytokines, but details of evidence for their role and success are lacking.

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

- This proposal provides an exciting framework with which to create, generate, and test new cytokines to potentially induce novel signaling outcomes in muscle stem cells (MuSCs), fibro-adipogenic progenitors (FAPs), and iPSCs. The significance of the work is very high, as developing new cytokines could open new avenues of research and therapeutics. However, chimeric cytokines may prove to be immunogenic, and this tempers some of the excitement behind the proposal.
- This is a highly ambitious project based on the use of recently developed tools for computational protein design to create novel growth factors/cytokines. The testing ground is muscle differentiation.
- Successful development of the technology could have profound implications for understanding stem cell biology and potentially equal practical impact in regenerative medicine.
- This is balanced against a significant risk from the underlying unproven assumption that the proposed cytokines, with each binding to a different receptor, will function effectively and generate novel and useful biological responses in stem and progenitor cells.
- The idea of regulating muscle stem cells for regeneration using cytokine engagement is unique. The outcomes of this work are difficult to determine due to the absence of experimental details and generated results, aside from the description of the goals and strategies that is reiterated several times.

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

- This is a very innovative and technologically advanced proposal, which takes advantage of cutting-edge approaches and analytical tools.
- Both the proposed platform for assessment of the activity of cytokines and the design of the cytokines are highly innovative. The former remains to be achieved. The feasibility of engineering cytokines with new signaling properties, both quantitative and qualitative, is supported by preliminary data from the PI's postdoctoral work, based on manuscripts still under review and available through bioRxiv.
- The technology development is unique, especially the modeling.
- The combination of computational and cytokine receptor biology is innovative. The absence of details and choices, and how data will be generated, makes the innovation and its impact difficult to assess.

**Rationale: Evaluate the scientific rationale in the proposal**

- The applicants frame the vision for their work as a means to develop a new language for how cells communicate with each other and induce differentiation programs. This rationale provides a clear vision for their proposed work.
- The rationale rests on assumptions about how growth factor signaling controls stem cell properties, particularly specific differentiation, which are speculative but grow out of established properties of receptors and signal transduction.
- The knowledge potentially to be gained amply justifies the investment of resources despite the risks of a project dependent on such a novel approach.
- Examples of changing receptor-ligand access and the use of new cytokines or factors in stem cells are missing. It is unclear how or why this is believed to work, and preliminary data would have helped support this concept by providing information that iPSC-generated cells or muscle stem cells could be affected in this manner.

**Plan & Design: Evaluate the project plan and design**

- The work will generate new fusions of cytokines and growth factors by taking advantage of advanced protein design approaches, which will be functionally tested for signaling outcomes and differentiation. The main strengths come from the innovative approach and track record in developing new tools and chimeric proteins.
- The main weakness of the proposal comes from not showing any preliminary results or proof-of-concept outcomes that use the proposed culture conditions and readout systems. Nevertheless, the PIs have extensive experience in the field, and their previous publications do lend some support to the feasibility of the complex and intricate research program.
- The first aim will set up the design of the cytokines, then Aim 2 will test the function of the cytokines, and Aim 3 will test their rational design for predicting differentiation outcomes. This last aim is the most aspirational and transformative if it indeed works out.
- The project is overly ambitious.
- The highly ambitious plan is presented at a conceptual level, with limited detail on some key elements. Overall, the plan does appear well organized. The team has world-class expertise in working with the relevant cells. Feasibility risks are noted under rationale.
- More specific discussion of the molecular details by which designed cytokines might signal is needed. For example, many growth factors function as dimers and act to bring 2 receptor subunits together as a

homodimer. Others have different molecular mechanisms. How does that relate to the design of the cytokines and expectations about their potential activity?

- The capabilities of the Co-Investigators are strong and complementary, providing confidence that both the protein engineering and biological aspects will be carried out with great expertise. If any team can accomplish this highly ambitious program, this one is appropriately constituted to do so.
- Specifically, the team pairs an outstanding expert on muscle stem cells with a new faculty member bringing state-of-the-art expertise in computational protein design.
- Aim 1A is the foundation of the application and has little to no detail. It indicates no cell type to be used and lists methods to "identify ligand receptor chains across major receptor families." The capacity to generate these and purify them seems massive, as stated in Aim 1B.
- It is unclear how candidate binders targeting individual receptor subunits central to stem cell signaling will be determined or prioritized. Which stem cells exactly will be used? Little to no information is provided, including which binders will be linked in Aim 1C. This is all being validated by a 20-core phospho-flow analysis (this is described in 3 different ways in the application, leaving it unclear which is being used or how), without a rationale for the choice or interpretation of these data to rank hits or prioritize them.
- The controls, positive or negative, and thresholds to evaluate fate output in Aim 2 are not described, nor are the iPSC lines or progenitor types to be tested or how these are to be compared. Despite the knowledge and description of the techniques, the experimental applications to biology are nearly absent.
- In a single line, the applicant indicates "... profiling of transcription factor occupancy with scRNA-seq to reconstruct transcriptional trajectories." This is not a trivial process, and more details of these experimental studies are needed to understand how this relates to the goals of the project.

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

- The impact of the work on affected populations is clearly outlined and considered.
- The population impact is significant.
- The applicants propose to use primary muscle stem cells and iPSC-derived progenitors from multiple donors varying in age and "disease-relevant states" and representing the diversity of the region's population.
- The project will use muscle stem cells from individuals of multiple ages and disease states, as well as iPSCs, for maximum inclusivity.

| Application# | DISC5-20042 |
|---|---|
| **Title**<br>(as written by the applicant) | Functional dissection of pathogenic splicing defects in neurodegeneration |
| **Project Objective & Impact**<br>(as written by the applicant) | Hundreds of disease-associated splicing defects and RNA-binding protein disruptions are observed in ALS/FTD and Alzheimer's disease, but it remains unknown which are causal drivers, preventing therapeutic prioritization. This project will identify pathogenic splicing events and regulators, enabling rational development of targeted therapies for ALS/FTD, Alzheimer's disease, and related dementias. |
| **Specific Aims**<br>(as written by the applicant) | • Identify causal disease-associated splicing events by pooled perturbation in transdifferentiated neurons<br>• Identify RNA-binding proteins and splicing factors that drive pathogenic splicing programs<br>• Validate causal splicing events and regulatory proteins by targeted correction of disease-relevant phenotypes |
| **Statement of Benefit to California**<br>(as written by the applicant) | Alzheimer's disease is projected to affect over 1.5 million Californians by 2030, with additional substantial medical and caregiving burden from ALS and frontotemporal dementia. By identifying disease-driving splicing defects in human neurons, this project will accelerate development of RNA-targeted therapies, strengthen California's leadership in regenerative and RNA medicine, and support future translational programs, biotech growth, and workforce development within the state. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 80

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

| | |
|---|---|
| **Mean** | 82 |
| **Median** | 80 |
| **Standard Deviation** | 3 |
| **Highest** | 88 |
| **Lowest** | 80 |
| **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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

**Key Strengths and Weaknesses**

- The work is supported by strong preliminary data, and the rationale for using aged neurons appears strong. However, concerns were raised in the discussion that the preliminary data show that WT neurons generated in the proposed manner exhibit stress and mislocalized TDP-43. Whether direct conversion of ALS cells could be tolerated given the baseline stress of WT cells, and then stressed further in future aims for study, was questionable. These concerns lowered enthusiasm.
- It is focusing on an area where the field is moving with innovative and therapeutic approaches.
- A weakness is having some of these cells double-stressed and then analyzed.
- The project is innovative and would be highly significant if successful, but some of the rationale and experimental plans have flaws that may prevent meaningful insights.

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

- The outcome of these studies will move the examination of how splicing changes in ALS/FTD and other TDP-43-related disorders impact disease pathogenesis from a one-by-one analysis to a higher-throughput interrogation using advanced methods.
- The findings will have implications beyond single disorders and define broadly which splicing factors may be imparting susceptibility or resilience.
- This knowledge will inform future therapeutics, and in fact, the ASOs developed as part of this work could be further advanced for therapeutic application.
- This proposal will reveal new paradigms for RNA splicing in neurodegenerative diseases (AD, ALS, and FTD) in human neurons. It appears that this proposal could be impactful in moving the field forward as it relates to RNA splicing and binding protein biology, pathology, and targets.
- The application focuses on RNA splicing defects associated with neurodegenerative disease and aims to identify splicing events that may promote neuronal dysfunction in human disease. Functional dissection of RNA splicing dysregulation is an important goal for understanding brain health and disease. The proposal uses stem cell technology to dissect disease mechanisms and might help to uncover therapeutic targets and/or biomarkers if successful.
- The approach involves several potentially impactful methods: pooled perturbation of 421 candidate disease-linked splicing events and targeting upstream RBPs in human neurons by a rapid chemogenetic protein degradation method.
- The direct differentiation model to generate neurons causes high levels of baseline cellular stress and splicing alterations, and it is not clear if the planned manipulations and readouts, which will cause further cell stress, will be feasible in this cell model and generate clear results that impact the field.

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

- This proposal uses new methods to understand key functions in aged neurons related to splicing and the misregulation of splicing in neurodegeneration.
- The pooled snRNA-based perturbation of splicing events together with the inducible HITAG-enabled degradation of target proteins provides a two-pronged approach that is innovative.
- It is innovative, as it relies on human neurons and genetic underpinnings. It is a novel framework, and preclinical animal models are deficient in translatability to humans.
- The proposed approach of inducing and screening various individual splicing changes and RNA-binding proteins linked to disease for their sufficiency in causing neuronal dysfunction is highly innovative.
- In Aim 1, the proposed manipulation of RNA splicing events in a targeted but relatively large-scale manner is innovative and likely to generate new and valuable insights.

**Rationale: Evaluate the scientific rationale in the proposal**

- The studies will address which mis-splicing events are causal drivers of neuronal dysfunction, which are compensatory, and which are neutral—key questions in many neurodegenerative disorders. The work is supported by a sound experimental plan and preliminary data, and the model system choice is excellent for addressing issues in aging neurons.
- The rationale makes sense for assessing these targets in human neuron cell lines from various diverse genetic backgrounds. One concern is that these are very specific to only RNA splicing and binding protein deficiencies, so other molecular players, pathways, and/or protein disturbances will not be studied.
- The evolution of siRNAs and other RNA approaches has been challenging in ophthalmic degenerative diseases, given the ability of the therapeutic to get into the cells and actually affect the cell DNA/RNA machinery. This is still a limitation that is not addressed here.
- The proposed trans-differentiation model to generate neurons has high levels of baseline cell stress, and it is not clear if the planned manipulations and readouts will be feasible in this model or cause major technical problems. Indeed, the preliminary data to establish feasibility were derived using standard iPSC-derived neurons.
- The project focuses on inducing additional targeted splicing defects (forced abnormal exon inclusion) and additional depletions in RNA-binding proteins in the trans-differentiated neurons, which already have baseline splicing changes and RBP depletions. It is therefore unclear if this approach can reveal if and which individual targeted perturbations cause neuronal stress and impairments in this cell system.

**Plan & Design: Evaluate the project plan and design**

- The experimental design is excellent, and the results will be a major contribution to the field. The team has addressed possible pitfalls and plans a two-pronged approach to the question, as well as ML-based learning, to improve the chances of success. The team, budget, and environment are excellent.
- The approach is reasonable and can yield meaningful insights but, again, is very specific to RNA-focused biology and pathology.
- Control trans-differentiated neurons have robust baseline TDP-43 mislocalization and disease-related phenotypes, and it is not yet tested how the proposed patient-derived lines with pathology will perform in this stress state, given that their viability and main phenotypes are unknown.

- It is possible that RNA splicing patterns and functional effects in patient-derived neurons will be altered in a way that is quite distinct from the splicing alterations found in the control cells through individual RNA splice-site screening. The proposed plan to focus on a few RNA targets in the patient-derived cells based on screening results in control cells may be an additional pitfall.

- Sorting stressed cells multiple times will further add to cell stress and possibly cause cell death, altering mitochondrial and other readouts and possibly confounding screening results.

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

- The team has developed a multifaceted plan for outreach, partnerships, and educational integration.

- There is a huge unmet need for effective therapies to address AD, but also a need to further understand the pathologies and targets.

- The design is reasonable in considering relevant factors and has potentially high applicability to various types of neurodegenerative disorders that impact diverse populations.

- The applicants describe good advocacy and outreach activities.

| Application# | DISC5-19929 |
|---|---|
| **Title**<br>(as written by the applicant) | Immunopathogenic Mechanisms of Anti-Hu Paraneoplastic Neurologic Disease |
| **Project Objective & Impact**<br>(as written by the applicant) | There is a critical gap in understanding how immune responses triggered by cancer damage human neurons in anti-Hu PNS. By leveraging patient-derived stem cells, this project advances regenerative medicine-driven discovery to inform targeted therapies and reduce irreversible neurological disability. |
| **Specific Aims**<br>(as written by the applicant) | • Develop patient-derived iPSC neuron models to study susceptibility to Hu-specific immune injury.<br>• Identify immunodominant Hu-derived peptides presented by neurons and B cells in Anti-Hu PNS.<br>• Define the molecular mechanisms by which patient-derived $CD8^+$ T cells selectively target Hu antigens in neurons in Anti-Hu PNS.<br>• Engineer Hu-specific $CD8^+$ CAR-T cells to selectively eliminate Hu-antigen–presenting B cells in Anti-Hu PNS |
| **Statement of Benefit to California**<br>(as written by the applicant) | This project benefits California by advancing precision immunotherapy and stem cell–based disease modeling to address severe untreatable paraneoplastic neurological syndromes. By leveraging patient-derived iPSC models and antigen-specific immune engineering, the work strengthens California's leadership in regenerative medicine, trains a skilled biomedical workforce, and generates broadly applicable tools and data that can accelerate innovation and improve neurological outcomes for Californians. |
| **Funds Requested** | $2,494,912 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 80

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

| | |
|---|---|
| **Mean** | 79 |
| **Median** | 80 |
| **Standard Deviation** | 5 |
| **Highest** | 87 |
| **Lowest** | 70 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 3 |

| | |
|---|---|
| **(1-84): Not recommended for funding** | 12 |

## FINAL COMMENTS

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

**Key Strengths and Weaknesses**

- The project is exciting and could lead to new understanding on how to target TAAs and treat autoimmune disease. However, greater clarity is needed on justification for the focus on B cells, the CAR T approach, and the risk for continued tumor control.
- Overall, this is a scientifically compelling, technically innovative, and potentially high-impact proposal that addresses fundamental questions of immune-mediated neuronal vulnerability and degeneration and, despite feasibility risks related to limited preliminary data and interdependent aims, has strong potential to establish important mechanistic frameworks and inform targeted immunotherapeutic strategies across neuroimmune and neurodegenerative diseases.

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

- While relatively rare, anti-Hu PNS is severe and poorly treated currently, and the field would certainly benefit from an effective therapy. This study could be foundational in generating such a therapy.
- This proposal, even if wildly successful, still needs to be coupled with early PNS detection in order to be useful.
- The project is exciting and could lead to new understanding on how to target TAAs and treat autoimmune disease.
- This project is high risk, but high reward. The CAR-T aspects are very confusing.
- Challenges in understanding the neuroimmune mechanisms underlying aberrant targeting of neuronal populations continue to limit therapeutic development. Although PNS is relatively rare, elucidating the mechanisms of neuronal MHC-I–based antigen presentation and developing therapies with cell-specific immune targeting could have broader implications for other neuroimmune-mediated autoimmune disorders, including multiple sclerosis, as well as neurotropic viral infections.
- This study could generate important mechanistic insight into how cytotoxic CD8+ T cells mediate neurodegeneration through emerging neuroimmune pathways. Given the growing evidence linking T cells to the pathogenesis of neurological disease, these findings could inform the development of targeted therapeutics and biomarkers relevant to neuroimmune disorders more broadly.
- These studies may provide important insights into the selective vulnerability of specific neuronal populations to distinct neuroinflammatory changes, an area that remains poorly understood in neurodegenerative disease. This could also improve understanding of how different neuronal populations respond to inflammatory or cellular stressors, potentially revealing mechanisms that mediate differential susceptibility to degeneration and neuroimmune injury across disease contexts.

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

- Coming into PNS laterally by killing off the Hu-presenting B cells is a cool, almost Judo-like move to destabilize the self-perpetuating inflammation and neuronal damage. Due to the rather unknown biology of the mechanism, this project could certainly fail.
- Identifying a mechanism for targeting paraneoplastic syndrome would be a valuable and innovative discovery effort.
- The integration of patient-derived iPSC-based neuronal models with autologous immune cell systems enables the investigation of neuroimmune interactions in a physiologically relevant context. This combined stem cell and immunology approach is particularly innovative in its ability to distinguish neuron-intrinsic versus -extrinsic mechanisms driving immune-mediated pathology.
- The proposal employs innovative genetic and immunopeptidomics approaches, including mechanisms enabling unbiased detection of naturally processed immunopeptides. These studies could provide important new insights into antigen presentation pathways and immune recognition mechanisms relevant to neurodegenerative diseases.

**Rationale: Evaluate the scientific rationale in the proposal**

- Humans are risk averse in suboptimal ways, a well-replicated psychological effect, which has documented effects on NIH (and probably CIRM) reviews, as people simply do not weigh upsides adequately vs downsides. In this case, there are minimal preliminary data in support of pushing PNS into a CAR-T engineering framework that is very malleable and generally useful. If the B-cells can be targets, that's huge. This seems like a plausible approach that would have a big practical effect if demonstrated.
- Strengths include unmet need. However, it is unclear why the applicant focuses on B cells and not other antigen presenting cells?
- The project is grounded in a strong scientific rationale supported by the investigators' expertise and a growing body of literature implicating neuroimmune interactions and antigen-specific immune targeting in neurological disease. However, the proposal remains highly exploratory, with no preliminary data provided to support feasibility. Given the rarity of the condition under investigation, the studies represent high-risk but potentially high-impact efforts.
- While the overall approach is compelling, the aims are highly interdependent. As noted in the proposal, "These findings [Aim 1 and 2] will directly inform mechanistic studies in Aim 3 and enable rational design of antigen-specific immunotherapeutic strategies in Aim 4." This raises concern that failure of earlier discovery-based approaches could substantially limit the feasibility or interpretability of downstream aims.

