AWARD NUMBER: W81XWH-19-1-0181
AL180126

TITLE: RNA-Directed Therapy for C9ORF72-Linked ALS

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REPORT DATE: OCTOBER 2021

TYPE OF REPORT: FINAL

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
OCTOBER 2021	FINAL	07/01/2019–06/30/2021

4. TITLE AND SUBTITLE

RNA-Directed Therapy for C9ORF72-Linked ALS

5a. CONTRACT NUMBER
W81XWH-19-1-0181

5b. GRANT NUMBER
AL180126

5c. PROGRAM ELEMENT NUMBER
N/A

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5d. PROJECT NUMBER
0011329395

5e. TASK NUMBER
N/A

5f. WORK UNIT NUMBER
N/A

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
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8. PERFORMING ORGANIZATION REPORT NUMBER
N/A

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

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

10. SPONSOR/MONITOR'S ACRONYM(S)
N/A

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
N/A

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES
None

14. ABSTRACT
In this reporting period, we have established and extensively validated a new mouse model of C9ALS that shows robust disease-relevant molecular and neuromuscular phenotypes. We have established a stem-cell based spinal and cortical differentiation paradigm that recapitulates development of repeat RNA foci and poly-dipeptide translation. We have also generated Cas13d-based adeno-associated viral vectors that robustly reduce these molecular phenotypes in cellular assays including stem-cell derived spinal and cortical organoids.

15. SUBJECT TERMS
NONE LISTED

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	13	USAMRDC

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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1. INTRODUCTION

C9ALS is caused by expression of RNA repeat expansions. Hexanucleotide (G_4C_2) repeat expansions in the first intron of the C9ORF72 transcript constitute the most common known cause of ALS (C9-ALS), accounting for 40% of familial ALS cases and as many as 10% of sporadic cases. Numerous pathological mechanisms have been proposed, including RNA-mediated pathogenesis via formation of RNA foci, RNA binding protein sequestration, RNA splicing alterations, translation of repetitive poly-dipeptides (mainly poly-(glycine-alanine) and poly(glycine-proline)), and alteration of nuclear-cytoplasmic transport. It is widely accepted that irrespective of the molecular pathway(s) perturbed by the repeat-containing RNAs produced from this locus, elimination of these toxic RNA species will provide therapeutic benefit 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, posing safety issues in the affected CNS tissues. Similarly, newly developed genome engineering strategies aimed at removing the mutated locus from the genome are liable to cause permanent genetic mutations at off-target sites. Building upon recent work in the Yeo lab that established the ability of a modified CRISPR/Cas9 genome editing system to target and degrade RNA (RCas9), we had demonstrated that RCas9 directed to repeat-containing RNAs, including C9ORF72 repeat RNAs, enables efficient and specific degradation of targeted toxic RNA species in cellular models of repeat expansion disease.

A new compact Cas-based repeat expansion targeting system. In the original grant proposal, we had proposed to use our modified Cas9-based RNA targeting system to target C9ORF72 hexanucleotide repeat expansions for degradation, via delivery into the CNS by adeno-associated viral vectors (AAV) using the subpial injection technique pioneered by co-investigator Martin Marsala. As the combined size of the RCas9 expression construct and the repeat-targeting guide RNA construct greatly exceeds the cargo capacity of an AAV, our strategy was to engineer reduced size RCas9 constructs that retain the ability to efficiently and specifically target RNA. We had also proposed to use the C9ALS transgenic mouse model developed by co-investigator Don Cleveland. These mice express a bacterial artificial chromosome (BAC) with a human expanded *C9ORF72* gene from a C9ALS patient with 450 G_4C_2 repeats. This mouse models recapitulates molecular hallmarks of the disease, including accumulation of repeat RNA foci and of cytoplasmic inclusions of poly-dipeptides, and also show age-dependent development of cognitive impairment. However, as was noted as a significant weakness by the grant reviewers, this model does not recapitulate motor dysfunction as a key clinical phenotype of ALS.

A robust and disease-relevant C9ALS mouse model. During the grant review and negotiations, we established a new mouse model, based on AAV-mediated delivery of a transcript expressing 66 G_4C_2 repeats, as initially reported by Petrucelli and colleagues, which we validated to recapitulate the molecular features of C9ALS and motor dysfunction. We also implemented a new Cas system, based on Cas13d, which targets RNA naturally and is sufficiently compact to be delivered in an AAV vector without size reduction. Therefore, in consultation with our DoD Grants Officer at the time, we developed a new strategy, using this more highly disease-relevant mouse model and the more readily implemented Cas13d technology (see revised SOW provided in the Attachment).

