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W81XWH-21-1-0323

TITLE:
RNA-Directed Therapy for C9ORF72-Linked ALS Using Engineered Zinc Finger Nucleases

PRINCIPAL INVESTIGATOR:
Yeo, Eugene, PhD MBA, Professor

CONTRACTING ORGANIZATION:
The Regents of the University of California San Diego, La Jolla, CA

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Fort Detrick, Maryland 21702-5012

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Yeo, Eugene, PhD MBA, Professor

E-Mail:

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

UNIVERSITY OF CALIFORNIA, SAN DIEGO
OFFICE OF CONTRACT & GRANT ADMINISTRATIO
S. CORTLANDT URQUHART, JD, LLM
9500 GILMAN DR
LA JOLLA CA 92093-5004

8. PERFORMING ORGANIZATION REPORT NUMBER

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U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

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12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

In this reporting period, we have established a panel of C9-ALS patient specific spinal organoids derived from patient iPSCs as well as neurotypical controls that exhibit key pathological features of disease including sense (G4C2) and antisense (C4G2) RNA foci. We have also generated human zinc finger fusion proteins that reduce these molecular phenotypes in C9-ALS spinal organoids. We have completed single cell RNAseq libraries from cohorts of spinal organoids and have established gene markers that delineate the cell type composition within our new *in vitro* preclinical model. Potential RNA off-targets of Z1 were also analyzed in human cell lines with enhanced cross-linking and immunoprecipitation.

15. SUBJECT TERMS

C9ALS, C9ORF72, spinal organoids, zinc finger, RNA foci

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	22	**19b. TELEPHONE NUMBER** *(include area code)*

TABLE OF CONTENTS

1. INTRODUCTION:

***ALS caused by mutations in the gene* C9ORF72.** GGGGCC hexanucleotide repeat expansions, $(G_4C_2)^{exp}$, in the first intron of the C9ORF72 gene is the most common cause of familial ALS (C9-ALS) and have been linked to RNA-mediated pathogenesis by the formation of toxic RNA foci formed from both sense $(G_4C_2)^{exp}$ and antisense $(C_4G_2)^{exp}$ C9ORF72 transcripts. Such intranuclear RNA foci have been attributed to the onset of disease by causing RNA-mediated pathogenesis including, RNA binding protein (RBP) sequestration, RNA splicing alterations, translation of repetitive polypeptides, and alteration of nuclear-cytoplasmic transport. Although the molecular etiology of C9-ALS remains unclear, it is widely accepted that elimination of the repetitive RNA produced by this locus provides a universal therapeutic principle for patients suffering from C9-ALS. While therapeutic approaches involving antisense oligonucleotides (ASOs) have shown promise in preclinical studies, ASOs must be continuously re-administered for the life of the patient which can pose safety issues in the affected CNS tissues. The difficulty associated with delivery to the CNS has thwarted countless therapeutics, but there is a recent resurgence in virally-delivered therapeutics to many tissues including the CNS. Our ZNF-based approach is composed entirely of human protein that can be encoded in viral particles and targeted to the CNS or delivered throughout the spinal cord via subpial administration, to support long-lived (10 years or more) expression of the therapeutic system. This unique combination of tissue-targeted delivery and efficient cleavage of toxic RNAs positions us to generate an entirely new class of therapeutics with a first application focused on C9-ALS.

2. KEYWORDS:

C9ALS, C9ORF72, spinal organoids, zinc finger, RNA foci

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1. Evaluation of ZNF fusion proteins to eliminate repeat expansion RNA in patient-specific spinal organoids.
Major Task 1: Generation and characterization of spinal organoids from a panel of C9ALS patient iPSCs and neurotypical controls (6 months). ***(100% complete)***
Major Task 2: Validation of an ZNF fusion proteins for targeted cleavage of G_4C_2 and C_4G_2-containing RNAs in C9-ALS spinal organoid models (9 months). ***(100% complete)***

Aim 2. *In vivo* safety studies of the RNA-targeting ZNF fusion protein system.
Major Task 3: Subpial injection of AAV9-packaged ZNF fusion protein system into mice (Timeline 12 months) ***(20% complete)***

Aim 3: *In vivo* efficacy studies of the scAAV9-delivered ZNF fusion proteins in C9-ALS mouse models.