**Plan & Design: Evaluate the project plan and design**

- Either this will work or it won't. Most projects don't have such a binary outcome, but the value and possibility of 100% success in this project is actually higher than multi-faceted studies of comparatively more incremental improvements to our knowledge. Basically the odds and payoff warrant funding.
- Strengths include unmet need and strong approaches in first 3 aims. It's unclear if this approach could adversely affect immunologic tumor control. It is also unclear why the project utilizes so much effort to specifically eliminate these B cells. Couldn't the same be achieved with CD19 CAR T cells?
- The project is designed to yield potentially meaningful mechanistic and translational insights into neuroimmune targeting of neuronal populations, with the possibility of identifying therapeutically relevant targets for a currently untreatable, though rare, neurological condition.
- The studies are ambitious and high-risk, and feasibility remains a significant consideration.

| **Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations** |
| --- |
| • The applicant is using an all-human iPSC setting. There is mention of drawing these lines from diverse populations.<br>• More detail should be included on the exact breakdown of lines to “prove” they’re really going to be used.<br>• The project considers the potential impact of successful outcomes across affected populations.<br>• Although the condition under study is rare, the proposed approaches may provide important proof-of-principle insight into mechanisms underlying more common autoimmune-mediated neurodegenerative and neuroimmune disorders. As such, the potential applicability of discoveries could extend beyond the specific proposed disease context. |

| Application# | DISC5-20198 |
|---|---|
| **Title**<br>(as written by the applicant) | Systematic Discovery of Next-Generation Reprogramming Factors |
| **Project Objective & Impact**<br>(as written by the applicant) | Reprogramming adult cells into induced pluripotent stem cells has revolutionized regenerative medicine. But reprogramming remains slow, costly, and inefficient, limiting basic research and clinical translation. We will comprehensively evaluate natural and synthetic human transcription factors for reprogramming capacity, ultimately developing more simplified high-efficiency reprogramming cassettes. |
| **Specific Aims**<br>(as written by the applicant) | • Comprehensive Discovery of Natural Reprogramming Factors.<br>• Construction of Improved Synthetic Human Reprogramming Factors.<br>• Simplification and Dissemination of Improved Reprogramming Resources.<br>• Generalized Application of Natural and Synthetic Gene Discovery. |
| **Statement of Benefit to California**<br>(as written by the applicant) | The significance of improving reprogramming efficiency for Californians extends across multiple domains. For basic research, more efficient methods would enable routine generation of iPSCs from limited clinical samples, rare cell types, and from larger and more diverse patient populations. For therapeutic development, improved efficiency could reduce manufacturing costs and timelines while potentially improving product consistency. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

### Final Score: 80

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

| | |
|---|---|
| **Mean** | 79 |
| **Median** | 80 |
| **Standard Deviation** | 3 |
| **Highest** | 80 |
| **Lowest** | 70 |
| **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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

**Key Strengths and Weaknesses**

- Highly involved and innovative proposal, with little fear and lots of confidence in the capacity of the labs. Excellent track records in this area.
- Screen and design will be valuable for the wider field of transcription factor-based reprogramming.
- Good research group.
- Prioritization, features, and data collection from this screen should be laid out, as it is more likely the experiments will generate a large variety of data. The current pitfalls discuss failures, but it is likely the numerous successful combinations will be difficult or impossible to manage in the time frame of this grant as many of them are interdependent or will provide results to design the next screen.
- The design of the screen in stem cells is not well considered when compared to their T cell work.
- The specific targets for improvement here and for the generation of iPSCs are not as convincing.
- Validation of final cocktails and in-depth characterization of lines would be needed.
- Results for TF minimization come from T-cell CAR development. No data are provided to show similar concepts in reprogramming of iPSCs to this scale proposed. For Aim 2, cloning has not been done. The applicants show capacity and expertise to do so, but no evidence that this strategy is sound in iPSC generation. More preliminary data core to the proposal are required.

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

- Reprogramming via transcription factors is hugely impactful for regenerative medicine. Expanding the toolbox beyond the known factors would clearly be significant. Aim 1 and 2 would attempt to find these, while Aim 3 would make them easily accessible. Aim 4 is somewhat separate and attempts to use the screening capability towards factors for partial (therapeutic) reprogramming.
- Very significant if it works.
- Rapid control of cell fate with the proposed strategy could unlock new cell and gene therapies.
- The first screens hold the potential to have important improvements for the study of stem cells in terms of tools. As little molecular analysis beyond the screen is proposed, any additional impact would be in the future, and not proposed here. The second portion using partial reprogramming could have applications to the hematopoietic tissue and related pathologies as well as other tissues where the paradigm could be applied, but these are not proposed or discussed.
- Few mechanistic studies are proposed or discussed, but the findings could have implications for the field once reported/published and validated.

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

- The proposal has implications to all levels and directions of regenerative medicine based on iPSCs and the episomal single plasmid as well as minimal TF goals hold the potential to improve iPSC generation.
- The CRISPR-ALL and screens are very inspired, as well as the ideas of using TF motifs and combinations that have yet to be explored. The techniques and approaches themselves, along with the investigators are unique in this idea and approach, and unlikely to be attempted elsewhere.
- The screening strategy is unique and novel.
- The screens and vector systems are clearly innovative and the synthetic TFs (and insights gained) in Aim 2 can be quite powerful in other contexts.
- The track record, expertise, and background of the investigators are outstanding and the team is well positioned.
- Very innovative, but risky; their screening program may come up with nothing.

**Rationale: Evaluate the scientific rationale in the proposal**

- Whether the reprogramming efficiency is truly the major concern is debatable. That said, whatever the motivation, the search for other factors or the general rules (and specific domains) is valuable and logical.
- It is unclear or debated at least, that the “bottleneck” of iPSC applications is the lower frequency at 0.01-0.1% as cited from a 2010 paper. Other methods such as pre-selection of subsets vulnerable to reprogramming such as the progenitors or stem cell fractions are not incorporated here, along with the challenges of characterizing large numbers of iPSC candidate colonies initially generated.
- The first three aims are discovery-oriented, but the metric for success in the screen may not be entirely relevant to the biology.
- The rationale to investigate other reprogramming factors has merit, e.g., redundancy of Nanog and removal of Myc, however the idea that other TFs can substitute and combinations are limited is somewhat unclear or unfounded. This is an interesting idea, but evidence beyond T-cell findings from the applicants is absent.
- Some of the targets for improvement are not clear and whether these are then also truly "fully" reprogrammed stable lines is not described.
- As only quantity and not quality is assessed e.g., not differentiation potential, it is unclear if the goal is worthy of such massive efforts.

**Plan & Design: Evaluate the project plan and design**

- The screens in Aim 1 and 2 are well designed and the team appears to have the required setup. Aim 3 is largely designed for the community transfer and clearly an asset if Aim 1 and 2 produce relevant factors. Aim 4 is a bit more speculative and whether this is now finding factors similar to the many partial reprogramming studies or factors that specifically convert cells to CLPs is not entirely clear.
- Core to this proposal is the use of T-cells for improved CAR-T therapy as evidenced by two unpublished papers, references 6 and 7. This includes the use of the CRISPR-ALL platform, and results to show use and TF minimization for use in T-cell CAR development and that new TF function can be discovered. However, no data is shown that this approach works for reprogramming of iPSCs to this scale as proposed.

- For Aim 2, the factors have yet to be cloned and this represents a considerable amount of work and testing, e.g., are these lethal or oncogenic. Despite the applicants' capacity and expertise, no evidence that this strategy is applicable to iPSC generation is provided.

- The decision-making based on the results from so many combinations and TFs being tested is unclear. Will stats or mathematical tests be applied and how this is going to be decided or narrowed down for the next sequential steps of the study? This is poorly discussed and in some cases absent. Prioritization and features most important for iPSC generation, along with data collection from this screen should be laid out as it is likely that TFs, natural or otherwise will have many biological effects. How will this be interpreted?

- The current pitfalls discuss failures, but it is likely the numerous successful combinations will be difficult or impossible to manage in the time frame of this grant as many of them are interdependent or will provide results used to design the next screen.

- Aim 3 seems premature, and the applicants seem to be preparing for phase 0 human-ready testing. No animal studies or functional differentiation is suggested to characterize the lines or their biological behavior. It is understandable this might be episomally expressed but the epigenetic landscape may lead to other vulnerabilities or alterations in the pluripotent setting that are ignored here. A disproportionate emphasis is placed on public sharing of these reagents, e.g., how, where, when, etc..

- Stage 2 assessment in Aim 2 may lead to blocked or preferential differentiation of lineages, in which case combinations need to be re-assessed. Although the frequency of iPSC generation may be increased, the quality and oncogenic properties of the resulting cells need to be evaluated. This includes somatic cancer formation that may be masked in pluripotent or early germ layer states.

- Aim 4 is unclear regarding the intended epigenetic shift; scATAC is not used for NK to common lymphoid progenitors (CLPs), and why not just HSCs vs CLP? No in vivo or co-culture support is used which is known to be important to CLP genesis.

- Very little mechanistic understanding or design to understand the biology at play is included, making the findings limited to discovery based observations via massive screening efforts.

- Very ambitious without enough attention to epigenomic stability and partial reprogramming.

- Over-optimistic aims for 3 years work.

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

- The grant is well designed to have maximal impact and community access to the generated insights and resources.

- This is well documented and considered in the core use of iPSC lines and novel reprogramming.

| Application# | DISC5-19717 |
|---|---|
| **Title**<br>(as written by the applicant) | Integrating cardiac organoids and in silico models to define the mechanistic roles of sex hormones in atrial fibrillation |
| **Project Objective & Impact**<br>(as written by the applicant) | The critical knowledge gap is the lack of a human-relevant, causal understanding of how sex hormones reprogram atrial remodeling and trigger formation in atrial fibrillation. The impact of this project is to identify mechanism-based interaction drivers that enable hormone-state-informed targets, biomarkers, and precision therapies. |
| **Specific Aims**<br>(as written by the applicant) | • Determine cell-autonomous (hiPSC-aCM and hiPSC-CF) responses to sex hormones and use mechanistic in silico modeling to identify causal mechanisms<br>• Determine how sex hormones reshape atrial myocyte-fibroblast interactions in organoids and identify interaction drivers using multiscale computational modeling. |
| **Statement of Benefit to California**<br>(as written by the applicant) | This project will benefit Californians by advancing human stem cell-based atrial organoid and computational platforms to uncover sex- and hormone-dependent mechanisms that drive atrial fibrillation, a major cause of stroke and heart failure. The resulting insights will support more precise risk stratification and therapy selection across California's diverse population, and enable scalable drug-response and cardiotoxicity testing in human tissues. |
| **Funds Requested** | $2,479,659 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

**Final Score: 80**

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

| | |
|---|---|
| **Mean** | 77 |
| **Median** | 80 |
| **Standard Deviation** | 5 |
| **Highest** | 85 |
| **Lowest** | 70 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 1 |
| **(1-84): Not recommended for funding** | 14 |

# FINAL COMMENTS

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

**Key Strengths and Weaknesses**

- This proposal has extensive supportive preliminary data and a clear plan/means to achieve the aims. There are no fatal flaws in this proposal.
- The proposal addresses an important and understudied problem in sex difference in atrial fibrillation but there are concerns about the ability of the iPSC-derived cells to recapitulate this disease in vitro.
- This proposal is reasonable, but has several flaws that decrease its potential impact:
    - the use of iPSC-derived CMs and FBs without demonstrating their maturity
    - the use of only one cell line in the organoid aim
    - apparently failing to match cells with ECM by sex
    - apparently failing to ensure the atrium used for ECM is the affected atrium of the patient
- The translator is really unique and could be awesome; how they will get to it is unclear.

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

- The project would provide insight into the role of sex and sex hormones in atrial fibrillation. This could guide development of novel treatments and patient-specific therapies for atrial fibrillation.
- The study would link inflammasome with electrophysiology in atrial cardiomyocytes and activation/signaling in cardiac fibroblasts.
- There are concerns that the iPSC organoid model does not recapitulate the sex and sex hormone effects on atrial physiology found in adult human hearts, which would reduce significance and impact.
- Atrial fibrillation is the most common arrhythmia and a major driver of stroke, heart failure, and mortality. The critical knowledge gap is the lack of a human-relevant model to understand how sex hormones reprogram atrial remodeling and trigger formation in AF. The impact of this project is to identify mechanism-based interaction drivers that enable hormone-state-informed targets, biomarkers, and precision therapies.
- The underlying hypothesis is that the inflammasome is uniquely positioned to connect inflammatory stress to both cardiomyocyte calcium handling/electrical instability and fibroblast pro-fibrotic signaling and does so in a sex-specific, hormone-dependent manner.
- Tested using hiPSC-derived atrial cardiomyocytes and fibroblasts (n=3 males / 3 females of differing genetic backgrounds) either alone or in a co-culture model on decellularized human atrial appendage ECM. Biologic data are primarily used to evaluate predictions from mechanistic multi-scale in silico models.
- Proposed creation of unique organoid to human translators whereby drug data generated in an organoid made of hiPSCs can be "translated" to its normal or diseased human atrial equivalent. Personalized medicine model and human drug discovery tool.

- If successful, this application would provide therapeutic targets not only for evaluating atrial fibrillation by sex but also potential new therapeutic targets able to be translated to human-based in vitro or rodent studies.

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

- The integration of human atrial organoid models with in silico electrophysiology and signaling models is innovative.
- The translator framework to map drug responses in the organoid model to humans is innovative.
- By themselves the organoid and model structures are fairly standard in the field, but the combination is potentially powerful.
- The project combines engineered human atrial organoids with computational modeling to study how sex hormones contribute to atrial fibrillation. It will establish a novel and more physiologically relevant setting compared to tradition cell model if successful.
- Use of both in vitro and in silico models to evaluate mechanisms and therapeutic targets of atrial fibrillation by sex, first in a cell autonomous manner and then in a co-culture model, is a strength.
- Use of genetic algorithms (model inversion) in hiPSC-derived human atrial cardiomyocytes is unique. It not only allows systemic variation of model parameters (sex, hormone exposure inflammatory stress) it allows simulation of parameters that can be targeted then in vitro such as ionic conductances and Ca-handling protein expression and function. The investigators have used models like these previously.
- If the organoid to human translator is successful, it would create a strong tool that likely would be widely used in academia and potentially industry. It is impossible in the current proposal to determine the degree to which this model would be available more broadly. It is not mentioned in data sharing and the required inputs, etc., are not elucidated to a degree that this can be evaluated.

**Rationale: Evaluate the scientific rationale in the proposal**

- The project is supported by sex differences in atrial fibrillation outcomes in patients.
- The proposal contains preliminary data demonstrating the ability to generate the organoids and models proposed.
- There are concerns about the relevance of the iPSC model system. The cardiomyocytes are immature with different ion channel expression and electrophysiological properties than adults. The cardiac fibroblasts are less prone to activation than adult primary fibroblasts.
- The proposal provided substantial preliminary data to support the central hypothesis that sex hormones may contribute to atrial fibrillation progression through coordinated effects on inflammation, fibroblast activation, and atrial electrophysiology. The integration of atrial cardiomyocytes and fibroblasts into the organoid platform may provide a biologically relevant system to study multicellular interactions that are difficult to capture in conventional 2D culture models.
- The rationale is strong - creating both in silico and in vitro models of human atrial fibrillation. Sex differences exist and the authors propose to evaluate the role of two specific cell types in these differences: atrial cardiomyocytes and atrial fibroblasts.
- By using hiPSCs from 3 males and 3 females of differing genetic backgrounds and then evaluating by sex, it is difficult to know if genetic effects will be seen, will dominate, or will overpower sex effects. Increasing

the n value would help address this. The investigators acknowledge a power analysis requiring n= 8 per group, which they will not have.

- Plans to validate the cell autonomous effects in Aim 1 rely on the iPSCs accurately reflecting in vivo cells. Until demonstrated, this cannot be assumed.
- Using n=1 hiPSC cell line in aim 2 greatly decreases the potential impact. How will the investigators choose which line to use? Based on what criteria and why?

**Plan & Design: Evaluate the project plan and design**

- The project is well-designed to assess effects of sex and hormone treatment on the cell systems. Experiments are clearly described with appropriate treatment, analysis, and interpretation.
- The integration of the in vitro organoids and in silico models is strong.
- Plans to validate mechanistic interpretations of the models in Aim 1 are insufficient, relying on pathway analysis rather than perturbation.
- There are concerns about the use of only one cell line of each sex in Aim 2. While these are complicated experiments, this does not provide sufficient power to draw conclusions regarding sex effects.
- The use of pharmacologic prediction and intervention in Aim 2 is a strength of the approach.
- The project lacks a validation of the identified mechanism in models that may better recapitulate adult atrial fibrillation.
- The experimental design is logical and well organized. The proposal first examines direct hormone effects in atrial cardiomyocytes and fibroblasts separately (Aim 1). The study then extends these findings into engineered human atrial organoids composed of hiPSC-aCMs and hiPSC-CFs under inflammatory and profibrotic stress conditions relevant to AF (Aim 2). They will also establish a computational framework linking organoid phenotypes with adult human atrial responses.
- A major concern is the use of immature iPSC-CMs and -Fbs that are known not to reflect adult cell phenotype typically in vitro. The investigators do not fully show their plan to mature the cells and that they can.
- The use of decellularized atrial appendage from atrial fibrillation patients is a strength. However, the investigators do not acknowledge or discuss that post surgical availability typically only applies to right atrial appendage and that may or may not be from an affected atrium.
- In the constructs utilized in Aim 2 there is no indication that sex/gender of the ECM will be matched to the cells nor is there any indication that cell phenotype alignment and maturation will be evaluated prior to the use as a disease model. How will atrial mechanics be modeled?

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

- The project effectively accounts for sex and age-related hormone status in atrial fibrillation.
- Partnership with American Heart Association programs at the applicant organization for outreach is a strength of the proposal.

- The proposal uses six age-matched male and female hiPSC donor lines spanning diverse ancestries, with standardized differentiation protocols and rigorous quality control to study hormone-dependent phenotypes.

- In Aim 1 the investigators will use three male and three female hiPSC lines of differing genetic backgrounds - one each of Caucasian, African American and Latino of each sex. By comparing then by sex the impact of genetics will likely be lost. That said, if a difference were seen there is no indication it would be evaluated or followed.

- In Aim 2, the investigators will only use one hiPSC line. it is not clear if it will be male or female, or its genetic background, or what the criteria are for choosing that line.

| Application# | DISC5-20237 |
|---|---|
| **Title**<br>(as written by the applicant) | Defining the Neural Stem Cell Nexus: A High-Resolution Proteomic Atlas of Convergent Dysfunction in Autism Spectrum Disorder |
| **Project Objective & Impact**<br>(as written by the applicant) | Autism spectrum disorder (ASD) research is limited by poor understanding of how diverse risk genes converge at the protein level in human neural stem cells (NSC). Our project defines NSC-specific interactomes, protein dysregulation, and functional consequences in human models, enabling mechanism-based target discovery and accelerating therapeutics for ASD and related neurodevelopmental disorders. |
| **Specific Aims**<br>(as written by the applicant) | • Map the wild-type and variant-associated NSC interactome of highly convergent ASD risk genes<br>• Define the impact of ASD variants on NSC proteomic landscape<br>• Identify the functional consequences of NSC Nexus perturbations in 2D and 3D human cortical models |
| **Statement of Benefit to California**<br>(as written by the applicant) | Autism affects 5.3% of California's children, exceeding the national average and creating a massive healthcare and economic burden. Our research targets the "NSC Nexus"—the protein machinery driving early brain development—to identify novel therapeutic targets. By translating genetic risk into precision interventions, this project aims to accelerate the delivery of regenerative therapies, ultimately improving quality of life for California's neurodiverse citizens and their families. |
| **Funds Requested** | $2,499,998 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

**Final Score: 80**

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

| | |
|---|---|
| **Mean** | 77 |
| **Median** | 80 |
| **Standard Deviation** | 6 |
| **Highest** | 80 |
| **Lowest** | 60 |
| **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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

**Key Strengths and Weaknesses**

- This proposal is technically sophisticated and supported by strong preliminary data, but concerns regarding scope, feasibility, reliance on a single iPSC background for key experiments, and the likelihood of achieving deep mechanistic insight across all aims resulted in a final score below the fundable range.
- There are not enough advances in this proposal, which is focused on a relatively small number of genes and one cell line.
- The rationale does not adequately consider progress from other groups, and some aims are not well developed.