New cell- based preclinical models. As an important addition to our originally proposed work (which was based upon monolayer cortical neurons), we have recently established a robust spinal and cortical organoid differentiation paradigms representing more highly physiologically relevant systems that monolayer models.

Potential and limitations of Cas protein therapeutic strategies in C9ALS. In our in vivo studies, we identified the Cas9 protein (and not the AAV capsids) as a major contributor to immune-response related toxicity when delivered at doses required to ameliorate cellular and motor deficits. While this could certainly be considered a somewhat disappointing outcome, it is nevertheless a critical insight that will guide future ALS therapeutic strategies: Cas protein-based therapeutic applications must address and solve the problem of significant immunogenicity elicited by this bacterial protein. Leveraging the technologies and preclinical models that were developed in this project, we are now testing the potential of a human protein based therapy, specifically the use of human endogenous zinc finger proteins engineered to target C9ALS G_4C_2 sense and antisense RNA repeat expansions. This strategy is now being pursued in a DOD ALSRP project.

Thus we have established a robust framework of the potential and also limitations of RNA-targeting strategies in the treatment of C9ALS.

2. KEYWORDS

AAV, C9ALS, C9ORF72, Cas13d, cerebral organoids, RNA-targeting Cas, spinal organoids.

3. ACCOMPLISHMENTS

What were the major goals of the project?

Specific Aim 1: Evaluation of the ability of RCas9 to eliminate repeat expansion RNA in patient cell lines

Major task 1: Generate "disease-driving" and "therapeutic" AAV constructs (**Timeline:** 3 months)

Major task 2: Development and validation of an RCas13 endonuclease for targeted cleavage of G_4C_2 repeat-containing RNAs (**Timeline**: 9 months)

Specific Aim 2: Optimization of in vivo delivery regimen

Major task 3: Subpial injection of AAV9-packaged Cas13d into mice (**Timeline:** 12 months)

What was accomplished under these goals?

Successful design and generation of a repeat RNA expression system. Our 'disease-driving' AAV construct expresses the guide RNA from a U6 promoter; Cas13d is driven by a modified EF1alpha promoter (**Fig. 1a**). Our "CUG_3" construct efficiently (~50%) degrades G_4C_2 repeat RNA in a co-transfection assay in HEK293T cells (**Fig. 1b,c**). We successfully generated high-quality, high-titer (10^{13} gc/ml) AAV9 viral vectors for delivery of Cas13d targeting G_4C_2 repeats and control non-targeting viral constructs (which are based on a sequence derived from the lambda bacteriophage that has no match in the human genome).

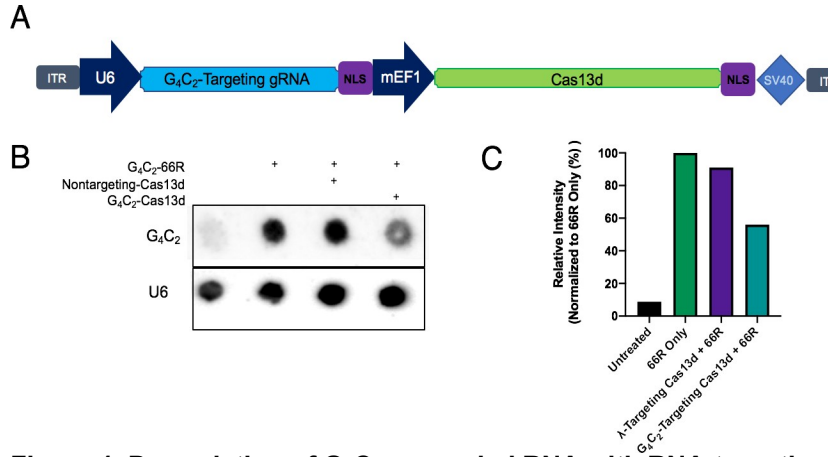


Figure 1. Degradation of G_4C_2-expanded RNA with RNA-targeting Cas13d. (A) Diagram of G_4C_2-targeting Cas13d adeno-associated viral vector (Cas13d- AAV). **(B)** RNA dot blot of expanded G4C2 RNA levels in COS-M6 cells transfected with a transcript containing 66 G_4C_2 repeats (G_4C_2-66R), a non-targeting or G4C2-targeting Cad13d AAV. **(C)** Quantification of expanded G_4C_2 RNA signal observed in RNA dot blot.