Major Task 4: Generate AAV9 titers that express RNA transcript containing 66 sense and antisense C_4G_2 repeats.

Major Task 5: Evaluation of AAV9-delivered ZNF fusion protein to reduce RAN dipeptide repeat protein and RNA foci in C9-ALS mouse model.

What was accomplished under these goals?

1. Successful demonstration of reduction of molecular hallmarks of C9-ALS in a panel of patient iPSC-derived spinal organoids, *relevant to Major Tasks 1+2.*

We generated cerebral and spinal organoids from several C9-ALS patient iPSCs with varying repeat expansion RNA lengths, as well as healthy control lines, in order to test whether these cellular models recapitulate the hallmark presence of repeat expansion RNA foci and to test the efficacy of our Z1 therapeutic candidate. Using our spinal organoid differentiation protocol we developed, Organoids were characterized at three developmental time points, corresponding to (1) the specification of neuronal progenitors, (2) the establishment of mature cortical or motor neurons and (3) the emergence of glia. As illustrated in **Fig. 1**, using our high-titer Z1 AAV preparations, we ***demonstrated reduction (by ~50%) of G4C2 and C4G2 repeat foci*** in patient iPSC-derived C9ALS cortical organoids.

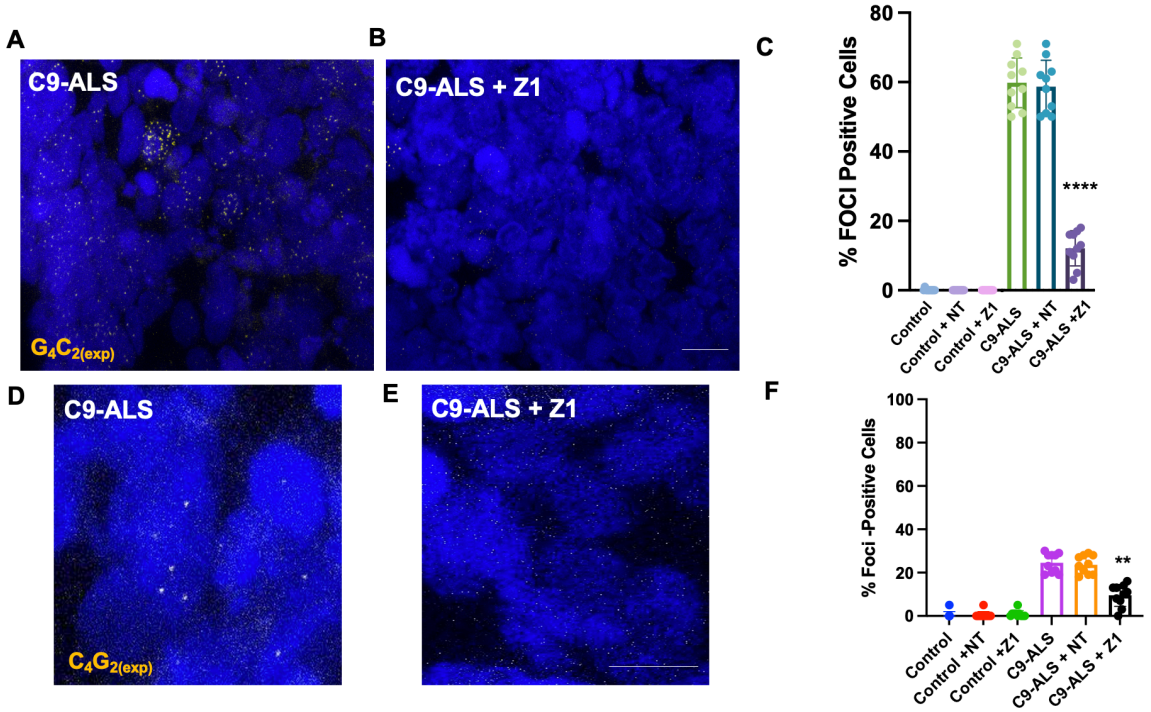

Figure 1. Repeat-targeting ZNF (Z1) constructs reduce levels of G_4C_2 and C_4G_2 repeat expansion RNAs in C9ALS spinal organoids. (**A-B**) Images of RNA-FISH with antisense probe recognizing G_4C_2-expanded RNA expansions in 2-month old C9-ALS spinal organoids, with and without introduction of the Z1 system. Scale bar = 50 µm at 20x. (**C**) Bar plots summarizing the frequency of G_4C_2 foci-positive cells across organoid lines. Graph