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

- This project addresses a major gap in ND biology: how genetically diverse autism risk genes converge on shared molecular mechanisms in NSCs. By generating a high-resolution protein–protein interaction map in human iPSC-derived NSCs, the work has a strong potential to advance foundational understanding of how early stem cell dysfunction drives cortical development.
- The proposal introduces the concept of an "NSC Nexus," reframing autism pathogenesis as protein-level convergence within stem cell regulatory networks rather than gene-centric or transcriptomic effects. If validated, this could represent a meaningful, but rather incremental shift with implications across NDDs.
- A successful outcome will generate additional proteomics data that will further help in the interpretation of mechanisms underlying the function of 15 high confidence risk genes and variants.
- A list of the specific 15 genes that will be prioritized is not provided, and the advantages over existing proteomics datasets from iPSC-neural cells including NPCs and neurons (e.g., from PMID: 36950384, with 13 high-confidence ASD genes profiled by IP-MS, published 3 years ago) are not well articulated.
- The current application will investigate 15 variants in 5 genes, which will provide valuable information about which interactions are disrupted by specific genetic variants. There is also additional value from the functional characterization in 2D neurons and organoids. However, overall, the profiling of only 15 high-confidence genes and 15 variants in 5 of these genes is perhaps too incremental from a significance and impact perspective.
- The proposed study will generate large proteomic datasets for NSCs with ASD risk variants. The study will address whether NSCs are the converging point for key pathology in ASD. NSCs in early neurodevelopment may not be druggable targets.

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

- Multi-scale approach—from protein networks to tissue-level phenotypes—represents strong interdisciplinary synergy. A central innovation is the "NSC Nexus," which reframes autism as a disorder of protein-interaction network convergence in neural stem cells rather than gene-level or transcriptomic dysregulation.

- The application extends the application of an approach previously employed, at larger scale (100 genes and 54 variants), in HEK cells, to iPSC-based models. The addition of subcellular proteomics and post-translational modification analysis (in Aim 2) is novel in this context.
- The proposed study will test the hypothesis that NSCs represent an unrecognized key driver for ASD pathology in neurodevelopment. The hypothesis that genetic risk of ASD functionally converge on molecular pathways at PPI level. Both NSCs and brain organoids from iPSCs will be used.

**Rationale: Evaluate the scientific rationale in the proposal**

- The rationale is strong and well supported by previous work — autism risk genes converge at the level of protein-interaction networks. The project is well supported by preliminary data.
- The rationale is sound and compelling; however, the scale and breadth in light of existing data is limited. The experimental approach is justified, as the human iPSC progenitor cells, neurons, and organoids are more likely to recapitulate protein-protein interaction networks present in the human brains compared to HEK cells.
- Human iPSCs will be edited for selected genes. However, there is no clear description which genes will be selected. Preliminary data are provided to support that ASD risk genes are highly expressed in NSCs.

**Plan & Design: Evaluate the project plan and design**

- Design is well aligned with project aims. Key risks are identified and appropriate mitigating strategies described. The project is ambitious, raising concern that the work could become largely descriptive.
- Team is a major strength, with exceptional expertise in proteomics and neuropsychiatric genetics, and previous collaborative track record. Working of the team is well thought through.
- Only one iPSC line will be used which is one of the major weaknesses.
- A list of the specific 15 genes that will be prioritized is not provided.
- The data generated will likely provide mechanistic insight into the protein level function of the prioritized genes in human neural cells. However, because a list of the specific genes is not provided, it is not possible to evaluate this appropriately.
- There is no specific plan to integrate or compare to other datasets.
- It is unclear how the 2D and organoid functional data will be compared or co-analyzed.
- The leadership, integration, resources and staffing are appropriate.
- The team communication and management plan are effective.
- NSCs and brain organoids will be used for functional testing. This aim appears to be less developed.

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

- There is limited consideration of population-level variability. The applicants aim to identify shared molecular mechanisms across genetically diverse autism cases, potentially increasing generalizability. There is good engagement with patient and advocacy groups.

- Only one iPSC line will be used. Validation of key results in additional lines would have added more confidence in the results.
- The outreach and educational efforts plans are appropriate.
- ASD has higher prevalence in California, and effective treatments are still lacking.
- It seems that only one founder line will be used.
- Both PIs show clear commitment to patient advocacy and engagement.

| Application# | DISC5-19569 |
|---|---|
| **Title**<br>(as written by the applicant) | When “young” cells meet old tissues: mechanistic impact of host senescence on human iPSC-derived musculoskeletal tissues |
| **Project Objective & Impact**<br>(as written by the applicant) | Stem cell therapies hold enormous promise, yet aging tissues often block their success. This project uncovers how aging environments influence human stem-cell repair and reveals new biological insights that could unlock more durable regeneration, paving the way for more effective treatments for age-related musculoskeletal disease. |
| **Specific Aims**<br>(as written by the applicant) | • Define the impact of SASP on the viability, phenotype, function, and senescence of human iPSC-derived MSK cells in vitro.<br>• Determine how senescence-rich host environment impair human iPSC-derived implants in vivo and if removal of senescence cells can reverse this effect<br>• Integrate in vitro and in vivo datasets to identify SASP-implant interaction mechanisms and predictive biomarkers |
| **Statement of Benefit to California**<br>(as written by the applicant) | California’s population aged 65+ is projected to exceed 8.6 million by 2030, and more than 50% of older adults experience chronic musculoskeletal conditions that limit mobility and quality of life. By revealing why regenerative therapies may fail in aging tissues, this research can enable more effective stem cell–based treatments, reduce healthcare costs, and benefit millions of Californians. |
| **Funds Requested** | $2,499,998 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

**Final Score: 75**

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

| | |
|---|---|
| **Mean** | 76 |
| **Median** | 75 |
| **Standard Deviation** | 4 |
| **Highest** | 82 |
| **Lowest** | 65 |
| **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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

**Key Strengths and Weaknesses**

- Strong team. Very important question with major practical implications.

Senolytics may be too crude and more refined understanding of the signals would be desirable.

Aim 3 too short and not convincing.

- In general, this proposal is seen as preliminary with high potential in future submissions.
- This project has potential significance but needs refinement to achieve the stated aims.

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

- The project addresses a very significant issue in regenerative biology. Step 1 in stem cell-based regeneration is the generation of target cell types, which is now widely accomplished, including for the various cell types on the proposal. Step 2 requires the transplantation and successful engraftment. Here "young" cells are exposed to "older" or "injured" environments which may impair the success of tissue repair.
- The proposed project will clarify the impact of senescent cells and explore solutions with senolytics as well as the identification of biomarkers.
- This project investigates the effects of using young stem cells on older individuals. Specifically, the project examines how the senescent host environment influences iPS-derived musculoskeletal cells. The team is outstanding and has the expertise required to develop a high-impact project.
- This project is among the first to investigate how aging affects skeletal muscle using iPS-derived musculoskeletal cells as a potential therapy. In particular, it investigates the paracrine effect of senescent cells on recently implanted cells. Preliminary data suggest that, in murine models, aging negatively impacts Achilles tendon repair, and this can be improved with senolytic therapy, suggesting that senescent cells impair the regeneration of musculoskeletal tissue after injury.
- As the population ages, there will be an increased need for musculoskeletal (MSK) tissue regeneration strategies aimed at return to function. This proposal addresses a key issue, that current iPSC-based MSK tissue repair is not robustly successful in older patients.

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

- The proposal spans a number of innovative in vitro and in vivo approaches and dissects a relevant question in a very systematic way. The results will clearly provide insights of value to the specific application but also more generally for transplantation of regenerative cells.

- The concept and use of conditioned media from senescent cells have been employed to demonstrate the paracrine effect of senescent cells on neighboring cells, including local stem cells, which is not very novel. The novelty lies in the effect on iPS-derived cells, making this a moderately novel proposal.
- By focusing on the potential of the biology/physiology of the host to affect MSK iPSC implant-based regeneration success, the project expands current knowledge.

**Rationale: Evaluate the scientific rationale in the proposal**

- The rationale is very sound and the objectives clearly address a problem in the field.
- There is increasing evidence that instead of being a “zombie cell,” senescent cells are active participants in tissue degeneration. They secrete inflammatory cytokines, chemokines, proteases, and growth factors known as the senescence-associated phenotype (SASP). This secretion can modify the extracellular matrix, disrupt immune function, and ultimately impair regenerative capacity.
- Focusing on the implant environment in aging patients and understanding how this environment affects the success of MSK regeneration is valuable.
- As the environment encompasses both the local site of implantation and the signals present in circulation, more emphasis needs to be placed on how the compartments interact to affect success of repair.
- There are panels of SASP biomarkers already identified; a stronger rationale is needed for why additional biomarkers are useful.

**Plan & Design: Evaluate the project plan and design**

- There are some concerns about the models, and Aim 3 is too short and vague.
- Aim 1 is simple and descriptive. Aim 2 will generate the appropriate preliminary data for this type of proposal. Aim 3 is very preliminary and poorly developed.
- The team has demonstrated success in producing iPSC MSK tissue implants and in using many sophisticated methodologies to examine iPSC mediated repair of MSK.
- For bone regeneration in aging, the use of a calvarial defect model is not well justified as the skull does not lose bone mass with age so the environment will not mimic that of osteoporotic fracture.
- Aim 3 is scant on specifics making it hard to determine if useful information will come out of successful completion.

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

- Results are expected to be generally applicable.
- Musculoskeletal degeneration disproportionately affects aging populations and is a leading cause of disability among California residents. Degenerative conditions of tendons and cartilage are driven by biological aging, injury history, and environmental exposures. The project includes outreach presentations to high schools and community colleges, collaboration with orthopedic surgical teams, and community outreach and engagement.
- Broad populations are considered.

| Application# | DISC5-19787 |
|---|---|
| **Title**<br>(as written by the applicant) | Unlocking the Brain's Hidden Immune Regulatory Cells: A New Path to Alzheimer's and Parkinson's Cures |
| **Project Objective & Impact**<br>(as written by the applicant) | Bottlenecks: (1) mouse models of neurodegeneration lack human-specific biology, causing translation failure; (2) Stem cell-based cultures miss critical brain-immune interactions; and (3) oligodendroglia dysfunction is overlooked in neurodegeneration research. Our project uses a novel humanized mouse model to decode oligodendroglial dysfunction and uncover untapped therapeutic targets. |
| **Specific Aims**<br>(as written by the applicant) | • Aim 1. Construct the first time-resolved map of human oligodendroglial state transition in Alzheimer's disease and Parkinson's disease<br>• Aim 2. Define the causal role of SERPINA3 and MHCI signaling in oligodendroglia-driven neuroinflammation<br>• Aim 3. Investigate how Alzheimer's disease and Parkinson's disease-risk genes act within oligodendroglia to accelerate pathology |
| **Statement of Benefit to California**<br>(as written by the applicant) | Alzheimer's and Parkinson's diseases affect over 800,000 Californians, costing $51+ billion annually. Our humanized mouse model—using human stem cell-derived glia and immune cells—will reveal oligodendroglia-targeted therapies, an unexplored frontier with high translational potential. This research will accelerate drug development, establish California as the global leader in next-generation disease modeling, attract biotech investment, and deliver novel treatments for devastating diseases. |
| **Funds Requested** | $2,498,937 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 75

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

| | |
|---|---|
| **Mean** | 74 |
| **Median** | 75 |
| **Standard Deviation** | 7 |
| **Highest** | 85 |
| **Lowest** | 65 |
| **Count** | 14 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 1 |
| **(1-84): Not recommended for funding** | 13 |

# FINAL COMMENTS

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

**Key Strengths and Weaknesses**

- Overall, this is a scientifically compelling and potentially high-impact proposal that addresses an important unmet need through innovative modeling of oligodendroglia-mediated human neuroimmune interactions in neurodegeneration. Despite concerns regarding scope, model complexity, and variability in humanized systems, it is supported by preliminary data and has the potential to generate important mechanistic and translational insights.
- A strong team and a genuinely valuable humanized in vivo platform are paired with an oversold hypothesis, an experimental design whose directionality is baked in such that the central causal claim cannot be tested and an immune-readout vulnerability in the xenogeneic model that is left entirely unaddressed. On balance the weaknesses outweigh the strengths, but the platform and team keep the application clearly in revisable territory.
- There are a number of problematic flaws reflecting an inadequate understanding of the limitations in the model, the differentiation of the transplanted cells into astrocytes as well as oligodendrocyte lineage cells, how to deal with variability between mice, interactions with the murine innate immune system, and what one would do next even if experimental outcomes were favorable.

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

- This proposal addresses a critical gap in knowledge regarding the role and contribution of oligodendroglial lineage cells to disease etiology and progression, and how disease-relevant mutations impact their functions in vivo.
- Better understanding the role of oligodendrocyte lineage cells in immune system cross talk in neurodegenerative diseases such as Alzheimer's and Parkinson's may shed new light on useful interventions in the disease process.
- This work could establish a new conceptual framework for how oligodendrocytes and OPCs mediate immune cell recruitment and neuroinflammatory changes relevant across multiple neurodegenerative conditions.
- There is strong potential for novel mechanistic discoveries related to early oligodendroglial alterations that could serve as biomarkers of early disease or identify new druggable therapeutic targets.
- The proposal is pitched as fundamentally reframing AD/PD pathogenesis by repositioning oligodendrocytes as primary disease drivers, but the cited literature itself shows most of the conceptual scaffold is already in place; the true significance lies in the platform, not the hypothesis.
- The "graveyard of failed clinical trials" framing is essentially true for any disease and adds little justificatory weight.
- The framing of this as a stem cell grant on the grounds that no good stem cell models exist is not the strongest argument for the mechanism.

- The scope spanning multiple diseases and mechanisms increases the potential impact and broad applicability of the findings, though the overall approach may be somewhat too ambitious.

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

- This project will utilize a highly innovative chimeric mouse model integrating donor-matched humanized immune and glial systems to enable investigation of human cell–cell interactions in vivo, representing a unique convergence of stem cell biology, neuroimmunology, and translational disease modeling.
- Novel model.
- The innovation lies in combining two types of mice, one in which the mouse immune system has been replaced with a human immune system and one in which human glial progenitor cells are used to populate the central nervous system.
- The animal model and the proposed perturbation of 10 oligodendroglia-enriched risk genes is a genuinely innovative experimental approach.
- The platform has strong potential to support future early-stage clinical translation by enabling testing of human-relevant disease mechanisms and therapeutic hypotheses in a preclinical setting, and could provide a valuable screening platform for evaluating candidate pathways, biomarkers, or therapeutic strategies in models that more closely recapitulate human biology.
- The genuine innovation is the glia-immune humanized mouse platform itself: a humanized in vivo system that mechanistically tests human cells, with donor-matched human glia and a human immune system reconstituted in a single animal.
- This work leverages the advantages of intact in vivo systems, including the blood–brain barrier, lymphatic pathways, and immune infiltration, while incorporating human-relevant cell populations to better interrogate mechanisms underlying disease pathology.
- It has long been known that myelin damage occurs early in Alzheimer's disease and that oligodendrocytes are active participants in crosstalk with the immune system.
- The hypothesis being tested with that platform is less innovative than advertised, since most of the conceptual scaffold is already in the literature.

**Rationale: Evaluate the scientific rationale in the proposal**

- The project is highly innovative and supported by compelling proof-of-concept preliminary data using a newly developed human chimeric rodent model incorporating human immune and glial populations. The overall scientific rationale is strong and addresses an important unmet need in modeling human neuroimmune interactions in neurodegeneration.
- Preliminary data demonstrate successful engraftment of both immune and glial populations, supporting the technical feasibility of the proposed approach. Studies provide evidence that the model can capture human oligodendroglial responses, including upregulation of interferon-induced MX1 in an unrelated HIV model. However, it remains unclear whether similar responses will be observed in the proposed AD and PD paradigms.
- The proposed experimental systems allow temporal assessment of disease-associated cellular and inflammatory changes, increasing the likelihood of identifying dynamic mechanisms relevant to disease initiation and progression.
- The inclusion of both 5XFAD and α-synuclein pre-formed fibril models broadens the potential impact of the work and may enable identification of convergent mechanisms across neurodegenerative diseases, though

the overall scope may be somewhat ambitious. Both models have limitations that may restrict their ability to fully recapitulate age-related human disease, making them better suited for mechanistic interrogation of pathology.

- One problem with the rationale is that the glial precursor cells only provide variable and incomplete repopulation, which means comparison between mice is more challenging and interpretation of data is more difficult. The incomplete repopulation means that there is cross talk going on between murine and human glial cells, with great potential for species-specific cytokine mismatch (let alone the cross-talk of human cells with other types of murine cells).
- The idea that the transplanted cells only replace oligodendrocytes is flawed as these transplant cells also generate astrocytes (which partake in many aspects of cross-talk with the immune system).
- The immune system is a hybrid one in which the murine innate immune cells (which express disease-causing genes in these models) remain in place. Those innate immune cells would be expected to include myeloid populations that populate the CNS to make microglia during early development.
- The central scientific problem is cause and effect: in both the AD model and the PD model, the disease driver sits in the neuronal/extracellular compartment and human oligodendrocytes are downstream responders, so the design structurally cannot test the central claim that they are initiating drivers; this directionality is baked in.
- A much more powerful design would put the disease lesion into the oligodendrocytes themselves (e.g., iPSC-derived OPCs from LRRK2, GBA, or familial AD donors) and ask whether pathology propagates into otherwise healthy neurons; Aim 3 gestures at this, but the "sensitized background" framing in the pitfalls uses neuronal pathology to amplify weak oligodendroglial phenotypes, which inverts the causal claim.
- The mechanistic rationale for the SERPINA3/MHC-I arm is thinner than presented: MHC-I antigen presentation by oligodendrocytes comes mostly from demyelination studies rather than AD/PD, SERPINA3-as-granzyme-B-inhibitor evidence comes from the vascular system, and the key B2M reference in PD is 30 years old on bulk striatal tissue.
- It is unclear how the continued presence of endogenous mouse glial populations or interactions with mouse neurons may influence or confound interpretation of human cell–specific mechanisms. While alternative strategies are discussed, the extent to which experimental depletion or knockout approaches could alter engraftment efficiency or cellular behavior remains insufficiently addressed.
- It is unclear how differences in engraftment efficiency across different donors and genetic backgrounds may influence the interpretation of mechanistic or behavioral outcomes, particularly if variability is substantial between animals.