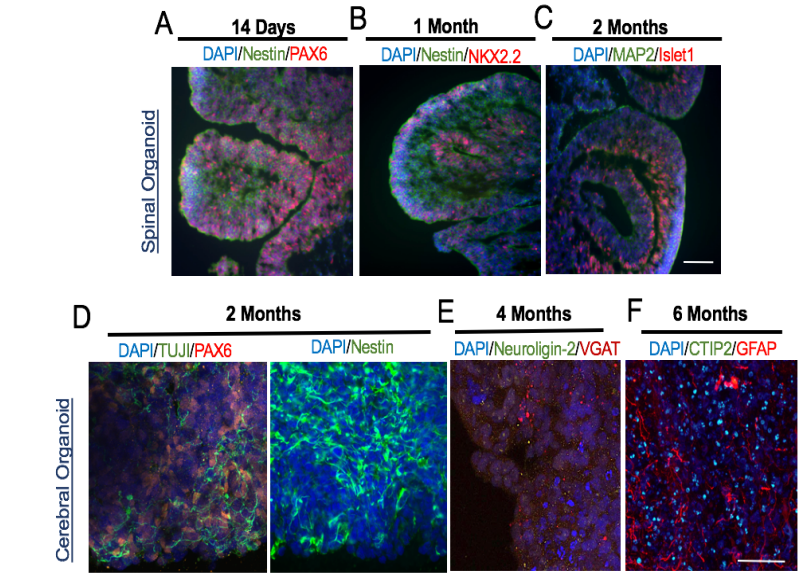


Figure 2. Spinal & Cerebral Organoid Differentiation. (A) Neural progenitor (PAX6+, Nestin+ cells) are observed within the developing spinal organoids as early as 14 days post-differentiation. **(B)** Over time, neural progenitors start to differentiate into interneuron & oligodendrocyte precursors (NKX2.2) and **(C)** eventually into mature motor neurons (Islet1+ cells). At the beginning of differentiation, an abundance of proliferative neural progenitor cells (Nestin+ & PAX6+) are observed in both Control and C9-ALS cerebral organoids. Progressively, the cerebral organoids **(E)** develop mature synapses marked by Neuroligin (postsynaptic marker) and VGAT (GABAergic synapse marker) as well as **(F)** mature neurons (CTIP2+) and astrocytes (GFAP+).

Successful generation of cerebral and spinal organoids. We generated several lines of cerebral and spinal organoids, differentiated from C9-ALS patient iPSCs and control lines (**Fig. 2**). Samples of each line were successfully collected and 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.

Successful demonstration of molecular hallmarks of C9ALS in iPSC-derived C9ALS cerebral organoids.
We identified G_4C_2-expanded RNA foci in C9-ALS cerebral organoids at 1 month post-differentiation – an early developmental stage where neural progenitors are the dominant cell type in this model, as well as toxic sense G_4C_2-encoding poly-GR dipeptide aggregates at 120 days post- differentiation – a time point where mature synaptic structures begin to form (**Fig. 3**).

Successful demonstration of reduction of molecular hallmarks of C9ALS in iPSC-derived spinal organoid models of C9ALS.

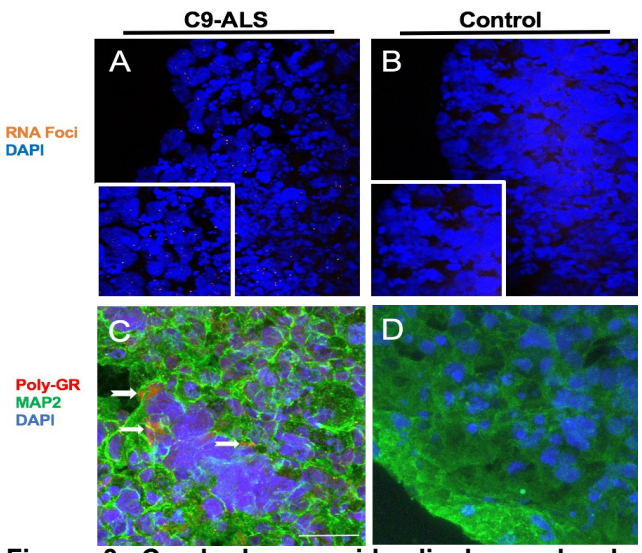


Figure 3. Cerebral organoids display molecular hallmarks of C9-ALS. (A-B) C9-ALS cerebral organoids display G_4C_2-mediated RNA foci detected through RNA fluorescent in situ hybridization as early as 1 month post- differentiation, a time-point when cerebral organoids mainly consist of early neural progenitors. **(C-D)** Toxic poly-GR dipeptide proteins detected via immunofluorescence staining with anti-poly-GR antibodies are present in 4-month- old C9-ALS organoids (MAP2+ = mature neuronal processes).