shows mean ± SEM (n= 10 individual organoids per line; 3 individual C9-ALS lines and 3 individual control lines; *N* = 2; one-way ANOVA, Tukey's Multiple Comparison Test; **** $p < 0.0001$). (**D-E**) Images of RNA-FISH with antisense probe recognizing C_4G_2-expanded RNA expansions in 2-month old C9-ALS spinal organoids, with and without introduction of the Z1 system. Scale bar = 25 µm at 40x. (**F**) Bar plots summarizing the frequency of G_4C_2 foci-positive cells across organoid lines. Graph shows mean ± SEM (n= 10 individual organoids per line; 3 individual C9-ALS lines and 3 individual control lines; *N* = 2; one-way ANOVA, Tukey's Multiple Comparison Test; ** $p < 0.01$).

Successful profiling of spinal organoids cell composition with single-cell RNA-seq, *relevant to Major Task 3.*

To begin safety and efficacy studies at cell-type resolution, we performed single-cell RNA-seq to identify cell types represented in our organoids. Unsupervised clustering was implemented on the combined dataset of ~6,000 cells from cortical organoids that consisted of ~1865 cells per organoid line and ~17,575 reads per cell to identify cell type-specific clusters in control spinal organoids at 2-months post-differentiation. The type of cell represented by each cluster was determined by the identification of established cell type markers within the 50 highest-expressed genes (**Fig. 2**). Future directions include using these cell markers to quantify cell type composition within C9-ALS organoids and compared them to control lines. We will also conduct cell-type specific differential expression analyses to assess cell-type specific molecular biomarkers of disease and off targets of Z1 in the human transcriptome.