**Plan & Design: Evaluate the project plan and design**

- The overall approach is innovative and thoughtfully designed to yield meaningful mechanistic insights into neuroimmune and glial contributions to neurodegenerative disease. While the inclusion of both AD and PD models increases the ambition of the project, the rationale for identifying convergent disease mechanisms is well justified and could substantially broaden the impact of the findings.
- The proposal demonstrates careful consideration of biological complexity through the use of knock-in versus knockout strategies tailored to the type of allele being modeled, rather than relying exclusively on loss-of-function approaches. This strengthens the biological relevance of the experimental design and enables investigation of more fundamental mechanistic questions.
- The glial precursor cells transplanted do not express the disease-exacerbating genes, and thus the core hypothesis is not being tested because disease processes will start in the mouse cells.

- There are no experiments that differentiate between early changes in oligodendrocyte lineage cells that represent bystander effects due to changes in other murine cells.
- There are no indications as to what outcomes would confirm the core hypothesis, or what one would do next with such information.
- There is no consideration given as to whether disease-causing genes in the murine cells would alter transplantation outcomes (for example, pushing glial precursor cells into reactive astrocytes due to pre-morbid disease processes and/or inhibiting oligodendrocyte generation and differentiation).
- The single biggest unaddressed risk is the authenticity of the xenogeneic model for the immune readouts: the HSC-only NSG/5xFAD TKO host has no human thymic tissue, so human T cells are educated on mouse thymic epithelium and mouse MHC, producing well-documented distortions (skewed TCR repertoire, abnormal CD4/CD8 selection, poor antigen-specific responses) that directly undermine the MHC-I/B2M arm in Aim 2 and the field has moved to other NSG variants precisely for this reason.
- Combined with the cause-and-effect problem, Aim 2 is the riskiest aim: if effects don't appear, it will be hard to disentangle whether the hypothesis is wrong from whether the humanized T cells in this model can do physiologic antigen-specific recognition of human MHC-I at all. Cytokine cross-reactivity issues (IL-7, GM-CSF, M-CSF, IL-15) compound this.
- The scope is unrealistic: two disease models, multiple time points, snRNA-seq, IHC, flow, Luminex, EM, and behavior, plus two CRISPR knockouts in Aim 2 and 10 risk gene perturbations in Aim 3, all constrained by donor matching; this is worth multiple grants compressed into a single application and needs more realistic prioritization.
- Over ambitious for 3 years.
- Potential pitfalls are generally well recognized, and the proposal includes reasonable alternative approaches. However, variability in engraftment efficiency, disease heterogeneity, and the complexity of humanized in vivo systems may still present challenges for the interpretation of experimental outcomes.

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

- The diseases of interest cross ethnic boundaries.
- As the transplanted cells do not express disease-causing genes, obtaining them from different ethnic groups makes little difference.
- Fetal tissue is donated through a named institution and is claimed to reflect a diverse background, but no further documentation is provided.
- Partnerships with patient and community organizations are mentioned, but nothing is specifically stated about training in inclusive research and education.
- Research outreach is claimed but nothing is specified in particular.
- The proposal does an excellent job of incorporating genetic and cellular factors into the experimental design to study mechanisms relevant to neurodegeneration. The approach is structured to capture effects across multiple cell types, aging states, and disease-related conditions.
- Use of patient-derived samples strengthens the ability to address genetic heterogeneity and increases the translational relevance of the proposed studies, while complementary investigation in intact in vivo systems enables assessment of complex multicellular and environmental interactions.

- Validation of discoveries in human patient tissue further strengthens the likelihood that the identified mechanisms are biologically meaningful and relevant to human disease populations.

| Application# | DISC5-19580 |
|---|---|
| **Title**<br>(as written by the applicant) | Population-wide deimmunization of gene therapy cargos |
| **Project Objective & Impact**<br>(as written by the applicant) | We are addressing the immunogenicity of gene therapy cargos, a fundamental challenge that often causes efficacy and safety issues. If successful, we will immediately improve the genetic modification of immune cells against cancers, as well as provide a platform for comprehensively deimmunizing therapeutic cargos against an immune-diverse population. |
| **Specific Aims**<br>(as written by the applicant) | • Develop a computational platform to deimmunize any gene therapy cargos while maintaining their function.<br>• Establish MHC class I and II antigen screen platforms to examine population-wide immunogenicity of therapeutic cargos.<br>• Integrate the experimental and computational platforms to deimmunize binders for CAR-T therapies. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Patients everywhere including those in California with numerous diseases could benefit from gene therapies, yet a fundamental limitation is that the proteins produced from gene therapies often trigger immune responses, which reduces the efficacy of the therapies or even cause severe side effects. Our work will provide the first platform that makes it possible to minimize such immunogenicity and help deliver the promise of gene therapy for Californians. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

**Final Score: 75**

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

| | |
|---|---|
| **Mean** | 73 |
| **Median** | 75 |
| **Standard Deviation** | 3 |
| **Highest** | 75 |
| **Lowest** | 65 |
| **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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

**Key Strengths and Weaknesses**

- This proposal is innovative in that it plans to systematically profile both HLA class I and class II antigen presentation across diverse HLA backgrounds to attempt a larger-scale population-scale immunogenicity assessment.
- This proposal addresses a potentially quite interesting and important problem of immunogenicity of protein therapeutics while taking into account a wide range of HLA molecules. However, the proposed focus on CD19 CAR-T cells is suboptimal, as the unmet need is less clear here than for other potential applications.
- Immunogenicity is a concern.
- Major concern: the problem of immunogenicity in the context of CAR-T cells is a bit overstated (both antidrug and T-cell-mediated immunity) as it has not appeared to affect clinical outcomes in CD19 (or other) CAR-T cells with the scFv the applicants use as proof of principle; thus, even if addressed, this would not necessarily reveal a new paradigm and one-step improvement in CAR-T efficacy, given that efficacy and persistence are multifactorial.
- As the investigators point out, not every presented antigen is immunogenic and it is possible that the ESCAPE-identified peptides may fail to elicit T-cell responses in vitro, and may not be reflective of human dynamics.

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

- The proposed work addresses an important barrier in regenerative medicine and gene therapy: immune recognition of non-human or engineered therapeutic proteins. A successful outcome could advance foundational knowledge by providing a framework to map how therapeutic cargo peptides are presented across diverse HLA backgrounds and by defining sequence features associated with population-wide immunogenicity.
- A major strength is the potential generalizability of the platform. The proposal aims to establish tools applicable to antibody fragments, CRISPR-related proteins, AI-designed binders, and other non-human cargos. This platform could have broad implications for the development of durable cell and gene therapies.
- The proposal focuses on the important problem of immunogenicity of therapeutic protein cargoes, which is applicable to multiple types of therapies from CAR-T cells to gene/stem cell therapies. To address this problem, the applicants aim to establish a novel computational algorithm to "deimmunize" proteins by removing potentially immunogenic sequences from the cargos while maintaining their functions.
- The applicant is using AI protein design and synthetic biology to create a deimmunization toolkit. The toolkit would apply to any non-human cargos (from antibody fragments to base editors to de novo binders). They are creating this toolkit with broad HLA types in mind (relevant to diverse human populations). The approach could allow safer, more effective gene therapy delivery.
- One weakness is that the significance of reducing immunogenicity in anti-CD19 CAR-T cells may be overstated. Although [redacted Ab] immunogenicity has been reported, the proposal does not fully reconcile this rationale with clinical observations of long-term CAR-T cell persistence in some patients,

including reports of persistence for many years. CD19 CAR-T cells induce profound B-cell aplasia and the likelihood of a sustained anti-CAR B-cell response is low.

- The chosen example of deimmunizing CAR-T cells is not ideal since the mouse scFvs used for CARs can readily be humanized or made fully human so they are not immunogenic. Immunogenicity of CRISPR editors with repeated dosing and of AI-generated proteins may be a more significant unmet need to address. Similarly, anti-drug antibody responses against protein replacement therapies or biologic therapeutics are appealing. Thus, the significance of the immunogenicity problem for CAR-T is unclear.

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

- This proposal is innovative in that it plans to systematically profile both HLA class I and class II antigen presentation across diverse HLA backgrounds to attempt a larger-scale population-scale immunogenicity assessment.
- Developing a MHC-II-focused library for high-throughput evaluation of class II presentation and CD4 T cell recognition would be a significant innovation.
- The preliminary MHC-I ESCAPE-seq platform appears powerful, with the ability to profile tens of thousands of peptide-HLA combinations and benchmark against mass spectrometry immunopeptidome datasets.
- The immunopeptidomics approaches and combination of computational and experimental work are innovative.
- The proposal is not particularly innovative.

**Rationale: Evaluate the scientific rationale in the proposal**

- Overall, the scientific rationale for this technology is strong. Therapeutic cargo immunogenicity driven in part by MHC-I and MHC-II presentation can be predicted and proteins can be modified to reduce immunogenicity while maintaining structural properties and function.
- The rationale of attempting to better understand what makes therapeutic cargos immunogenic across diverse populations and how this knowledge can be practically applied has a strong scientific basis.
- The proposal is well supported for the MHC-I and computational components. The applicants present preliminary data that ESCAPE-seq can identify MHC-I-presented peptides across multiple alleles and that ProVADA can substantially reduce predicted MHC-I presentation in TEV protease and [redacted Ab] while largely preserving predicted structure.
- The rationale is weaker for MHC-II ESCAPE-seq feasibility. The proposal describes an elaborate system involving invariant chain fusion, cathepsin processing, CLIP displacement, HLA-DM-mediated loading, and FLAG-based surface detection, but does not provide direct preliminary data demonstrating that the system works for even a small set of MHC-II alleles or known binders.
- As described above for significance, the rationale for focusing on immunogenicity of CAR-T cells is not particularly compelling, and there may be other bigger areas with unmet need to address immunogenicity.
- Missed opportunity to focus on other applications that could be more generalizable and relevant, including those the investigators include in the rationale for this work (repeat dosing gene editing, other AI-designed cargo, etc).
- There is also a lack of consideration of immunogenicity that may be T-cell-independent (antibody responses). This should be discussed.

| **Plan & Design: Evaluate the project plan and design** |
| --- |
| • Experienced team with capabilities to carry out plan. The applicant lays out how they will develop a computational platform to deimmunize CAR-T cargos while maintaining their function.<br><br>• The plan to validate ESCAPE-identified epitopes using HLA-matched PBMCs and T-cell stimulation assays strengthens the biological relevance of the work, given that investigators have access to those samples.<br><br>• The project depends heavily on successful completion of the MHC-II platform. The MHC-II assay is central to the claim of reducing humoral immunogenicity, but the lack of feasibility data creates a significant execution risk. The proposed alternatives are not fully sufficient because they primarily adjust biological processing conditions rather than offering an independent, validated backup method for scalable MHC-II epitope mapping. If the initial tethering to Ii does not work, the aim fails.<br><br>• The team has appropriate expertise but may benefit from additional cell therapy expertise. There is one line added at the end of preliminary data for Aim 3 that suggests experience with CD19 CAR-T cells, but feasibility is not demonstrated. |
| **Population Impact: Evaluate the extent to which the project considers the potential impact of successful outcomes across affected populations** |
| • The proposal addresses HLA diversity, intentionally focusing on more than HLA-A*02:01. The plan to screen 30 common class I alleles and 20 common class II alleles, with stated broad global population coverage, is well aligned with the goal of improving relevance across genetically diverse populations.<br><br>• The applicants discuss how analysis of large-scale biobank data (about 1 million people in total) will include cohorts that have ethnic and socioeconomic diversity both within and outside California and the US, also taking into account age, sex, and socioeconomic status, with additional germline genetic data from which genetic ancestry can be inferred.<br><br>• Missed opportunity to focus on other applications that could be more generalizable and relevant, including those the applicants include in the rationale for this work (repeat-dosing gene editing, other AI-designed cargo, etc.). |

| Application# | DISC5-19851 |
|---|---|
| **Title**<br>(as written by the applicant) | 3D Bioprinted Cortical Organoids with Human-Large Animal Chimeric White Matter for Exploring Evolutionary Constraints on Human OPC Maturation Capacity |
| **Project Objective & Impact**<br>(as written by the applicant) | This project addresses whether human OPCs have a species-specific deficit in maturation capacity, limiting remyelination. This could explain why pro-remyelinating clinical trials have repeatedly failed and provide a new perspective on promoting remyelination in human demyelinating disease, ultimately identifying gene therapy candidates to enhance remyelination capacity. |
| **Specific Aims**<br>(as written by the applicant) | • Establish a 3D bio-printed cortical organoid platform that recapitulates deep cortical layers and adjacent white matter architecture.<br>• Elucidate intrinsic differences in OPC maturation capacity using a 3D bio-printed cortical organoid incorporating chimeric WM.<br>• Evaluate the functional consequences of species-specific differences in OPC maturation capacity on remyelination under basal and inflammatory conditions. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Approximately 100,000 Californians are affected by multiple sclerosis. With annual per-patient costs at ~$90,000, this represents a multi-billion-dollar economic burden for the state. Age of onset for MS is during working and family rearing years, impacting a patient's health and contribution to the local economy and stability of communities' social fabric. This project will accelerate the development of therapies that improve function, independence, and quality of life for Californians. |
| **Funds Requested** | $2,499,999 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 75

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

| | |
|---|---|
| **Mean** | 73 |
| **Median** | 75 |
| **Standard Deviation** | 5 |
| **Highest** | 80 |
| **Lowest** | 65 |
| **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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| **Key Strengths and Weaknesses** |
|---|
| • There is little originality.<br>• This proposal aims to address an important knowledge gap regarding whether hOPCs possess an intrinsic evolutionary limitation in maturation capacity that contributes to incomplete remyelination in human disease; however, the approach of mixing human and large animal OPCs may be complicated by confounding factors such as differences in growth rate, survival, competitive fitness, and developmental timing, which could also influence outcomes in the chimeric organoid experiments.<br>• Substantial concerns with respect to the design and feasibility of the bioprinted organoids reduce enthusiasm for this award. |
| **Significance: Evaluate the project's significance and potential for impact** |
| • The project is potentially important.<br>• This proposal addresses a knowledge gap regarding whether human oligodendrocyte progenitor cells (hOPCs) possess an intrinsic evolutionary limitation in maturation capacity and whether this contributes to incomplete remyelination in human disease.<br>• By comparing human and large animal OPCs, the study may improve understanding of species-specific differences in oligodendrocyte maturation and identify evolution-related genes that could serve as future therapeutic targets to enhance remyelination.<br>• A concern is the potential confounding factors associated with cross-species comparisons. Factors other than intrinsic maturation capacity may contribute to the observed differences between species, such as differences in cell growth rate, survival, competitive advantage, or developmental timing. These variables may complicate data interpretation and could potentially affect the success of the chimeric organoid experiments.<br>• The reasons for failure of remyelination in human disease are poorly understood. The overarching question is why humans exhibit relatively poor remyelination capacity compared with other species. Multiple sclerosis is characterized by inefficient remyelination, which has been linked to impaired OPC proliferation and recruitment, as well as an apparent block in differentiation that limits the generation and survival of newly formed oligodendrocytes.<br>• The datasets generated in this application are not anticipated to substantially contribute to the field at large, beyond the inclusion of NHP OPC/OL profiles.<br>• The successful completion of these studies will provide a detailed molecular catalog of the differences between human and large animal OPCs when cultured as organoids in different cellular environments. The stated aims, if successful, are not considered likely to provide a meaningful impact on current therapeutic strategies or provide insight into novel biomarkers for multiple sclerosis. |
| **Innovation: Evaluate the project for innovation relative to the current state of research** |

- There is little innovation, although it might be useful to collect data on large animal OPCs.
- This project is innovative both technically and conceptually. The investigators propose to develop a bioprinted, spatially organized cortical organoid containing an adjacent white matter compartment, which represents a more physiologically relevant system for studying human oligodendrocyte biology.
- Another innovative aspect of the proposal is the incorporation of human-large animal chimeric white matter within the same organoid system. This approach allows direct comparison of species-specific OPC maturation and lineage progression under shared environmental conditions, if successful.
- The PI focuses on the potential for species-specific intrinsic differences in the oligodendrocyte lineage. The PI proposes that the human brain undergoes a uniquely prolonged development of myelin and that this slowed process of differentiation represents an Achilles' heel following demyelination, thereby limiting repair.
- The applicant overstates the central knowledge gap that human cells have not been compared with other species. Multiple studies have shown successful myelination by human cells transplanted into various host species, including mouse and large animal models. These studies establish that the rate of differentiation is, at least in part, determined by species-specific differences. For example, human OPCs can remyelinate the entire mouse brain, although this process takes more than a year to accomplish.
- In addition, human OPCs have been shown to robustly remyelinate the mouse brain following induction of cuprizone-induced demyelination (Windrem et al., 2021; Cell Reports). These observations reduce the conceptual novelty of the proposal and raise questions regarding both the premise and potential significance of the application. It is also concerning that these studies, which at minimum provide important context for the proposal, were not discussed.
- The application uses iPSC-based culture systems, 3D bioprinting, and various molecular profiling approaches that provide a comprehensive approach to compare human and large animal cells.
- The use of 3D bioprinting to establish the cytoarchitecture of organoids is an innovative approach that could improve the translational impact of organoid-based human models.

**Rationale: Evaluate the scientific rationale in the proposal**

- It has been assumed that differences between human and animal myelination are a problem in human disease. Alternatively, the slowing of myelination in humans might be a good thing.
- There is strong interdependence of the aims.
- The scientific rationale is sound. Remyelination therapies have repeatedly failed in clinical trials for demyelinating diseases such as multiple sclerosis (MS), despite showing strong efficacy in animal models. In many MS lesions, abundant OPCs are still present, suggesting that the failure of remyelination may not simply result from OPC depletion, but rather from an impaired maturation process in human OPCs.
- The preliminary data support the feasibility of the proposed work. The investigators demonstrate successful differentiation of several relevant neural cell types, including layer 5 neurons, layer 6 neurons, astrocytes, and OPC-related populations. They also demonstrate technical expertise in spatial transcriptomics, single-nucleus multiome analysis, and 3D bioprinting workflows, providing a strong technical foundation for the proposed experiments.
- Understanding the species-specific cellular and molecular underpinnings of remyelination failure in human disease is an important unanswered question.
- There are major concerns with the overall approach. It is unclear how bona fide white matter can be established without encouraging axonal outgrowth. The general process seems to be to seed OPCs first,

then layer on top of that layer 6 and then layer 5 neurons. However, OPCs and oligodendrocytes express proteins that can repel axons, making it unclear whether or why axons would extend into the OPC-containing layer.