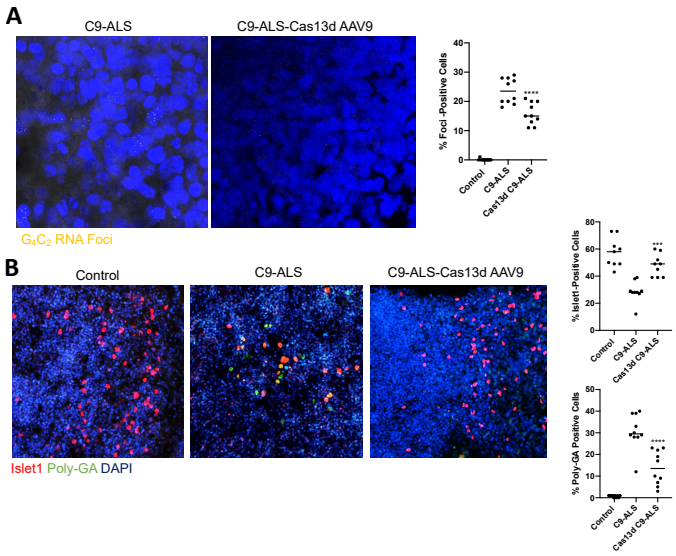


Figure 4. Reduction of molecular hallmarks of C9ALS in iPSC-derived spinal organoid models of C9ALS. (A) RNA-FISH targeting G_4C_2 repeats in C9ALS patient iPSC-derived spinal organoids without (left) and with (middle) transduction of AAV9 viral particles delivering out G_4C_2 targeting Cas13d system, and quantitation from 10 fields per condition (right). **(B)** Immunofluorescence staining for the motor neuron marker Islet1 (red) and poly-glycine-alanine (poly-GA) poly-dipeptides. For all panels: "control" = non-ALS (normal) iPSCs; *** p<0.001, **** p<0.0001; 10 fields per condition.

Using our high-titer Cas13d/gRNA AAV preparations, we demonstrated reduction (by ~50%) of G_4C_2 repeat foci in patient iPSC-derived C9ALS cortical organoids (**Fig. 4a**). We have also demonstrated reduction of poly-GA poly-dipeptides (by ~60%) in our spinal organoid models (**Fig. 4b**). Significantly, we observe an increase in the number of Islet1-positive cells (motor neurons) to near control levels, indicating that Cas13d/gRNA AAV-treatment reduces motor neuron death.

Successful demonstration of hallmark C9ALS molecular phenotypes and neuromuscular dysfunction induced by subpial injection of AAV vectors expressing 66 repeats of C_4G_2 RNA.

We established a new C9ALS mouse model by AAV-mediated subpial delivery of C9ALS C_4G_2 RNA repeats. As shown in **Fig. 5**, C9ALS molecular phenotypes and neuromuscular dysfunction induced by lumbar subpial injection of AAV vectors expressing 66 repeats of C_4G_2 RNA. Our model recapitulates key molecular and functional C9ALS phenotypes: the emergence of dipeptide and repeat RNA foci (4 weeks post-injection) (**Fig. 5A,B,E**); loss of motor neurons, reduced axonal diameter and glial activation (**Fig. 5C,E**) at disease onset; dramatic reduction in motor neuron numbers and persistence of glial activation (**Fig. 5D, E**) and fibrillation at the paralysis stage (13 weeks post-injection) (**Fig. 5F**). Critically, we also observe weakening of hindlimb grip strength starting at disease onset (**Fig. 5G**). We also observe disruption of neuromuscular junctions at disease onset. In contrast, we do not observe loss of sensory neurons in the dorsal horn at either the disease onset or paralysis stages. These data demonstrate that our model recapitulates motor neuron vulnerability as a cardinal feature of ALS.

Successful delivery of AAV9-packaged Cas13d into the spinal cord.