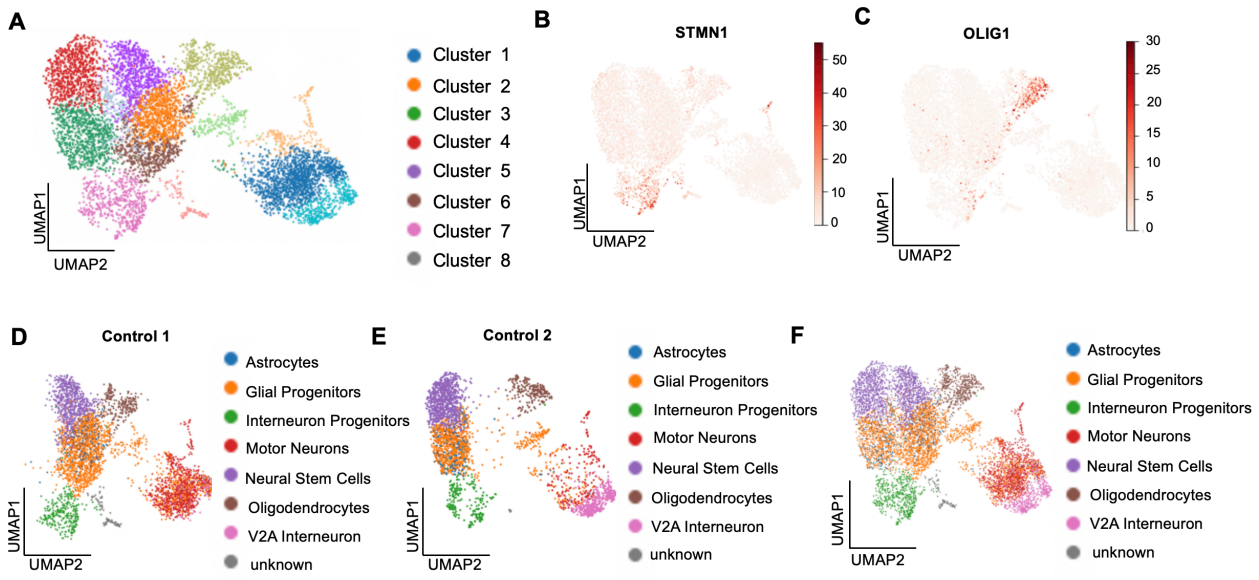

Figure 2. (**A**) Uniform Manifold Approximation and Projection (UMAP) of 2-month old spinal organoids from two individual neurotypical iPSC lines color-coded by sample following Harmony batch correction. (**B-C**) UMAPs representing two examples of cell type specific markers; (B) Same UMAP as in (A) but color-coded based on the expression of representative genes corresponding to interneuron progenitors (STMN1) and (C) oligodendrocytes (OLIG1). (**D-E**) UMAP color-coded based on the expression of representative genes corresponding to all different cell-types expected to be present at 2 months post-differentiation in two individual control spinal organoid lines. (**F**) UMAP of both control lines combined color-coded based on the expression of representative genes corresponding to all different cell-types expected to be present at 2 months post-differentiation in two individual control spinal organoid lines.

Successful analysis of Z1 RNA off-targets in human cell lines with enhanced cross-linking and immunoprecipitation, relevant to *Major Task 3.*

To determine potential off-targets in the human transcriptome of the Z1 ZNF fusion protein, we overexpressed flag-tagged zinc finger motif within the Z1 ZNF fusion protein in HEK293 and performed enhanced crosslinking and immunoprecipitation (eCLIP) with an IP-grade anti-FLAG antibody. Z1-FLAG eCLIP libraries were performed in biologically independent duplicates, with each replicate experiment consisting of a Z1-FLAG (IP) and a paired size-matched input (SMInput) library. We used CLIPper to identify clusters of reads representing regions in the transcriptome significantly associated with Z1-FLAG binding in HEK293, only 19 significant overlapping RNA interactions (peaks) were detected in protein-coding transcripts in both biological replicates. These data suggest that Z1 fusion proteins will have limited off-target RNA interactions. Formal off-target analyses will be conducted in each *in vivo* and *in vitro* models in upcoming experiments.

What opportunities for training and professional development has the project provided?

This project was not intended to provide training and professional development opportunities. However, it has provided the postdocs working on this project (Kathryn Morelli, Yeo lab; Mariana Bravo-Hernández, Marsala lab) with an opportunity to expand their skill sets, co-lead a multi-disciplinary project, and present work in departmental and institution-wide meetings.

How were the results disseminated to communities of interest?

All postdocs have presented this project at departmental, UCSD-wide meetings. Dr. Morelli is currently seeking a faculty position and has presented these data at her faculty interviews.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

1. Development of a panel of patient-specific pre-clinical model of C9-ALS: Given the rapid progress in using stem-cell derived organoids models to study mechanisms of disease and for screening therapeutic candidates, we anticipate that this demonstration will have a major impact on preclinical research in the field of ALS and other nervous system diseases.

2. Development of a new RNA-targeting therapeutic based on human RNA-targeting zinc finger motifs: Our compelling data from our newly developed organoid cellular model of ALS constitutes a highly promising new therapeutic modality to treat repeat expansion diseases. We are excited to validate these findings in animal models of ALS and perform the proposed in vivo safety and efficacy studies .

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Nothing to report

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to report

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report

Significant changes in use or care of human subjects

Nothing to report

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Nothing to report

6. PRODUCTS:

Publications, conference papers, and presentations

Journal publications.
Dr. Morelli is currently preparing a manuscript for submission that describes the spinal and cortical organoid preclinical paradigms.

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Drs. Yeo and Morelli have presented this work at several departmental seminars.