- Conversely, OPCs are highly migratory and will likely migrate into the neuronal layers. While this migration would not invalidate the model, it would undermine the rationale for establishing a distinct "WM" compartment.

- Another substantial concern is the use of thrombin-containing hydrogels for cross-linking during bioprinting. Thrombin directly activates PAR1, which is expressed by OPCs and is known to regulate neural stem cell behavior while impairing OPC differentiation (Choi et al., 2018, Scientific Reports). Thus, a thrombin-containing hydrogel may reasonably be expected to block oligodendrocyte differentiation and confound the interpretation of the proposed experiments.

- The lack of preliminary data with printed neural cells increases the risk of this application. Neural cells are far more fragile to shear stress and injury. Migration of neurons and glial cells after establishment of 3D organoids is not considered.

- Primary demyelination in organoids will need to be established prior to the proposed experiments in Aim 3a. Demonstrating myelin loss, preservation of demyelinated axons, and subsequent remyelination is fundamentally different from measuring new myelin synthesis following exposure to lysolecithin.

**Plan & Design: Evaluate the project plan and design**

- 3D bioprinting of the cytoarchitecture of organoids is novel.

- The project is well planned and logically designed. The overall goal is to determine whether human OPCs possess species-specific limitations in maturation capacity and whether these limitations contribute to impaired remyelination. The proposal is organized into three specific aims that progressively build upon one another.

- In Aim 1, the investigators will establish a 3D bioprinted cortical organoid platform designed to recapitulate deep cortical layers and adjacent white matter architecture, which looks feasible based on the preliminary data presented in the proposal.

- In Aim 2, the investigators will generate chimeric white matter organoids containing a 1:1 mixture of human and large animal OPCs. This experimental design allows direct comparison of human and large animal OPC behavior within the same microenvironment.

- However, factors other than intrinsic maturation capacity may contribute to the observed differences between species, such as differences in cell growth rate, survival, competitive advantage, or developmental timing. These variables may complicate data interpretation and could potentially affect the success of the chimeric organoid experiments.

- In Aim 3, the investigators will evaluate the functional consequences of species-specific OPC maturation differences on remyelination under both basal and inflammatory conditions. This aim is dependent on the success of Aim 2.

- Aim interdependence is a major concern. If the layer-specific printing fails to produce a cytoarchitecture that is distinguishable from typical self-organizing organoids, then Aims 2 and 3 will not be worth pursuing.

- The number of biological replicates for each experiment is unclear. It appears that individual organoids are being treated as experimental replicates, which raises a concern regarding rigor and reproducibility. The population impact statement mentions the use of six iPSC lines, but it is not clear if all of these lines will be used in each experiment or how many times a given experiment will be repeated.

- Distinguishing human vs. large animal model cells is not yet developed, but the investigator will explore antibody-based approaches followed by incorporation of genetic reporters if they are not appropriate.

Molecular profiling approaches will also be used to compare these cell types. However, there appears to be no plan to determine functional differences in myelin production, internodal length, or myelin sheath thickness between species.

- The budget/timeline is appropriate.
- The team is appropriate for the research proposed. The communication plan is described and reasonable.

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

- The population impact could potentially be high.
- The proposal will use 12 iPSC lines comprising 3 male and 3 female lines per species. The human iPSC lines will be selected from diverse ethnic backgrounds to capture a range of donor backgrounds. The team will utilize 3D bioprinting to improve the uniformity and quality of 3D organoids across lines and experiments to minimize technical variability associated with spontaneous differentiation.
- Six human iPSC lines will be used, balanced for sex. The applicant mentions that these represent diverse backgrounds, but these are not described.
- MS broadly affects the California population, with demographics that are generally similar to those observed in other Northern Hemisphere populations.
- The PI has a history of outreach at a previous institution involving undergraduate trainees of diverse backgrounds. The Co-Investigator is heavily involved in outreach, with a clear track record of involving and mentoring students from diverse backgrounds.

| Application# | DISC5-19822 |
|---|---|
| **Title**<br>(as written by the applicant) | Elucidating and targeting $\alpha$-syn and neurodegeneration in patient stem cell models of Parkinson's disease with AI, chemical biology and robotics |
| **Project Objective & Impact**<br>(as written by the applicant) | Parkinson's disease (PD) is the fastest growing neurogenerative disease but no therapies exist that slow the PD because the causes are not well understood. If successful, this project could address this knowledge gap and overcome research bottlenecks to the discovery of the structure of a whole class of disease-causing proteins and target them with small molecules that might become therapies. |
| **Specific Aims**<br>(as written by the applicant) | • Predict and evaluate compounds that prevent pathogenic oligomerization<br>• Predict and evaluate compounds that promote tetramer formation and prevent neurodegeneration<br>• Predict and evaluate compounds that disrupt $\alpha$-synuclein binding to membranes<br>• Predict and evaluate compounds that promote $\alpha$-synuclein clearance |
| **Statement of Benefit to California**<br>(as written by the applicant) | Parkinson's disease (PD) is the fastest growing neurodegenerative disease, and California has more people with PD than any other state. If successful, the project could eventually lead to therapies for Californians. It also could create an innovative new strategy for targeting proteins with similar properties that cause other major neurodegenerative diseases, including Alzheimer's disease and ALS. It also will support jobs and further establish the California as a leader in biotechnology and AI. |
| **Funds Requested** | $2,499,996 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

### Final Score: 70

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

| | |
|---|---|
| **Mean** | 72 |
| **Median** | 70 |
| **Standard Deviation** | 3 |
| **Highest** | 75 |
| **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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

**Key Strengths and Weaknesses**

- The proposal pairs an exceptionally rigorous patient-derived iPSC platform with an innovative AI-driven intrinsically disordered protein (IDP) ensemble model. However, its central scientific risk — that the AI model's validated capabilities are confined to monomeric IDPs in solution while every aim requires multi-chain, assembly-state, or membrane-bound predictions that the technical report itself lists only as roadmap items — substantially outweighs the strengths of the wet-bench design and places the application below the funding threshold.
- Preliminary data do not sufficiently support the feasibility.
- Strengths: Highly experienced lab with a well-scoped disease-model and great use of AI/ML technology.
- Weaknesses: limited testing across different cell lines. The AI technology is not discussed as extendable to multimers as discussed in the application. Scope seems very ambitious, to the point where any failures between the two sides may cascade forward and push several goals out of reach.

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

- This application would advance foundational knowledge on neurodegeneration around α-synuclein oligomerization and phosphorylation. It will not directly advance foundational knowledge of stem cell biology or regenerative medicine per se. For this research, the applicant is using patient-derived iPSC lines (stem cells) from patients with familial and idiopathic forms of synucleinopathies.
- If successful, this research would identify compounds or other strategies to mitigate accumulation and toxicity of synuclein proteins to treat synucleinopathies, which are neurodegenerative diseases without effective mitigating therapies.
- A successful project outcome would inform development of future therapeutics and/or biomarkers and identify treatments for a range of disorders called synucleinopathies, but could be applied to any disorder that arises from intrinsically disordered proteins (IDPs). While this is not regenerative medicine per se, it's an important approach as IDPs are difficult to model towards developing therapies.
- Chemical tools that selectively engage distinct α-synuclein conformational states would establish a conformation-selective therapeutic strategy for Parkinson's disease and related synucleinopathies.
- Characterizing the downstream cellular phenotypes elicited by these tools in patient iPSC-derived neurons using high-content robotic microscopy will elucidate how conformation-specific modulation of α-synuclein translates to neuronal function across genetic backgrounds.
- AI models capable of predicting intrinsically disordered protein (IDP) conformational ensembles would reduce the computational cost of molecular dynamics (MD) by orders of magnitude and open IDPs to rational drug discovery.
- The central hypothesis has been tested many times already with disappointing results; a long list of prior fibrillization inhibitors has advanced in one version or another covering all the major hypotheses in this proposal.

- The hypothesis is very hard to falsify: if Aim 1 fails, the oligomer state was wrong; if Aim 2 fails, tetramers don't matter; if Aim 3 fails, membrane binding wasn't pathogenic; if Aim 4 fails, the clearance pathway wasn't rate-limiting.
- Aim 2 is the most differentiated aim scientifically, specifically testing a longstanding hypothesis the field has been unable to resolve; Aim 3 has the strongest mechanistic chain to neurodegeneration; Aim 4 is the most de-risked aim with the cleanest therapeutic logic.

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

- Provides a good use of modern AI/ML tools to predict transient structures of disordered proteins with well-characterized patient iPSC models of synucleinopathies. The use of the novel methods is innovative especially as applied to these diseases.
- The AI model is a generative neural network producing all-atom conformational ensembles for intrinsically disordered proteins, specifically built to fill the gap left by AlphaFold-2/3, etc, which are all trained to predict single dominant structures.
- The AI model predicts conformational ensembles directly from sequence without experimental input, replacing weeks of restrained MD with minutes of inference.
- Combining AI model ensembles with virtual screening and de novo design allows structure-guided design against defined α-synuclein conformers rather than the aggregate ensemble.
- Coupling conformation-selective compounds to patient-derived neuronal phenotyping turns these probes from candidate drugs into causal tools for asking which α-synuclein conformers drive/relieve disease-relevant phenotypes in human neurons.

**Rationale: Evaluate the scientific rationale in the proposal**

- The AI model demonstrates state-of-the-art performance on IDP ensemble prediction with rigorous benchmarking, including excellent α-synuclein agreement with reference molecular dynamics.
- The applicant's robotic microscopy platform has extensive published validation for longitudinal single-neuron tracking in neurodegeneration models.
- The system is claimed to be extremely fast and can determine possible configurations orders of magnitude faster than conventional molecular dynamics.
- Preliminary data on α-synuclein looks good, with only 3.9% mean absolute error in comparison to other structural data, and the scaling curve shows a power-law improvement trajectory.
- This is a highly innovative project and has great promise overall for the field of neurodegenerative medicine. Overall the rationale for employing in silico models of IDP is reasonable except for the fact that these are multimer models that would need to be built -- not sure how that would be addressed.
- The AI model predicts a single-chain α-syn conformation. However, it is unclear how this result informs oligomer and tetramer formation. Finding compound hits is likely much harder than AR NTD, which only requires monomer ensemble generation.
- No supporting data are provided to indicate that the AI model can predict membrane-bound α-synuclein conformational states and validation has been performed only on a soluble IDP in dilute solution.
- The generative chemistry component is described without disclosed validation data. The in-house ML-MD software introduced in Aim 4 is also presented without disclosed validation data.

- The scientific rationale is sound at its baseline, but the width of the proposal scope is a concern if the model would need to be rebuilt or approximated for multi-protein assemblies and structure stabilization under that type of model. It seems very ambitious, though both organizations have adequate experience.

**Plan & Design: Evaluate the project plan and design**

- There is a mismatch between where the model has been validated and what it needs to do for the aims: the technical report is almost entirely about monomeric IDPs in solution, but the aims require oligomeric a-synuclein (Aim 1), tetramers (Aim 2), membrane-bound conformations (Aim 3), and ternary complexes (Aim 4). None of these capabilities are demonstrated in the technical report and are only listed as roadmap items.
- The technical report is a white paper, not a peer-reviewed publication; the grant-described 49% hit rate is actually a correlation coefficient, and the AI model is only shown to correlate with potency on the androgen receptor with no a-synuclein demonstration.
- The oligomer-competent monomer feature is doing a lot of work in Aim 1 and is the weakest conceptual link: The AI model generates monomeric ensembles, but Aim 1 needs to predict which monomeric features predispose to oligomerization. The model is being asked to do something it has not been benchmarked for, and this same gap applies to Aim 2 and Aim 3.
- The phenotype battery in Aim 1 is generally strong, the SNCA allele ladder is very strong, the OPL clearance readout for compounds that aren't supposed to affect clearance is the right way to go, and the active learning framework is technically appropriate.
- Numbers are vague in places where it matters: 6-8 biophysically validated tool compounds per cycle * 4 cycles = 20-30 compounds total in primary cell assays, which is very small given the chemical space being searched (10E5–10E6 in silico), and the dosing scheme is under-specified.
- The prediction risk is concentrated in the early cycles, pharmacokinetics and effects within cells feed back into the training model and may impact things in an unpredictable way, and Aim 4 has a dependency on Aim 1 that is not explicitly de-risked.
- The proposed platform is among the world's leading iPSC phenotyping systems and is well-suited to the proposed work. The AI team has appropriate computational credentials, though no demonstrated drug discovery track record.
- There are two different organizations, a lot of moving parts that require exploratory simulation of the IDPs with compound/ligand binding simulations, an advertised model (company website) that is based on single-protein models, mechanism validation/study, bioassay development, compound sourcing/synthesis and screening, and then assay hit analysis. Even if everything went smoothly, this may be a lot to accomplish in three years to the detail discussed in the application.
- The proposed timeline appears tight, particularly given dependencies on compound purchase and synthesis turnaround and on in vitro evaluation cycles. The plan to complete at least three design–make–test cycles for each aim in 3 years is ambitious, because several of the assays and methods appear not yet established in their lab.
- Over-ambitious timeline.

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

- α-synuclein is one of the major drivers of PD, and successful targeting would benefit a large fraction of the affected patient population.

- If the IDP-targeting workflow generalizes, it can be extended to other IDP-driven diseases, broadening the potential impact substantially beyond PD.

- By design, there isn't a lot of genetic, environmental and external factors to influence research findings in the first half (simulations). In the lab they are using a limited number of patient-derived iPSCs (looks like one), so there are a limited number of confounders but also not much in the way of reproducibility guarantees outside of a limited number of models. Variance will come from assay analysis as well as the experimental platform.

- The same iPSC line carries the entire project; while the Population Impact section mentions secondary validation in other cell lines, the primary mechanistic readout is always the same cell line and its isogenic control, creating risk if the line has a phenotypic quirk.

- Lipid composition specificity is not addressed in Aim 3, which limits the generalizability of any membrane-binding findings across membrane environments relevant to different patient populations and disease contexts.

| Application# | DISC5-19844 |
|---|---|
| **Title**<br>(as written by the applicant) | Minimal invasive approach for monitoring stem cell and gene therapy |
| **Project Objective & Impact**<br>(as written by the applicant) | Quantifying cell survival, gene expression, integration, and function in vivo remains a significant challenge. Our goal is to develop tools that are minimally invasive, cost-effective, and highly robust for monitoring and quantifying transplanted or targeted cells in vivo. Developing this capability would greatly enhance our ability to assess therapeutic efficacy and optimize treatments. |
| **Specific Aims**<br>(as written by the applicant) | • Systematically characterize RMA in the retina.<br>• Monitor cell transplantation and gene augmentation-based therapy in vivo through RMA.<br>• Apply RMA in the non-human primate (NHP) retina. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Vision plays a critical role in learning, working, and recreation, and vision impairment is one of the most feared disabilities. Around 747,000 California residents reported serious difficulty seeing even when wearing glasses. The objective of this project is to develop an innovative, minimally invasive approach for monitoring cellular states, functionality, and survival within the retina following cell and gene therapy, which offer transformative potential for the treatment of visual disorders. |
| **Funds Requested** | $2,499,989 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

**Final Score: 70**

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

| | |
|---|---|
| **Mean** | 70 |
| **Median** | 70 |
| **Standard Deviation** | 5 |
| **Highest** | 80 |
| **Lowest** | 60 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 15 |

# FINAL COMMENTS

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

| **Key Strengths and Weaknesses** |
|---|
| • Its unlikely in its present form that the work here would guide the development of future therapeutics or biomarkers. Unless one can demonstrate to these agencies that the RMAs reflect improvements in the cells transplanted or treated, this will be limited to screening methods in preclinical work, and therefore not meeting the goals set out by the applicants.<br>• Target good. As an adjuvant way of monitoring cell survival but it would need to be combined wight other methods such as OCT and ERG<br>• Use the RMA tool in animal models to discover new biology.<br>• Clinical use is not well motivated, given the likely lack of correlation or RMA with disease course.<br>• The immune response to the editor with constitutive expression is not well considered. |
| **Significance: Evaluate the project's significance and potential for impact** |
| • The overarching goal of the application is to apply to novel technologies to non-invasively monitor in blood cell and gene therapies after treatment for retinal disorders.<br>• If successful, that is, the monitoring of drug product engagement remotely via a blood test, would be an advantageous to advance therapies, particularly if that product was not foreign, did not result in innate or acquired immunogenic responses peripherally (given its systemic exposure), and provided insight into the activity of the gene or cell therapy product.<br>• The work will make it easier to understand if transplanted cells are still present and if cells treated with gene therapy are still expressing the gene products encoded by the vectors.<br>• If the tools were used to understand distinct properties that made some cells survive and others not, that may have broader application, however that is not the goal here. The goal is to augment standard ERG, OCT and other methods to assess outcomes.<br>• Its unlikely in its present form that the work here would guide the development of future therapeutics or biomarkers.<br>• Very significant.<br>• Establishing a clinically relevant biomarker for CGT would enable the translation of more therapies. |
| **Innovation: Evaluate the project for innovation relative to the current state of research** |
| • The project builds on what has already been advanced, that is, the expression of secreted luciferase proteins after gene transfer to tissues, or cell transplant after gene transfer to express the secreted luciferase reporters. |

- While exciting, the proposal falls short by relying on reporter proteins based on secreted luciferase or Cre-inducible systems, neither of which would find utility in the clinical setting. The collaborator has experience in advancing work to clinical trials using FUS-induced release of biomarkers into the circulation.
- Given the extensive single-cell experience of the team, the advanced knowledge of the genetic changes that occur in the retina with disease, and the clever capabilities of the team, it's unfortunate that the project will spend such an enormous effort on reporters that will ultimately not be useful in the clinical setting.
- While fine for preliminary data and understanding limitations, etc., luciferase and Cre are so ultra-sensitive that they become unrealistic when replaced with proteins and reporters that are not foreign to humans. What we really need to know is how this will translate to something that can be used to advance therapies that will move beyond studies in mice and the proof of concept that you can detect secreted luciferase reporters in NHP blood.
- Innovative.
- RMA is highly unique and innovative.

**Rationale: Evaluate the scientific rationale in the proposal**

- Assess the fundamental soundness of the scientific rationale for conducting the proposed research. The idea is exciting. The preliminary data and earlier published work by the group demonstrate that the methods and tools are in place.
- Evaluate the scientific rationale for the choice of proposed experimental approaches (including non-human models). The RMAs being tested are secreted luciferase analogs, which provide for high sensitivity and low background. However, because this would likely not be used clinically or allowed by the FDA, it would have been reasonable to propose advancing to non-foreign RMAs that were both reflective of drug product engagement and did not pose a risk due to being foreign.
- There may be immune problems.
- The use in humans is unlikely to be approved by regulatory agencies (e.g., FDA).