We used our AAV9-packaged Cas13d system for subpial delivery into naïve mice. As shown in **Fig. 6**, at the highest AAV doses used, we observe expression of Cas13d in the ventral horn of the spinal cord 24 days post-injection, as visualized by immunofluorescence for the HA tagged Cas13d. As is evident from AAV9-packaged Cas13d, the AAV dose used leads to significant cell death, and we have also observed a significant degree of immune response (glial activation). Thus, at therapeutically relevant doses, AAV9-packaged Cas13d elicits a strong degree of immunotoxicity. We further discuss this issue (and solutions) Section 5 CHANGES/ PROBLEMS.

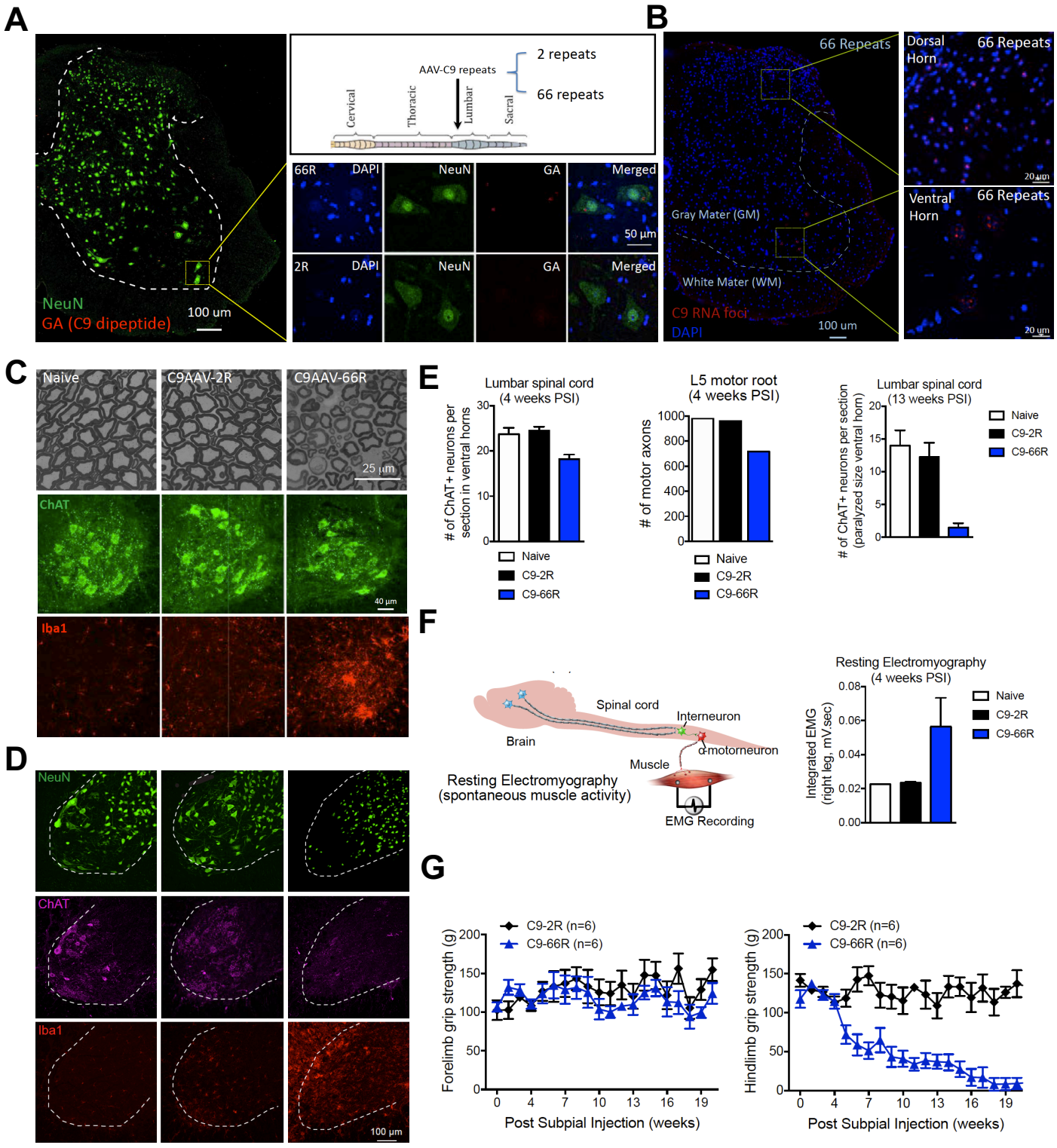
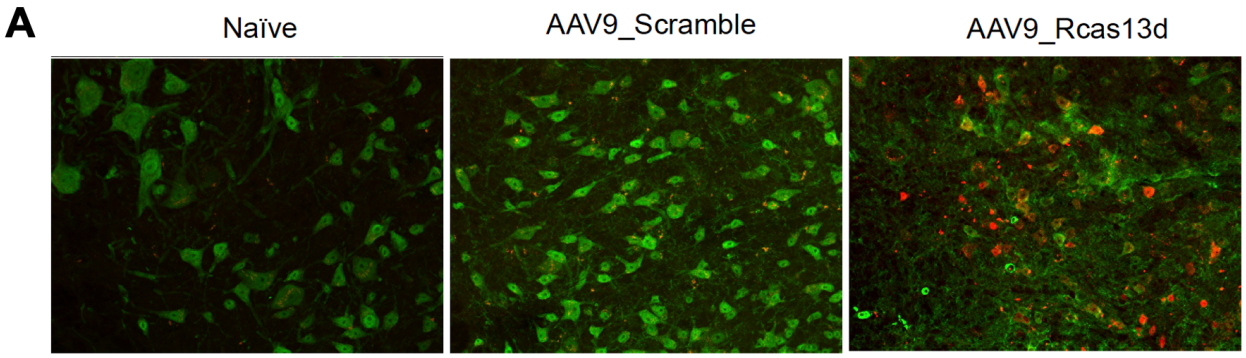