Website(s) or other Internet site(s)

Nothing to report

Technologies or techniques

We have demonstrated the proof-of-principle of a new RNA-targeting therapeutic modality engineered with human zinc finger domains. We have also validated stem cell models of spinal development as new tools for preclinical research in ALS.

Inventions, patent applications, and/or licenses

Several inventions have been disclosed to our Technology Transfer Office at UCSD and are under review.

Other Products

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name	Gene Yeo
Project Role	PD/PI
Researcher identifier	ORCID 0000-0002-0799-6037
Nearest person month worked	1
Contribution to project	Project lead
Funding support	This grant

Name	Martin Marsala
Project Role	Co-Investigator

Researcher identifier	ORCID 0000-0001-5048-6422
Nearest person month worked	1
Contribution to project	Lead of subpial injections
Funding support	This grant

Name	Kathryn Morelli
Project Role	Postdoc
Researcher identifier	ORCID 0000-0002-3123-5369
Nearest person month worked	9
Contribution to project	Design/generation of AAV constructs, in vitro stem-cell based assays
Funding support	This grant

Name	Mariana Bravo-Hernandez
Project Role	Postdoc
Researcher identifier	ORCID 0000-0001-8762-0357
Nearest person month worked	3
Contribution to project	Animal husbandry, *in vivo* characterization
Funding support	This grant

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

YEO

Closed:

RNA-directed therapy for C9ORF72-linked ALS

DoD AL180126	07/01/2019-06/30/2021
	0.12 calendar
U.S. Department of Defense	(annual direct costs Yeo)

Application of RNA-targeting Cas9 to Fuchs' dystrophy

NIH R01EY029166	04/01/2018-03/31/2022
	0.30 calendar
	(annual direct costs Yeo)

Dissecting splicing factor mutations in iPSCs

NIH R01HL137219	04/05/2017-01/31/2021
	0.95 calendar
	(Yeo annual direct costs)

Analysis of functional genetic variants in RNA processing and expression

NIH U01HG009417	02/01/2017-01/31/2021
	0.45 calendar

(annual direct costs Yeo)

Using single-cell RNA-seq to interrogate host immunity to pathogens
NIH R01AI123202 09/23/2016-08/31/2021
0.60 calendar
(annual direct costs Yeo)

Reconstruction of cardiovascular regulatory networks from large-scale single-cell analyses of cardiovascular lineages
NIH R01HD085902 03/01/2016-02/28/2021
1.00 calendar
(annual direct costs Yeo)

Collaboration on preclinical autism cellular assays, biosignatures, and network analyses (Copacabana)
NIH U19MH107367 09/21/2015-06/30/2021 (NCE)
1.80 calendar
(annual direct costs Yeo)

The Role of Alternative Splicing in Neurodegeneration
NIH 1R56AG055824-01A1 09/15/2019-08/31/2020
0.45 calendar
(annual direct costs Yeo)

Inhibition of UBAP2L as a treatment of fragile X syndrome
SFARI Pilot Award 668241 02/01/2020-01/31/2022
0.12 calendar
(annual direct costs)

SARS-CoV-2 proteome interaction with host transcriptome
UCSD/UCOP R00RG2636 04/15/2020-10/14/2020
0.01 calendar
(annual direct costs Yeo)

Targeting RNA binding proteins (RBPs) in Fragile X Syndrome: small molecule inhibitors of UBAP2L
UCSD 20202249 02/25/2020-02/24/2022
(Takeda Millennium Pharmaceuticals) 0.01 calendar
(annual direct costs)

Development of SG-PROTACs to target stress granule-associated protein aggregation
Internal UCSD Seed Grant 9/01/2019-09/01/2020
0.01 calendar
(annual direct costs Yeo)

New:

RNA-Directed Therapy for C9ORF72-Linked ALS Using Engineered Zinc Finger Nucleases

(THIS GRANT)

Major Goals: The goal of this project is to develop adenoviral vector (AAV)-delivered RNA-targeting zinc finger protein effectors as therapeutic candidates for treatment of amyotrophic lateral sclerosis caused repeat expansion in C9ORF72 (C9ALS). We will evaluate the ability of to eliminate repeat expansion RNA in patient cell lines and perform in vivo safety and efficacy studies in mice.