**Plan & Design: Evaluate the project plan and design**

- In the initial aims, they will use the secreted luciferase to determine the minimal number of cells transduced or transplanted that they can detect, the kinetics of that expression, and whether or not the mice mount immune responses in the tissues or peripherally to the protein. The most likely response is an anti-protein antibody, but mice are not good proxies for translational studies, and if immune-incompetent animals are used, the data will be uninformative.
- The final aim will be to repeat the studies in the NHP retina, again using secreted luciferase reporters, which will also likely mount immune responses to the proteins, but the extent of that immune response may be limited.
- Well planned.
- The work is more akin to technology development than discovery science.

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

- No concerns

| Application# | DISC5-19646 |
|---|---|
| **Title**<br>(as written by the applicant) | Stem cell-based approaches to dissect the neuro-immune axis in aging-associated peripheral neuropathies |
| **Project Objective & Impact**<br>(as written by the applicant) | Past 65 years of age, 25-50% of individuals develop tingling, burning, pain, and other unwanted sensations due to peripheral neuropathy. We do not yet understand what makes aging one of the strongest risk factors for peripheral neuropathy, but we believe stem cells will be key to unraveling this question and may be used in the future as cellular therapies for peripheral neuropathies. |
| **Specific Aims**<br>(as written by the applicant) | • Define how myelin triggers macrophage senescence to drive peripheral neuropathy.<br>• Define how HIV-1 compounds the effects of macrophage senescence and aging.<br>• Define mechanisms by which macrophage-secreted factors drive Schwann cell breakdown. |
| **Statement of Benefit to California**<br>(as written by the applicant) | By 2040, the number of Californians >65 is expected to increase by over 50% (to ~10 million). Peripheral neuropathies are estimated to affect up to 20% of these older adults and to decrease their quality of life. In addition, patients with peripheral neuropathy have an increased risk of falls and higher healthcare utilization. Thus, understanding the causes of peripheral nerve changes in older adults and developing new therapies will directly benefit the State of California and its citizens. |
| **Funds Requested** | $2,499,943 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

### Final Score: 70

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

| | |
|---|---|
| **Mean** | 69 |
| **Median** | 70 |
| **Standard Deviation** | 2 |
| **Highest** | 70 |
| **Lowest** | 65 |
| **Count** | 15 |
| **(85-100): Exceptional merit and warrants funding, if funds are available** | 0 |
| **(1-84): Not recommended for funding** | 15 |

# FINAL COMMENTS

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

**Key Strengths and Weaknesses**

- This is a potentially meritorious proposal, but some of the experimental approaches may not be feasible for the work proposed, which limits enthusiasm for the work.
- There are substantial weaknesses in this application. There is extensive research on the importance of pro-inflammatory macrophages on peripheral neuropathies, and whether there is a functional difference between other pro-inflammatory macrophages is not made clear.
- The Schwann biology is problematic, as Schwann cells need to be induced to differentiate to make true myelin in vitro.
- There is a paucity of in vivo experimentation.
- In summary, it was a strong application. The impact this may have on peripheral neuropathy is the key driving factor for enthusiasm. However, the link between aging and HIV is somewhat tenuous, and more data to support Aim 1 would have strengthened the application. The lack of a detailed discussion regarding Schwann cell biology was the major criticism.
- The case for protecting Schwann cells from potentially multiple toxic substances is not well made in contrast with focusing on macrophage function.

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

- The proposed work may provide new and valuable insights as to the role played by aged and inflammatory macrophages in mediating peripheral neuropathies.
- There is the possibility of uncovering novel macrophage-derived mediators that can directly impact Schwann cells or peripheral nerves.
- Peripheral neuropathies have an increased propensity in the elderly and with people living with HIV.
- Peripheral neuropathy is a frequent problem as a consequence of aging, certain types of viral infections, multiple cancer treatments, and for many unknown reasons. It can particularly impair quality of life and there are not only no suitable treatments but a poor understanding of the causes of these syndromes.
- Thus, developing better insights into the causes of peripheral neuropathy could lead to improved treatment strategies.
- Peripheral neuropathy (across all types) is a profound clinical problem that currently has no established treatment other than symptomatic relief. This proposal seeks to use iPSCs and hPSCs to investigate the effects of aging and HIV on peripheral neuropathy in an attempt to identify targets for future therapeutic development.
- The proposal has the potential to translate to other peripheral neuropathies. The approach can certainly be applied to other pathologies to identify targets that may not overlap.

- With an aging population, peripheral neuropathy is likely to become more prevalent. With improvements in HIV treatment, HIV-DSP is also going to become more prevalent. Studying the diseases in tandem will determine if they have a cumulative effect.
- Aging is associated with increased incidence of multiple diseases, which makes it difficult to consider as an independent risk factor for developing peripheral neuropathy.
- Macrophage polarity is widely accepted to contribute to peripheral neuropathy.

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

- The work is based on recent innovative work examining aged macrophages for their senescence-associated secretory phenotype, which can drive expression of pro-inflammatory cytokines.
- Innovation is relatively limited, as the role of macrophages in peripheral neuropathy is well established.
- The fundamental difference between the present studies and those of others is the focus on macrophage senescence. That said, the differences between functional outputs of senescent and pro-inflammatory (M1-like) macrophages seem minimal. Although most previous studies have focused on macrophage polarization in respect to pro-inflammatory and pro-reparative phenotypes, resemblances between senescent macrophages and classically studied pro-inflammatory macrophages are not sufficiently delineated.
- It is also well established, in both the central and peripheral nervous system, that phagocytosis of myelin debris by macrophages can trigger a phenotypic switch to a pro-inflammatory phenotype. Thus, the hypothesis that myelin debris triggers a pro-inflammatory phenotype in macrophages is not novel.
- Linking aging and macrophage senescence to peripheral neuropathy would be considered novel and using iPSCs/hPSCs has greater potential for translation.
- The concept that secreted factors from dysregulated macrophages drive PNS neurotoxicity is novel.

**Rationale: Evaluate the scientific rationale in the proposal**

- Important strong preliminary evidence is provided to support the rationale for the proposed experiments.
- The experimental approach makes use of stem cell approaches for the generation of different types of macrophages, being derived from human HSCs vs iPSCs. However, differentiation towards Schwann cells may be challenging.
- The focus of this application is on the possibility that the chronic inflammation frequent in multiple peripheral neuropathies is related to macrophage senescence.
- The rationale for the proposed experiments is sound, in that it is known that myelin debris can trigger pro-inflammatory changes in macrophages. Whether the change is studied or truly indicative of a senescent phenotype is unclear, and whether that matters is unclear.
- HIV infection is frequently associated with peripheral neuropathy, so this is also a reasonable thing to study. Whether aging exacerbates the effects of HIV infection may be worth confirming but would not in and of itself alter views on causes and treatment strategies. Moreover, most peripheral neuropathies in aging populations are not associated with HIV infection and most HIV infection is not confined to aged individuals.
- Aging is not specific to peripheral neuropathy; this is where enthusiasm is diminished for this proposal. There are many confounders that occur with aging that cannot be separated; the incidence of diabetes

increases with age, the chronic toxicity of alcohol occurs over time and individuals are more likely to develop cancer and need neurotoxic chemotherapy.

- The preliminary data presented confirms several key points which are convincing. Centrally, the investigators propose that aging results in increased myelin debris which propagates macrophage differentiation to M1. The supporting data for this claim are not presented clearly.
- The concept of age + viral infection making peripheral neuropathy more severe is tenuous; additional detail would be appreciated in the context of other, more frequent viral illnesses, and while HIV is undoubtedly important, it is less prevalent and an argument needs to be made. It is appreciated the applicants have a track record in the HIV VPR field, but the relevance needs to be transferable.
- With regard to the development of Aims, Aim 1 appears to be the least developed. More data needs to support the claim that myelin breakdown drives macrophage senescence. Aim 2 is robust, and Aim 3 is an appropriate goal targeting a mechanism.

**Plan & Design: Evaluate the project plan and design**

- The proposed work is clearly outlined and provides a broad set of potential pitfalls and alternative approaches.
- Strengths:

Aim 1.2 performing multiomic analyses is supported by the recently published preliminary work.

- Weaknesses:
    - Aim 1.1 provides a minor incremental set of experimental approaches to test the changes in macrophage function and expression of inflammatory mediators.
    - Aim 1.3 posits that deleting p53 will lead to macrophage apoptosis, which is very counter-intuitive and would need some preliminary results to support this claim, in which a loss of a tumor suppressor leads to cell death.
- The proposed work is valiantly justified, and the connection to PLWH and aging is meritorious. However, some of the approaches do go a bit beyond applicability, such as the use of radiation to induce senescence.
- Aim 3 will define conditioned media (CM) components that mediate Schwann cell apoptosis, which might be a tall task given that only about 2% of the Schwann cells show clear apoptotic caspase-3 cleavage.
- The preliminary studies indicating that HIV-infected macrophage secretomes provoke apoptosis in Schwann cells may be interesting, although it is well known that pro-inflammatory macrophages are capable of causing cell death in multiple cell types. The proposed experiments to determine how secretomes from senescent and HIV-infected macrophages might cause Schwann cell dysfunction and toxicity are standard experiments, and what they might reveal that is new is not a case that is well made.
- Even accepting the idea that the effects of HIV infection, aging, and cholesterol loading produce a stable senescent phenotype in macrophages, the indications as to why this would be different than a non-senescent pro-inflammatory phenotype are not well presented, which raises the question of what new will be added in terms of mechanism-related insights in what has been a well-studied problem.
- In terms of experimental design, there is another problem to consider, which is the superficial description of Schwann cell biology. Multiple aspects of these studies rest their novelty on the generation of myelin debris from iPSC-induced Schwann cells. Such cells grown in culture, however, are not myelin-generating

Schwann cells and only produce a limited myelin-related repertoire. The induction of differentiation along a myelinating pathway does not seem to be described at all.

- Whether this is an oversight, or a fundamental lack of understanding of Schwann cell biology, is not clear.
- An additional problem is that it is not clear what the consequences would be even if they are correct in all regards.
- It is also not clear how different this proposal is from preventing pro-inflammatory macrophages (a heavily studied topic). It is also not clear whether the goal is to prevent senescence/M1-like changes or to drive cells to a pro-repair phenotype. It is also unclear whether it is more effective to control the macrophages than it is to protect the Schwann cells.
- Finally, all the assays are conducted at short time points, and there are no in vivo experiments.
- The approach cites the applicants' own publications, which supports that there is appropriate expertise for conducting the experiments. The three-year timeline appears appropriate for this setting of PSC research.
- There was limited detail provided regarding Schwann cell biology, and some concern was raised regarding the lack of in vivo data. Schwann cells behave differently in vitro and have to be induced to produce myelin. It is unclear if the myelin debris will behave the same in vivo.

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

- Population impact considerations are well articulated and broadly impactful, in particular, the affected aging population and people living with HIV.
- HIV infection and aging are problems for all populations, with HIV being somewhat more of a problem for minority populations. Thus, a broad impact would be possible.
- This has a high population impact as this proposal focuses on aging and HIV. A recent study suggests that by 2040 ~350,000 Americans will be >65 years old with HIV.
- There is a high likelihood that the findings from this proposal will be incredibly relevant to other peripheral neuropathies (diabetes, alcohol, chemotherapy, etc.), which may be potentially more profound.

| Application# | DISC5-19927 |
|---|---|
| **Title**<br>(as written by the applicant) | The Virtual Stem Cell |
| **Project Objective & Impact**<br>(as written by the applicant) | Human induced pluripotent stem cells underpin much of regenerative medicine, yet no accurate, precise, and complete model of the human stem cell currently exists. We will construct a virtual cell that unifies protein composition, spatial organization, and functional state of the stem cell, enabling mechanistic insight, predictive perturbation analysis, and rational therapeutic discovery. |
| **Specific Aims**<br>(as written by the applicant) | • Construct an annotated model of the human induced pluripotent stem cell.<br>• Validate and analyze the Virtual Stem Cell for mechanistic insight and predictive hypotheses.<br>• Disseminate and translate the Virtual Stem Cell as a CIRM-enabling resource. |
| **Statement of Benefit to California**<br>(as written by the applicant) | This project will benefit California by delivering an open, predictive Virtual Stem Cell that accelerates regenerative medicine research, therapeutic discovery, and collaboration across the state. By enabling efficient interpretation of stem cell data and disease-associated variants, it will lower barriers to innovation, support California's biomedical research ecosystem, and strengthen the state's leadership in stem cell science and data-driven medicine. |
| **Funds Requested** | $2,499,998 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

**Final Score: 70**

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

| | |
|---|---|
| **Mean** | 68 |
| **Median** | 70 |
| **Standard Deviation** | 4 |
| **Highest** | 70 |
| **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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

**Key Strengths and Weaknesses**

- Interesting idea. Large data and likely some meaningful partial prediction models. Not enough data (and evidence) to build a true virtual cell.
- Preliminary data are lacking for proof of concept.
- A major concern for this project is the feasibility. This proposal lacks preliminary data showing it is possible to set up a computational model for human cells and human iPSCs.

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

- A fully "functional" virtual cell that enables the correct prediction and virtual testing of perturbations is clearly an interesting goal. If accomplished it may facilitate various studies though a refined articulation of which bottlenecks it solves and where the next one emerges as a result would be helpful. However, the significance of this more limited cell model is not clear.
- It's not clear why a virtual stem cell is needed.
- A new cutting-edge model could help fundamental stem biology.
- A computational model of iPSC is a major unmet need in regenerative medicine. A successful project outcome will advance stem cell biology field. However, this proposal may overstate the predictive capability of their Virtual Stem Cell model because human cells are inherently complicated and context-dependent (such as cell culture media and matrix).
- This proposal focuses on computational annotation of proteins inside of iPSCs and they do not plan to experimentally test biological hypotheses emerging from the iPSC model.
- It is unclear how scientists and engineers can use this model to design new cellular therapies, such as design new robust differentiation protocols to make a specific type of neuron, or pancreatic beta cells.

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

- Bringing together massive multi-layered data sets to build a meaningful cell model is not trivial and will certainly required innovation at various points. The general idea of a virtual cell has been floated many times but implementation is rather challenging given the scale of the problem (and the relatively sparse and imprecise data).
- The proposed new computational approaches using AI are clearly innovative.
- The proposal is innovative in its attempt to integrate structural biology, systems biology, AI/ML, perturbation genomics, cryo-ET, and protein interaction modeling into a unified multi-scale virtual cell framework.
- The proposal lacks rigorous benchmarking criteria to evaluate whether the integrated framework performs substantially better than existing approaches.

**Rationale: Evaluate the scientific rationale in the proposal**

- The rationale or implications could be articulated better. Several general points are raised, but some more details including some preliminary data/evidence would be helpful.
- The virtual stem cell model could help us understand differentiation processes at unprecedented resolution.
- Despite extensive preliminary computational work on proteins and protein network, there is relatively limited preliminary evidence demonstrating that the proposed Virtual Stem Cell model can accurately predict biologically meaningful stem cell behaviors
- Many feasibility claims depend on future generation of high-quality cryo-ET and multimodal datasets that may be technically difficult and variable.

**Plan & Design: Evaluate the project plan and design**

- The outlined plan in general is logical. Aim 1 will construct the virtual cell, Aim 2 will try to validate the model by predicting responses to perturbations and Aim 3 will disseminate the virtual cell to the community.
- The biggest concern is that the data (even the massive scale proposed here...Table 1 is still far away from a cell inventory) are still very limited to build a true virtual cell. It sounds good in theory, but since the application doesn't present much evidence it will be a rather risky endeavor at this stage.
- Validation of the model is underspecified.
- The costs and dissemination of running the model needs to be discussed.
- For Aim 1, this project proposed to construct an annotated model of the human iPSC. The plan to build an integrative computational workflow to assemble a model of iPSC composition, structure, and function across molecular, mesoscopic, and cellular scales. This aim may not achieved based on the availability of current data for building a human cell model.
- Aim 2 will benefit by incorporating experimental studies, such as using CRISPR system to do genetic perturbations in real human iPSC culture to see if the model can correctly predict what is happening in the cell culture.
- Aim 3 disseminating and virtual stem cell model as a CIRM-enabling resource. This aim does not address computational resource requirements and scalability challenges by the end users if other labs want to use this virtual stem cell model for their research.

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

- The cell model would be generally applicable. Parts of it may be influenced by the data sources.
- No concerns.
- The proposal explicitly recognizes the importance of incorporating genetic variation and disease-associated alleles into the Virtual Stem Cell framework.
- Most datasets appear derived from controlled iPSC systems rather than genetically diverse donor populations.

| Application# | DISC5-20093 |
|---|---|
| **Title**<br>(as written by the applicant) | AI-Guided Model Discovery for human stem cell differentiation |
| **Project Objective & Impact**<br>(as written by the applicant) | Human disease modeling is limited by the lack of interpretable, predictive rules linking stem cell state to differentiation and disease outcomes. This project will uncover invariant regulatory logic governing human stem cell differentiation, enabling robust disease modeling, biomarker discovery, and improved translational control of regenerative therapies. |
| **Specific Aims**<br>(as written by the applicant) | • To identify fundamental logical gene expression relationships in colon tissue<br>• To identify fundamental cell type specific biomarkers<br>• To develop new tools to connect stem cell differentiation to disease applications |
| **Statement of Benefit to California**<br>(as written by the applicant) | This project will advance California's leadership in stem cell and AI-driven biomedical research by generating new tools to improve human disease modeling, biomarker discovery, and regenerative medicine. The discoveries will accelerate translation to patients, support the state's biotech ecosystem, and train a workforce skilled in clinically relevant stem cell and AI technologies. |
| **Funds Requested** | $2,478,299 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

**Final Score: 70**

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

| | |
|---|---|
| **Mean** | 67 |
| **Median** | 70 |
| **Standard Deviation** | 4 |
| **Highest** | 70 |
| **Lowest** | 60 |
| **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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| **Key Strengths and Weaknesses** |
|---|
| • Key weakness: Neither the AI models nor the computational frameworks are sufficiently outlined. There is no validation or logical path to the promised application.<br>• The proposal generally lacks an explanation of the mathematical model's benefits and a plan to confirm functional relevance. |
| **Significance: Evaluate the project's significance and potential for impact** |
| • The proposal looks for specific gene expression signatures in large public datasets. The current focus is on colon/colon cancer. While the technical details of the analysis are somewhat outlined, the actual significance is less clear. The frequently mentioned NEJM paper is 10 years old, and the context is different.<br>• The main goal of this application is to use "AI" to discover genes that are uniquely expressed in specific cell types, starting with the colon. For that, the applicants aim to discover how genes can have mutually exclusive expression using a Boolean approach. The resulting knowledge will then be used to investigate the mechanisms by which stem cell differentiation can be connected with disease.<br>• There is little focus on stem cells.<br>• The relation to cancer is interesting, and the translational aspects will be supported by the clinician involved in the project.<br>• The study proposes advanced AI and mathematical modeling based on patient samples to infer mechanisms for stem cell differentiation.<br>• While data will certainly be obtained, it is not clear how this approach will be able to accurately identify factors guiding differentiation.<br>• Biomarker assessment is identified as a goal, but it is not clear how this will be applied or compared with other known biomarkers. |
| **Innovation: Evaluate the project for innovation relative to the current state of research** |
| • It appears the applicant would use a range of computational tools and established statistical methods to search through published datasets. Overall innovation is not clear.<br>• The manuscript that is frequently cited by the applicant was published in 2016 in the New England Journal of Medicine. Thus, the approach is not entirely new. The main conclusions of this manuscript regarding CDX2 are important. However, CDX2 is not currently used alone for clinical decisions. Identifying biomarkers remains a challenge. |

- The approach is definitely interesting, but it is difficult to understand what new results will be generated by this proposal. It seems that the main goal is to increase the number of datasets used and to identify new genes of interest.
- Some parts of the project are difficult to follow. The proposal is a succession of aims, but there is little explanation of how they will be achieved. For example, in Aim 2, the applicant wants to resolve cell-type diversity with an initial focus on human stem cell differentiation. How do they plan to achieve this objective, as the vast majority of datasets lack single-cell resolution?
- The project plans to apply invariant mathematical approaches to stem cell differentiation in the colon; this may allow differentiation between stable gene changes that define cell state.
- This is a new perspective and approach; however, it is not clear where deficiencies in current models have been demonstrated.

**Rationale: Evaluate the scientific rationale in the proposal**

- The rationale and how this relates to the title (human stem cell differentiation) are not clear. The grant is not well written, and the logic is not sufficiently clear.
- The hypothesis that a set of markers is expressed similarly between species is important and definitely interesting. However, human-specific mechanisms are also essential and probably more powerful. They could explain how disease can develop and the impact of population diversity.
- The project is divided into 3 aims, which are rational. However, there is a need for functional validation in vivo and in vitro to determine the real value of the findings. This lack of biology is a limitation.
- It is not clear why conservation across species is more relevant to human disease.
- The proposal is generally underdeveloped around known intestinal differentiation and discussion of predictions around specific lineages (i.e., “top of the crypt” contains multiple lineages).

**Plan & Design: Evaluate the project plan and design**

- All aims lack key details.
- Aim 1 is reasonably well outlined, while Aims 2 and 3 are less clear and rather short, especially for Aim 3. As a result, it is difficult to judge the project plan.
- Computational frameworks are suggested without any detail on how they are built. It is also not clear how these tools would deliver what is promised, for instance: “These tools will allow us to (i) link specific differentiation states or transition points to disease risk, progression, or treatment response.”
- The lead applicant is currently an associate professor at a CA state public university. They have published more than 10 papers in the last 10 years, including at least 5 papers as last author. They are definitely an expert in Boolean analyses. However, their expertise is strictly computational. The Co-Is will bring key expertise at the clinical level. The basic biology aspect could have been reinforced.
- Aims 1 and 2 make sense, as they are based on the core expertise of the lead PI. Aim 3 is more difficult to follow since different diseases involve different cell types, which might not be epithelial.
- It would have been useful to describe more precisely their new method to stain tissue using immunochemistry. It would help to understand the difference in expression observed with their old and new methods (Fig. 9A and B). Ultimately, immunofluorescence would be more relevant to study co-expression.

- The budget is relatively high for a proposal that seems mainly computational.
- Aim 1 proposes to identify fundamental logical gene expression relationships in colon tissue based on invariant relationships in the human colon. It is less clear how this will then effectively be applied to cell differentiation.
- Aim 2 proposes to identify fundamental cell type-specific biomarkers. Validation of these biomarkers will require reliance on known markers, which seems likely to skew interpretation.
- Aim 3 is where the goal is to link stem cell differentiation models to disease. This will certainly yield gene associations; however, it is not clear what differentiated cells in the colon are highly associated with specific diseases, so the rationale is weaker.

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

- It is unclear, but as outlined, the search would reveal ultra-conserved relationships, and hence they would likely apply to all.
- The paragraph on Population Impact is not really clear. The applicant could have explained how population diversity related to California is important for colon-related diseases and how this diversity will be considered in the program.
- It could have been useful to describe how the lead applicant plans to present their research to the general public.
- The study will incorporate large datasets that include many variables, such as genetic diversity and environmental exposures, so this will inherently be built into the model. Thus, it can be applied across distinct groups.

| Application# | DISC5-19876 |
|---|---|
| **Title**<br>(as written by the applicant) | Efficient Somatic Cell Reprogramming through AI-Guided Transcription Factor Control (FateFactor) |
| **Project Objective & Impact**<br>(as written by the applicant) | Current iPSC reprogramming lacks predictive control, leading to inefficient and unsafe outcomes. This project integrates a new AI framework for learning transcriptional control logic from millions of single-cell transcription data points with multiplex CRISPRa/i perturbations to identify and correct failed reprogramming paths, enabling safer, more reliable generation of human iPSCs. |
| **Specific Aims**<br>(as written by the applicant) | • Develop FateFactor, a deep-learning framework to predict transcriptional regulatory codes for safe and efficient somatic cell reprogramming.<br>• Iteratively refine transcriptional regulatory codes with CRISPR epigenetic programming to establish an efficient and safe somatic reprogramming protocol and quantify reprogramming outcomes |
| **Statement of Benefit to California**<br>(as written by the applicant) | This project will strengthen California's leadership in stem cell innovation by developing scalable technologies for safe iPSC generation. By testing reprogramming across genetically diverse donor cells reflecting California populations, the work will advance equitable regenerative medicine, support biotech translation, train a diverse workforce, and deliver openly shared data and tools for the state's research and clinical communities. |
| **Funds Requested** | $2,499,981 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that "The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG."<br><br>Patient advocate members unanimously affirmed that "The review was carried out in a fair manner and was free from undue bias." |

## SCORING DATA

**Final Score: 70**

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

| | |
|---|---|
| **Mean** | 64 |
| **Median** | 70 |
| **Standard Deviation** | 9 |
| **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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| **Key Strengths and Weaknesses** |
|---|
| • It could work.<br>• The team is strong and the contrastive-learning framing is a real conceptual contribution, but the multi-tier data integration is described as possibilities rather than a method, the reinforcement learning framing is overstated given that 30–80 trials cannot refine a deep model, and the headline 5-fold efficiency goal is not supported by the team's own 1.5-fold precedent on a much easier problem. On balance, weaknesses outweigh strengths.<br>• Although AI-driven approaches for identifying transcription factor combinations are novel and interesting, their ability to discover improved reprogramming strategies beyond the Yamanaka factors may be limited by the highly complex, heterogeneous, and noisy nature of cellular reprogramming. CRISPRa/i may not be an ideal validation approach because the activation strength is often insufficient to induce reprogramming. |
| **Significance: Evaluate the project's significance and potential for impact** |
| • There is reason to think that even a small improvement in the industrial manufacture of pluripotent cells will have massive benefits for the industrial manufacture of these cells.<br>• The premise that current reprogramming protocols are insufficient to generate clinical-grade cell lines is overstated, as there are already clinical trials using iPSCs generated with current protocols.<br>• The proposal does not articulate a baseline where AI would actually add value over rational guessing or simple alternatives such as literature mining (as Yamanaka did), gene regulatory network inference, or testing obvious transcription factor candidates.<br>• The current iPSC generation based on the Yamanaka factors is insufficient for producing consistent clinical-grade cell lines. This proposal integrates AI-driven modeling with multiplexed CRISPRa/CRISPRi perturbations to discover, validate, and refine transcription factor (TF) programs that may improve reprogramming efficiency, fidelity, and safety.<br>• It is an interesting and ambitious proposal. The reprogramming process is highly complex, heterogeneous, and noisy, which could make development of accurate AI-learning frameworks and trajectory prediction models challenging. However, if successful, the work could identify novel FateFactors or TF combinations that improve reprogramming efficiency and establish a broadly applicable framework for rational cell fate engineering.<br>• There are several concerns. First, somatic cell reprogramming has been studied extensively for nearly 20 years, and many groups have already screened large numbers of transcription factors and TF combinations. Despite these efforts, the canonical Yamanaka factors remain among the most robust and efficient reprogramming cocktails across diverse somatic cell types. It is uncertain whether the proposed approach will identify improved TF combinations beyond existing methods.<br>• A second concern is the experimental validation strategy using multiplexed CRISPRa/CRISPRi. Compared with direct TF overexpression systems, CRISPRa/CRISPRi-mediated endogenous activation or repression is generally weaker and more variable across loci. As a result, some predicted FateFactor combinations |

may fail to induce sufficiently strong transcriptional changes to meaningfully enhance reprogramming efficiency.

- Finally, the proposal may overstate the extent to which tumorigenic risk can be reduced solely through optimization of transcription factor programs. Tumorigenicity in iPSC generation is multifactorial and is not limited to TF factors.

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

- The project is relatively novel.
- The team has published essentially all the computational building blocks, and the collaborator's lab has established a CRISPR programmable platform; together, this is genuinely innovative work.
- The contrastive learning idea, explicitly modeling failed trajectories rather than just optimized endpoints, is a real conceptual contribution that goes beyond prior efforts, which typically treat transcription factor influence as static across contexts.
- However, parts of the toolkit are not new to this proposal: other groups have developed similar perturbation-prediction platforms, and the CRISPRa/i platform is itself prior work from the collaborator's group.
- This proposal is highly innovative in applying AI-driven modeling to the somatic cell reprogramming process. If successful, it could establish a new framework for identifying novel transcription factor combinations that regulate stem cell fate decisions and improve cellular reprogramming strategies.

**Rationale: Evaluate the scientific rationale in the proposal**

- There is uncertainty about the likelihood of AI learning being able to produce a “Fate Factor.” Lots of generated data may lead nowhere.
- The Manatee precedent is on a much easier problem (esophageal basal cell differentiation and target cells are developmentally adjacent) and yielded only a 1.5-fold improvement, yet the proposal targets at least a 5-fold improvement in reprogramming efficiency and a 50% reduction in timeline on a much harder problem.
- There is a methodological mismatch: Manatee modulated signaling pathway molecules, whereas this proposal activates and represses endogenous transcription factors via CRISPRa/i. These are very different modalities, and CRISPRa achieves only 2- to 10-fold activation, far below the supraphysiological levels of exogenous OCT4, SOX2, and KLF4 needed to break somatic identity.
- There is also an out-of-distribution problem: reprogramming intermediates are precisely the cell states least well represented in the pretraining atlases, and the proposal does not engage seriously with this issue.
- The scientific rationale is sound. The central hypothesis of this proposal is that somatic cell reprogramming can be made more efficient and reliable by identifying transcription factor (TF) programs that function dynamically across different stages of reprogramming, rather than relying on static transcription factor cocktails.

**Plan & Design: Evaluate the project plan and design**

- The main weakness is the relatively low efficacy of the CRISPR methodology in comparison to the Yamanaka protocol with transcription factors.

- The biggest weakness is data integration. The proposal says FateFactor will be focused on reprogramming using a weighted training scheme where the domain-relevance weight "could be" calculated, which is not a method but a list of possibilities; it does not specify how the >10 million-cell pretraining objective constrains the model toward reprogramming-relevant representations, nor what is actually transferred between tiers.

- Tier 3 is mislabeled as reinforcement learning: 3–4 cycles of 12–20 transcription factor combinations (30–80 trials total) cannot meaningfully refine a deep model with millions of parameters; real RL needs thousands to millions of trials, and what is described is closer to Bayesian active learning or few-shot fine-tuning.

- The five different TF search strategies (gradient-based perturbation, attention-guided prioritization, latent covariance and synergistic regulons, RL-based sequential perturbation, multi-criteria ensemble) read more as hedging than as a committed methodological choice.

- The proposal consists of two aims. Aim 1 focuses on developing the AI-based FateFactor. Aim 2 aims to validate FateFactor using CRISPRa/i-mediated somatic cell reprogramming. However, the proposed CRISPRa/i systems, such as dCas9-VP64, are generally much weaker than direct transcription factor overexpression approaches, which may limit their ability to induce sufficient reprogramming responses.

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

- There could be huge benefits for industrial manufacture, as noted above.

- The use of multiple human cell sources (fibroblasts, cardiomyocytes, hepatocytes, neurons) is presented as diversity, but this is cell-type diversity, not population diversity, and the proposal does not really address the latter.

- The data dissemination plan uses well-established platforms, and the data-sharing plan overall is thorough.

- This proposal incorporates diversity in both germline background and somatic cell origin. FateFactor will be trained and evaluated using single-cell datasets derived from fibroblasts, cardiomyocytes, hepatocytes, and neurons obtained from donors representing diverse genetic backgrounds reflective of California's population.

| Application# | DISC5-20094 |
|---|---|
| **Title**<br>(as written by the applicant) | Autoimmunity and Personalized Medicine: Can Blood Stem Cells Predict Future Disease? |
| **Project Objective & Impact**<br>(as written by the applicant) | Dysregulation of the immune system causes life-threatening autoimmune and fibrotic disease, but diagnosis usually occurs after tissue damage. We aim to will bring new ways to predict a person's risk to develop immune disease before tissue damage occurs. We expect to discover new therapies that can pre-emptively prevent progressive immune dysfunction or treat these disorders at an early stage. |
| **Specific Aims**<br>(as written by the applicant) | • Analyze signaling pathways turned on in blood stem cells using new technologies (multiomics) from patients with immune disorders compared to healthy subjects<br>• Study interactions between blood stem cells and the marrow environment that lead to abnormal immune activity using new technologies (spatial transcriptomics) from patients as in Activity 1<br>• Test the effect of targeted molecules on pathways identified in Activities 1 & 2 to block and/or reverse the behavior of blood stem cells that activate abnormal immune responses |
| **Statement of Benefit to California**<br>(as written by the applicant) | We study diseases of immune dysregulation which affect people of all ages, genetic and geographic backgrounds. These disorders cause acute and chronic illness impairing quality of life, school and work performance, family and social lives, and cause early death. We seek to bring new ways to predict who is at risk to develop these diseases to allow prevention and early treatment before tissue damage occurs. Mitigation of these illnesses will benefit the citizens of California and people worldwide. |
| **Funds Requested** | $2,500,000 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

**Final Score: 65**

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

| | |
|---|---|
| **Mean** | 66 |
| **Median** | 65 |
| **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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

**Key Strengths and Weaknesses**

- This is potentially meritorious work, but the notion that HSCs are themselves directly creating the inflammatory milieu is not supported by the preliminary data, and what is shown has some issues.

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

- The project title asks whether blood stem cells can predict future disease, but the study design primarily profiles patients with established severe disease undergoing HSCT. This design can identify disease-associated HSC signatures, but it does not directly test whether these signatures predict future disease onset in at-risk but unaffected individuals.
- The hypothesis that primitive HSCs encode molecular programs that drive—and predict—chronic autoimmune and fibrotic disease will be tested by leveraging paired pre/post-HSCT "reset" sampling in 3 diseases that share fibrotic phenotypes.
- If correct, the hypothesis could reframe how autoimmunity and fibrosis are diagnosed (predictive HSC signatures) and treated (HSC-directed interventions before overt disease).
- The clinical "reset" model—paired pre- and post-HSCT samples from the same patient—is a uniquely powerful within-person design.
- The three diseases span adaptive-immune, hybrid, and fibrotic mechanisms, allowing identification of common HSC-level drivers across distinct clinical syndromes.
- The work is relevant to large patient populations: approximately 4.6% of the US population has at least one autoimmune disease, and ~1 in 8 globally suffer from fibrotic-related diseases.
- There is no clear translational deliverable (e.g., a validated risk-prediction biomarker panel); expected outcomes are candidate signatures rather than a deployable test.
- Allogeneic-HSCT is already a standard for these diseases.
- The applicants posit that understanding the origins of autoimmune disorders could open the possibility of intervening before overt pathology is evident and lead to the development of novel treatment strategies, which would be a very significant achievement.
- The notion that immune dysregulation can act at the HSC level has been previously considered, but whether HSCs in turn help to amplify this feature would be a novel and significant finding.

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

- The proposal integrates sequencing, omics, and in vitro and in vivo bone marrow organoids using HSCs from three distinct disorders. There is interest in the biology of these cells, and hypotheses may be made from the findings.
- Integration of several methods of profiling on patient-derived disease-linked organoids may be powerful.
- Patient-derived (non-iPSC) BM organoids that preserve native disease biology, with NSG kidney-capsule transplantation, may be useful for in vivo validation.
- The hypothesis that HSC-intrinsic programs are causal—rather than reflective of upstream immune signaling—is hard to demonstrate even with the proposed organoid perturbations.
- Cross-platform integration is a major analytical challenge that is not fully addressed.
- The proposed study's innovation lies on the diverse nature of disorders being considered, which include, severe aplastic anemia (SAA), systemic sclerosis (SSc) and myelofibrosis (MF).
- The use of multiple distinct disorders is innovative but also complicates the proposed workflow and the potential underlying causes, which may not be shared across multiple conditions.

**Rationale: Evaluate the scientific rationale in the proposal**

- The applicant argues that all three selected diseases are curable by HSCT, suggesting that the HSCs are central to disease pathology.
- Preliminary data support the idea that HSCs can exhibit inflammatory or profibrotic programs. For example, the proposal shows inflammatory cytokine signatures in the marrow of NOD mice and transcriptional activation of innate immune pathways in HSCs. In MF models and patient samples, the proposal presents evidence for pathway-driven inflammatory and profibrotic programs, activation in stromal cells, and increased signaling in human MF HSCs.
- The key weakness in the rationale is the difficulty of distinguishing cause from consequence. HSCs from patients with severe disease may show inflammatory programs because of chronic inflammation, prior therapies, marrow stress, clonal selection, age, or transplant-related factors. The applicant did not discuss how to disentangle these complicating factors from the biology of the HSCs.
- Preliminary biology is strong: the NOD-mouse HSC inflammatory signature, a named signaling pathway as an MF/fibrosis driver in HSCs, and BM organoid data showing HSC marker preservation at 10 weeks.
- Standard-of-care sample collection at different time points post-HSCT is integrated into the clinical workflow, minimizing protocol burden.
- The selection of candidate inflammatory pathways is not strongly differentiated from prior literature and could benefit from clearer prioritization logic.
- HSC yields from BM aspirates of patients with SAA (a BM failure syndrome with severely reduced HSCs) are inherently limited and may compromise multi-omic profiling on a per-patient basis. This is the central feasibility risk.
- Sample-size calculations are provided per aim but assume uniform yield across disease contexts, which is unrealistic for SAA. SSc enrollment of approximately 3 patients per year is small for a three-year project; statistical power for SSc-specific signatures is likely inadequate even with multicenter access.
- The rationale for the work comes from the well-studied NOD model of T1D, which supports the notion that autoimmune disorders can affect HSC biology. However, the results shown in Figure 1a, as part of the

rationale support, are a bit confusing. If samples were normalized to WT controls, then why are there WT samples showing red or green changes?