Status of Support: Active

Project Number: DoD AL29052

Name of PD/PI: Yeo

Source of Support: U.S. Department of Defense

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/2021-12/2022

Total Award Amount (including Indirect Costs): (Yeo and Marsala)

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2022	00.12

Subcellular RNA dynamics in HD

Major Goals: We aim to define global mRNA localization landscapes in normal and Huntington's disease iPSC-motor and striatal neurons to evaluate mRNA localization in nuclear, cytoplasmic, and insoluble fractions.

Status of Support: Active

Project Number: 2021-235103 (5022)

Name of PD/PI: Thompson

Source of Support: Chan Zuckerberg Initiative

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available): 07/2021-06/2022

Total Award Amount (including Indirect Costs):

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	00.12

Genomic sequencing of SARS-CoV-2 to investigate local and cross-border emergence and spread

Major Goals: The goal of this contract is to develop and perform a COVID-19 genomic sequencing project to understand viral transmission dynamics. Dr. Yeo is responsible for development and expansion of open-source software for sample tracking and data analysis.

Status of Support: Active

Project Number: 5D30120C09795

Name of PD/PI: Andersen

Source of Support: U.S. Centers for Disease Control

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available): 08/2020-07/2022

Total Award Amount (including Indirect Costs):

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2022	00.24

Harnessing Technological Innovation and Community-Engaged Implementation Science to Optimize COVID-19 Testing for Women and Children in Underserved Communities

Major Goals: The goal of this supplement is to provide minorities and underserved communities in the San Diego area with facile, rapid and affordable access to COVID-19 testing.

Status of Support: Active

Project Number: P42 ES010337-19S1

Name of PD/PI: Tukey

Source of Support: NIH/NIEHS

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2020-08/2022

Total Award Amount (including Indirect Costs):

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2022	00.01

Identification of HTT interaction partners

Major Goals: The goal of this project is to use state-of-the art mass spectrometry and cross-linking immunoprecipitation assays in pluripotent stem cell-derived striatal neurons to identify protein and RNA interaction partners of mutant and normal huntingtin, and to validate these in postmortem brain tissue.

Status of Support: Active

Project Number: UCSD Proposal ID: 29557

Name of PD/PI: Yeo

Source of Support: CHDI Foundation

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available): 11/2020-10/2022

Total Award Amount (including Indirect Costs):

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2022	00.12

Effect of TDP-43 targeting biologics on stress granule formation

Major Goals: The Yeo lab will (1) generate lentiviral vectors from 4 proprietary plasmid constructs provided by ProMIS, and (2) perform stress granule resolution assays in iPSC-derived ALS and control motor neurons treated with the lentiviral vectors.

Status of Support: Active

Project Number: UCSD #2020111 (Lab service agreement)

Name of PD/PI: Yeo

Source of Support: ProMIS Neurosciences, Inc, Canada (**FOREIGN ENTITY**)

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available): 12/2021-12/2022

Total Award Amount (including Indirect Costs):

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2022	00.01

Modulation of RNA subcellular localization for the treatment of neurological diseases

Major Goals: The goal of this project is to generate comprehensive maps of RNA localization in cultured neurons and brain tissue at subcellular resolution and transcript isoform specificity, relevant to Parkinson's disease.

Status of Support: Active

Project Number: Roche ROADS Innovation Grant

Name of PD/PI: Yeo

Source of Support: F. Hoffmann – La Roche Switzerland (**FOREIGN ENTITY**)

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/2021-12/2022

Total Award Amount (including Indirect Costs):

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2022	00.01

Investigation of nuclear pore complex alterations in ALS/FTD

Major Goals: In this extension to our existing grant, we focus on a candidate protein we have identified to control aberrant nuclear pore complex integrity in ALS/FTD and perform a genome-wide screen for modulators of this protein.

Status of Support: Active

Project Number: NDCN Collaborative Science Extension

Name of PD/PI: Rothstein, Yeo

Source of Support: Chan Zuckerberg Initiative

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available): 03/2022-02/2023

Total Award Amount (including Indirect Costs):

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2023	00.12

Early Manifestations of Subcellular Defects in Neurodegenerative Diseases

Major Goals: In this project, we test the hypothesis that genetic mutations that cause late-onset neurodegenerative diseases lead to molecular and cellular defects in early development. We generate augmented stem cell models, to discover normal and aberrant changes in the protein and RNA components and identify the critical sub-cellular compartments and their components that protect the cell against degenerative disease.

Status of Support: Active

Project Number: Allen Distinguished Investigator Award

Name of PD/PI: Yeo

Source of Support: Paul G. Allen Family Foundation

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available): 08/2020-08/2023

Total Award Amount (including Indirect Costs):

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2022	02.40
3. 2023	02.40

Dissecting the role of FMRP in RNA processing using human stem cell models

Major Goals: The goal of this project is to elucidate fundamental molecular mechanisms of the RNA binding protein FMRP in relevant human cell types. Dr. Yeo is responsible for performing RNA-seq and eCLIP studies.

Status of Support: Active

Project Number: R01 HD101534-01A1

Name of PD/PI: Barrett

Source of Support: NIH/NICHD

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available): 09/2020-08/2024

Total Award Amount (including Indirect Costs):

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2022	00.23
3. 2023	00.23
4. 2024	00.23

Platforms to accelerate development of ASO therapeutics for haploinsufficiencies associated with autism

Major Goals: In this project, we develop technologies for treatment of genetic forms of autism, focusing on RNA-targeted antisense oligonucleotides (ASOs) to modulate gene expression and RNA processing.

Status of Support: Active

Project Number: SFARI 674442

Name of PD/PI: Sebat

Source of Support: Simons Autism Foundation

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available): 12/2021-11/2024

Total Award Amount (including Indirect Costs):

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	00.12
2. 2023	00.12
3. 2024	00.12

STAMP technology to enable single-cell and isoform-sensitive detection of RBP sites

Major Goals: The goal of this project is to develop a novel scalable technology, reagent resource, experimental protocols and a computational framework for detecting RBP-RNA targets and translation at the single-cell and single-molecule levels.

Status of Support: Active

Project Number: R01 HG011864

Name of PD/PI: Yeo

Source of Support: NIH/NGHRI

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available):

08/2021-07/2025 Total Award Amount (including Indirect Costs):

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	00.60
2. 2023	00.60
3. 2024	00.60
4. 2025	00.60

Enhancer Connectomes in Regulation of Gene Expression

Major Goals: The goal of this project is to study enhancer activation by liganded nuclear receptors, with a focus on ribonucleoprotein condensates assembled on estrogene receptor-occupied promoters. Dr. Yeo is responsible for providing expertise and analysis methods for real-time imaging experiments of enhancer bursting and single molecule studies.

Status of Support: Active

Project Number: R01 DK018477

Name of PD/PI: Rosenfeld

Source of Support: NIH/NIDDK

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/2022-12/2026

Total Award Amount (including Indirect Costs):

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	00.12
2. 2023	00.12
3. 2024	00.12
4. 2025	00.12
5. 2026	00.12

RNA-directed therapy for Huntington's disease

Major Goals: In this project, we will develop preclinical proof-of concept for therapeutic approaches for Huntington’s disease (HD) that are based on novel engineered proteins targeting the toxic HD repeat RNAs.

Status of Support: Active

Project Number: DISC2-13102

Name of PD/PI: Yeo

Source of Support: California Institute for Regenerative Medicine

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available): 04/2022-03/2024

Total Award Amount (including Indirect Costs):

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2023	02.40
2. 2024	02.40

A comprehensive binding and functional map of human RNA-binding proteins

Major Goals: This ENCORE (Encyclopedia of RNA Elements) project produces physical resources and datasets that form the reference annotation for RNA binding protein-RNA interactions in the human genome, providing a standard for insights into co- and post- transcriptional regulation in the short run, and consequences of expressed genetic variation in the long term, contributing to fundamental knowledge to benefit public health.