- The results shown for the mutant mice are interesting and could be, in themselves, an important area to pursue in further studies. However, the conclusion that mutant HSCs drive myelofibrosis and splenomegaly is perhaps an overreach, as it is likely not the HSCs that drive these outcomes but rather downstream cells that derive from HSCs.

**Plan & Design: Evaluate the project plan and design**

- Aim 1 will profile HSC-intrinsic disease programs with multiomics. Aim 2 will examine the marrow microenvironment spatially. Aim 3 will establish in vitro and in vivo bone marrow organoids to test HSC-niche interactions and secreted inflammatory programs.
- The main weakness is feasibility and focus. The proposal is very broad, spanning three diseases, multiple timepoints, rare HSC populations, spatial profiling, proteomics, organoids, and in vivo transplantation. The project could yield meaningful descriptive datasets but may struggle to deliver clear mechanistic conclusions within the project period.
- In Aim 3, it is not clear how the investigators will prioritize pathways, how many perturbations will be tested, or what criteria will be used to determine that an HSC program is causal rather than correlative.
- Three aims with distinct biological levels (cell-intrinsic, niche, functional organoid) are well organized.
- Statistical considerations and pitfalls are explicitly written for each aim, including power calculations and HSC neighborhood analysis.
- The multidisciplinary team is solid: The PI (HSC and autoimmunity), Co-I 1 (fibrosis and organoids), Co-I 2 (bioinformatics), Co-I 3 (clinical lead), and Co-I 4 (organoid expertise).
- Three aims spanning multiomics, spatial analyses, organoids, and in vivo work are challenging and create contingent risks.
- The organoid platform is acknowledged as lower-throughput than 2D culture and patient-specific (non-iPSC), limiting repeated perturbations from the same source.
- The proposed work is made up of three aims, which will 1) perform multiomic analysis of HSCs pre- and post-HSCT; 2) perform spatial transcriptomics and proteomics of bone marrow samples; and 3) develop BM organoids to study HSC/BM interactions and the secretome from the different disorders.
- Figure 4b appears to be manipulated and/or altered in an attempt to misrepresent results. This is a fatal weakness in the proposed work and sadly diminishes the potential benefits of the proposed work. The applicants argue that this is a placement error; however, it is a lot of effort to flip and reverse an image to use as a placeholder.
- Both Aims 1 and 2 are likely to provide useful and much-needed information, but Aim 3 is likely a challenging approach with a difficult model system, and it is not clear that it will provide clear results.
- Aim 3 is misplaced.

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

- The proposal addresses three diseases with disparities in incidence and outcomes.

- The disease epidemiology section is strong: it quantifies demographic disparities for each disorder (male/Black/Midwestern/rural; female 4–5:1, with earlier onset and higher mortality in Black patients; older White patients, but with worse outcomes in non-Hispanic Black and Hispanic patients).
- The planned collaboration and the partnerships with two patient advocacy organizations provide credible patient-engagement infrastructure that could inform the project.
- Multicenter SSc trial participation could meaningfully broaden geographic and demographic representation for that arm.
- Sample acquisition is concentrated at a single tertiary center for SAA and MF; selection bias (referral-eligible patients who completed HSCT) may limit generalizability and is not directly addressed.
- Diversity of healthy donors (n=4/year) is not addressed, which matters because they serve as the comparator for all disease-state inferences.

| Application# | DISC5-19805 |
|---|---|
| **Title**<br>(as written by the applicant) | Elucidating the impact of deleterious coding variants on serious mental illness phenotypes |
| **Project Objective & Impact**<br>(as written by the applicant) | The biologic underpinnings of serious mental illness (SMI) are poorly understood. Our recent identification of important genetic risk-variants provides an unprecedented opportunity to elucidate this biology, using stem cell models and assessments ranging from molecular to clinical levels. This new knowledge could provide a direct pathway to the development of biomarkers and new treatments. |
| **Specific Aims**<br>(as written by the applicant) | • Re-recruit participants based on their genotypes at ADAP1 and KDM5B, for studies to elucidate the biology underlying serious mental illness (SMI).<br>• Collect biological samples and perform studies to more precisely characterize the relationship between variants and SMI phenotypes.<br>• Conduct stem cell studies to generate cortical organoids to study the function of ADAP1 and KDM5B in human brain development and to characterize phenotype emergence in patient derived lines. |
| **Statement of Benefit to California**<br>(as written by the applicant) | Up to two million Californians live with serious mental illness (SMI) and are therefore at high risk for disability and premature death. The project will elucidate how “high impact” genetic variations recently discovered in a Latin American population, influence SMI — a key step towards development of biomarkers and new treatments. The results of the studies will be particularly relevant to individuals with Latin American ancestry; an underserved population that includes 15 million Californians. |
| **Funds Requested** | $2,499,995 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

**Final Score: 65**

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

| | |
|---|---|
| **Mean** | 64 |
| **Median** | 65 |
| **Standard Deviation** | 7 |
| **Highest** | 70 |
| **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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

| **Key Strengths and Weaknesses** |
|---|
| • Most of the proposal is further population studies of the unique data set. There is little stem cell work, or even the need for stem cells, to accomplish the goals of the experiments. Stem cell work does not correlate to Aims 1 and 2.<br>• An incredible clinical cohort with a generic and underdeveloped stem cell design. |
| **Significance: Evaluate the project's significance and potential for impact** |
| • The goal is to "elucidate biological underpinnings of serious mental illness (SMI)" which is a very ambitious goal.<br>• Understanding the function of these genes could aid in treatment (new drugs) or future gene-based therapies.<br>• We often treat brain disorders with overlapping drugs, so finding some common mechanisms across them could be helpful.<br>• The disorders are quite distinct, so understanding how a single gene contributes to them all requires contextualization of the surrounding molecular networks, which certainly could be done in the scope of this project but is neglected.<br>• Incredibly significant research cohort, Latin American ancestry in Colombia, built on a Paisa population of 9 million with a strong founder effect, and extensive electronic health records from all hospitals in the region. |
| **Innovation: Evaluate the project for innovation relative to the current state of research** |
| • The strength of innovation lies in this novel genetic cohort and proposed functional validation of two novel SMI genes (ADAP1 and KDM5B), one of which is highly understudied.<br>• Coding variants in a few genes common to multiple neuropsychiatric disorders is a rather unique lens, and probably the #1 strength of this project is this cohort which has those.<br>• The project merges expertise in psychiatry, genetics, and stem cell biology, but only in Aim 3.<br>• Massive missed opportunity from protein interaction networks to provide mechanistic details about how the effects of these genes are promulgated. Basically not leveraging the most unique aspect of the study with even a few computational or systems biology approaches.<br>• Major weaknesses are the lack of innovation in the stem cell approach or statistical approaches to integrate stem cell and clinical/genetic datasets. |
| **Rationale: Evaluate the scientific rationale in the proposal** |

- Further investigating what is occurring in patients who have variants of ASAP1 and KDM5B is sound, but it has nothing to do with stem cells (Aims 1 and 2).
- While many of the proposed analyses don't actually supply new or unexpected information about the two genes, the EHR might expand our understanding of their broader effects or maybe even supply variant-specific effects.
- Major Weakness: What is the rationale for reprogramming so many carriers? If variable penetrance is a concern, will recruitment consider and balance for the clinical phenotype of the carrier? Is a knockdown vs. splicing study of KDM5B more warranted? With the knockdown experiments complete, what is the value of the large-scale patient-specific study design?

**Plan & Design: Evaluate the project plan and design**

- Approaches appear sound, but are not using stem cells. The only exception is Aim 3, which used cortical organoids and this is a minor part of the proposal.
- Alternative experiments are only proposed for Aim 3.
- Thoughtful and feasible three-year plan that focuses on developing a future resource (hiPSCs and CSF) and preliminary gene-specific analyses using shRNA. The rationale for generating so many patient-specific mutations with the same variant is unclear. Is there substantial variability in clinical outcomes of carriers? A power analysis explaining whether a sample of 350 carriers is adequately or massively over-powered will be helpful.
- Limited details about how stem cell controls will be recruited; poorly defined computational approach; unclear justification for shRNA vs. CRISPR-editing.
- No power calculation is shown for the effect with the very small number of cell lines they propose to include - other studies with similar design suggest those Ns are very under-powered. It is understood that the applicant wants to demonstrate use of this cohort, but this small example that is unlikely to show an effect just isn't useful. The applicant proposes to use shRNA over CRISPR approach with no explicit justification. Maybe the applicant is trying for a graded effect, however from dealing with shRNA experiments, the off-target effects are often so widespread it makes interpreting results extremely difficult so CRISPR done in parallel is recommended.
- This study has a huge advantage over most complex disease genetics studies as it's mainly focused on coding variants. That means you can do a ton of computational exploration of the likely effects on binding partners, looking at exact interfaces disrupted and exploring all results in light of these putative effects but that is never done.
- Why *[proteomics platform redacted]* instead of some unbiased proteomics? There is the appeal of being guaranteed to get proteins in a particular system, but from dealing with their platform and others for many years it has a difficult background signal and another method may be preferable. This despite belief that this or that perturbation is mainly relevant to a particular system, oftentimes there are effects outside of it that are likely to be missed with the proposed platform, and you'll get just as many proteins within the system of initial interest with other platforms for the same price.
- Explicit examination of any LD structure is advised - is it really just these particular variants, or does the block extend into different genes?
- There are methods to predict brain molecular levels from plasma or CSF but these aren't utilized.

- From what is already known about these genes, it is highly likely they will affect organoids. What really does that mean more broadly for human cognition? How are the MEA parameters measured going to unfurl in broader networks? There are agonists for these proteins - those should be utilized.
- There is a small N, but in addition to what is proposed it would be interesting to compare ADAP1 in SCZ individuals who do not have symptoms (resilience).

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

- Have a very appropriate and diverse cohort lined up for this.
- Strong collection of genetic and environmental information, but no plan as to how to integrate these datasets with proposed functional analyses.
- The population impact statement makes no mention of stem cells and appears to be written only for a population studies application.

| Application# | DISC5-19543 |
|---|---|
| **Title**<br>(as written by the applicant) | Engineering Human Limb Gastruloids for Limb Morphogenesis and Congenital Malformation Modeling |
| **Project Objective & Impact**<br>(as written by the applicant) | Congenital limb malformations are the leading cause of infant physical disability. Model organisms do not recapitulate all aspects of human limb formation due to unique genome regulation, cellular architecture and toxicology. We will generate the first hPSC-gastruloid model of human limb formation enabling mechanistic interrogation of limb morphogenesis and inducers of human limb malformation. |
| **Specific Aims**<br>(as written by the applicant) | • Define how trunk-derived signaling centers coordinate human limb initiation in gastruloids.<br>• Elucidate the ex-utero culture mechanics necessary to enhance human gastruloid limb outgrowth and elongation.<br>• Use AI-enabled phenotyping and screening to define determinants of limb-bud morphogenesis and environmental-induced malformation in human gastruloid limb models. |
| **Statement of Benefit to California**<br>(as written by the applicant) | In California, ~17,000 babies each year are born with birth defects, costing the state ~$1.4B annually in medical care and special education. Medical care is largely surgical and supportive, with few options to prevent or reverse congenital limb defects. This grant will create human-relevant limb models to identify causes, predict risk, and enable safer drug and regenerative strategies evaluated in human models, helping to reduce long-term costs and improve lifelong outcomes for Californians. |
| **Funds Requested** | $2,499,997 |
| **GWG Recommendation** | **(1-84): Not recommended for funding** |
| **Process Vote** | All GWG members unanimously affirmed that “The review was scientifically rigorous, there was sufficient time for all viewpoints to be heard, and the scores reflect the recommendation of the GWG.”<br><br>Patient advocate members unanimously affirmed that “The review was carried out in a fair manner and was free from undue bias.” |

## SCORING DATA

Final Score: -

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

| | |
|---|---|
| **Mean** | - |
| **Median** | - |
| **Standard Deviation** | 8 |
| **Highest** | 60 |
| **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 based on the key questions shown below, which are also described in the PA/RFA. Following the panel's discussion and scoring of the application, the members of the GWG were asked to indicate whether the application addressed the key question and provide brief comments assessing the application in the context of each key question. The responses were provided by multiple reviewers and compiled and edited by CIRM for clarity.

**Key Strengths and Weaknesses**

- The team has no limb expertise. No preliminary data are provided demonstrating that the experiments will work. There is a fundamental flaw in the reasoning of the proposed experiments. The goal is to reproduce limb formation in vitro. Limb formation occurs during a very short window during embryogenesis. At this time of human development, it would be unknown (and unknowable) that there would be anything wrong with development.
- The goal of advancing gastruloid models to incorporate limb development is ambitious and potentially transformative, but the lack of convincing preliminary data to support that the team can induce early stages of limb formation makes the risk of this project very high.
- There is a need for an in vitro model for limb development in order to screen for risks, such as developmental toxicity of compounds. However, the grant is weakened by the paucity of preliminary data and the plan to develop such a model is mostly in the planning stages.

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

- Reactivating the "stalled" pathways for regeneration has been performed in vivo with mixed results (in vertebrate models). It is not clear why an artificial system would be better than in vivo systems that have been used for decades to investigate vertebrate regeneration.
- There is a fundamental flaw in the reasoning of the proposed experiments. The goal is to reproduce limb formation in vitro. Limb formation occurs during a very short window during embryogenesis. At this time of human development, it would be unknown (and unknowable) that there would be anything wrong with development. While it is essential to understand how the normal limb forms, regeneration/repair strategies focus on the postnatal, fully patterned limb.
- The only preliminary data are that the team has been able to "generate gastruloid models where the early upper limb bud signaling centers are just emerging." The data show "relative expression" of four genes, all of which are also expressed elsewhere in an embryo.
- If successful, the project would comprise a significant advance in the use of human pluripotent stem cells in modeling human development. To date, no models for human limb bud development exist.
- The models generated would provide new insights into mechanisms of limb bud development and suggest strategies to correct disease and malformations of limb development.
- The model would provide a platform for screening effects of drugs and other compounds on limb development.
- This research aims to develop a human limb organoid model, which would be the first. Such a model could allow the interrogation of mechanisms and insults that lead to limb morphogenesis during embryonic growth.
- Limb formation defects are a significant fetal problem. Better understanding and the ability to screen compounds for limb dysmorphogenesis risk could

reduce the incidence of limb birth defects.

- The single major weakness of this proposal is that key proof-of-concept preliminary data that would show that a limb development model is possible is not in place.

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

- Successful completion of the aim would require stem cells, bioengineering, and an experienced developmental biologist. The team lacks expertise in limb development/formation/regeneration.
- The advancement of gastruloids to limb bud development using trunk-derived signaling is innovative.
- The project employs innovative technologies to control the gastruloid microenvironment to generate and stabilize limb buds.
- The concept that the specific trunk-derived signals or the mechanical and oxygen signals influence limb bud development in gastruloids is not well supported.
- The use of spatial transcriptomics and high-content imaging along with AI provides some plan for integrating differing and complementary approaches.

**Rationale: Evaluate the scientific rationale in the proposal**

- The PI appears to switch between regeneration and limb development. These are two very different processes (even if some of the genes/pathways involved are the same).
- In the same vein, the PI discusses the idea of using hPSCs to uncover how human limbs develop and regenerate. Human limbs do not regenerate (exception: the tips of digits in babies). The inability of human limbs to regenerate is the very reason why humans (and even mice) are not studied in the context of limb regeneration (the exception being the spiny mouse, which has a remarkable ability to regenerate many of its body parts).
- Fundamental flaw in the thought.
- The premise of establishing limb buds in a human gastruloid is very ambitious but the means to do so are highly speculative. The concept of using FGF, oxygen, pressure, and shear is based in animal embryo development but it is not clear that these will be effective in the gastruloid models.
- The innovation and risk are balanced but the project would be significantly strengthened with a proof of concept of limb bud generation in the gastruloids.
- The use of a human system is warranted, since rodents are not affected by thalidomide, whereas humans are. This demonstrates that limb development in humans is at least partially different from rodents, and that rodent models are not sufficient.

**Plan & Design: Evaluate the project plan and design**

- The use of spatial transcriptomics and high-content imaging along with AI provides some plan for integrating differing and complementary approaches.
- The team is strong but would benefit from inclusion of a limb developmental biologist.

- The plan to first induce lateral plate mesoderm then generate and stabilize limb buds using developmental signals is well-considered. The hypotheses are clear and plans to evaluate them through molecular analysis are detailed and appropriate.

- The application suffers from the lack of a scientist in the field of limb development. It is highly suggested that the applicants review the paper: "Digits in a dish: An in vitro system to assess the molecular genetics of hand/foot development at single-cell resolution" from 2023 (https://doi.org/10.3389/fcell.2023.1135025). This report has come closest to inducing limb development in vitro (starting material was mouse limb bud mesenchyme).

- Aim 3 is a proof of concept of using the model to evaluate the ability of compounds to inhibit or induce limb buds. The use of reporters to assess different stages of limb development is a strength. The screens appear underdeveloped, lacking discussion of controls, throughput, and validation.

- The use of morphometric features in Aim 3 is a strength. It is unclear how AI will advance the goals of this aim, though.

- Aims 1 and 2 seem somewhat related, and could possibly be combined into a single aim-namely to achieve limb bud and limb growth in a gastruloid model.

Aim 3 will use the limb model to investigate mechanisms of limb dysmorphogenesis using AI and compound screening. Aim 3 is dependent upon the success of Aims 1/2.

- The goal of achieving a limb development organoid model (in this case starting with gastruloids) is ambitious. Though there is some preliminary work on working with gastruloids and also some evidence that neural ectoderm differentiation can be achieved, there are no strong proof of concept preliminary data to show that development of this model is well on the way.

- The limb organoid model is really only in the planning stages at this time. There is considerable risk that the model will not be realized as a consequence.

- The use of relevant reporter genes in the cells used will be helpful.

- A huge number of studies using model systems have recapitulated many of the limb defects observed in humans. It is difficult to come up with a human limb defect (in which the genetic cause is known) that has NOT been reproduced in a model system.

- The following statement is not well supported: "Model systems do not fully recapitulate most of the abnormalities seen in human limb malformations, nor do they replicate the regenerative responses (or lack thereof) seen in humans." The applicants provide no references to support this statement, and there are many examples in model systems that led to the discovery of the underlying genetic causes of human limb issues based on similar phenotypes.

- Pitfalls include switching FGF if outgrowth does not occur. As there does not appear to be much difference in the limb regarding how these FGFs function (see work of Gail Martin and Xin Sun from the 1990s, in particular their mouse KO studies), if one FGF does not work, using a different FGF will likely also not work.

- The pitfalls section of Aim 1 essentially states that the applicants anticipate succeeding, but with some plan to test FGF variants if the original plan fails to induce limb buds.

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

- This appears to be a new collaboration. No educational effects were described.

- The project has the potential to impact understanding of limb development and associated birth defects. Efforts to engage community stakeholders are appropriate.

- The grant will use a diverse set of hPSCs and also screen for compounds that might cause limb defects that diverse populations might encounter.