Status of Support: Active

Project Number: U24 HG009889

Name of PD/PI: Graveley, Yeo

Source of Support: NIH/NHGRI

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available): 02/2022-03/2026

Total Award Amount (including Indirect Costs):

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2023	01.20
2. 2024	01.20
3. 2025	01.20
4. 2026	01.20

MARSALA

Closed:

RNA-directed therapy for C9ORF72-linked ALS

DoD AL180126	07/01/2019-06/30/2021
	0.84 calendar
U.S. Department of Defense	(annual direct costs Marsala)

New:

RNA-Directed Therapy for C9ORF72-Linked ALS Using Engineered Zinc Finger Nucleases (THIS GRANT)

Major Goals: The goal of this project is to develop adenoviral vector (AAV)-delivered RNA-targeting zinc finger protein effectors as therapeutic candidates for treatment of amyotrophic lateral sclerosis caused repeat expansion in C9ORF72 (C9ALS). We will evaluate the ability of to eliminate repeat expansion RNA in patient cell lines and perform in vivo safety and efficacy studies in mice.

Status of Support: Active

Project Number: DoD AL29052

Name of PD/PI: Yeo

Source of Support: U.S. Department of Defense

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available): 01/2021-12/2022

Total Award Amount (including Indirect Costs): (Yeo and Marsala)

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2022	00.12

Porcine Center

Major Goals: To establish large animal models for pre-clinical testing of proposed human therapeutics and diagnostics for translation of stem cell discoveries to clinical trials and eventual clinical practice.

Status of Support: Active

Project Number: Porcine Center

Name of PD/PI: Marsala, Martin

Source of Support: Sanford Stem Cell Clinical Center at UCSD

Primary Place of Performance: UCSD

Project/Proposal Start and End Date: 03/2017 – 04/2024

Total Award Amount (including Indirect Costs):

* Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
5. 2022	1.3
6. 2023	1.3
7. 2024	1.3

Title: ***Determining stathmin-2 function and potential as a therapeutic target in ALS/FTD***

Major Goals: Characterization of the role of stathin-2 in the initiation and progression of familiar forms of ALS.

Status of Support: Active

Project Number: R01 NS112503

Name of PD/PI: Cleveland (contact), Lagier-Tourenne, Marsala

Source of Support: NIH/NINDS+NIA

Primary Place of Performance: University of California, San Diego

Project/Proposal Start and End Date: (MM/YYYY) (if available): 04/2020-03/2025

Total Award Amount (including Indirect Costs):

Person Months (Calendar) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2022	00.60
3. 2023	00.60
4. 2024	00.60
5. 2025	00.60

In vitro and in vivo characterization of human and porcine neural precursor

Major Goals: Development of in vitro-expandable neuronal precursors cell lines derived from re-programmed pluripotent stem cells.

Status of Support: Active

Project Number: 305864-00001

Name of PD/PI: Marsala, Martin

Source of Support: Sumitomo Industries, Ltd. (**FOREIGN ENTITY**)

Primary Place of Performance: UCSD

Project/Proposal Start and End Date: 09/2020 – 09/2025

Total Award Amount (including Indirect Costs):

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
2. 2022	variable

Year (YYYY)	Person Months (##.##)
3. 2023	variable
4. 2024	variable
5. 2025	variable

Cervical SCI cell-grafting efficacy study

Major Goals: Characterization of treatment effect after spinal human oligodendrocyte cell grafting in rats with spinal cervical traumatic injury.

Status of Support: Active

Project Number: 306395-00001

Name of PD/PI: Marsala, Martin

Source of Support: Lineage

Primary Place of Performance: UCSD

Project/Proposal Start and End Date: 03/2021 – 03/2022

Total Award Amount (including Indirect Costs):

Person Months (Calendar/Academic/Summer) per budget period.

Year (YYYY)	Person Months (##.##)
1. 2022	variable

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

(No Special Reporting Requirements stated in the Notice of Award.)

9. APPENDICES:

None