Figure 5. Hallmark C9ALS molecular phenotypes and neuromuscular dysfunction induced by lumbar subpial injection of AAV vectors expressing 66 repeats of C_4G_2 RNA. Top inset shows experimental design. **(A)** Production of poly(GA) dipeptide repeats 4 weeks post-injection (disease onset). **(B)** RNA foci in the dorsal and ventral horns of the spinal cord (insets) detected by FISH with a G_4C_2 sense probe 4 weeks post-injection. **(C)** Reduction of numbers and diameter of motor neuron axons glial activation 4 weeks post-injection **(D)** Motor neuron loss and glial activation at the paralysis stage (13 weeks post-injection). **(E)** Quantitation of images in (C) and (D). ALS-like fibrillation and abnormal electrophysiological phenotypes at the paralysis stage. **(F)** Progressive weakening of hindlimb grip strength at disease onset.



B

	Naive	AAV Scramble	AAV Rcas13d
No Tacro	2	4	4
One Tacro	2	4	4
Two Tacro	2	4	4
Total n	6	12	12

No immunosuppression (**No tacro**)
Single dose Depo-Medrol,15mg/kg IM + Single dose Tacrolimus microsphere, 20mg/kg S.C. (**One Tacro**)
Single dose Depo-Medrol,15mg/kg IM + Two doses of Tacrolimus microsphere, 20mg/kg S.C. (**Two Tacro**)
1st Tacrolimus dose 1h before subpial injection
2nd Tacrolimus dose 12 days after subpial injection

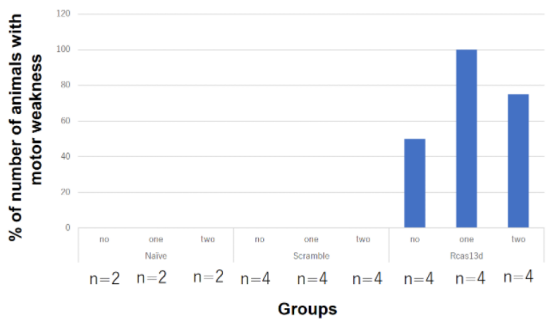


Figure 6. Subpial injection of AAV9-packaged Cas13d causes widespread motor neuron death and motor weakness. (A) Immunofluorescence study. Naïve C57BL/6 mice were injected (bilateral lumbal subpial) with 10 µl per side of AAV9-scramble or AAV9-RCas13d (10^{11} gc), or remained uninjected. Twenty-four days post-injection, spinal cords were isolated, dissected and stained for NeuN (marks neurons) and HA (marks Cas13d). Pyknotic neurons were evident in spinal cords from the AAV9-RCas13d but not naïve or AAV9_Scramble injected animals. **(B)** Motor function study. *(Left)* Study design. Animals were subpially injected as above (No tacro), or additionally with single (One Tacro) or double (Two Tacro) intramuscular doses of the immunosuppressant drug tacrolimus (20 mg/kg). (*Right*) Twenty-four days after the subpial injections, animals were scored for muscle function by observation of animals in their home cage. Motor weakness was observed in 9/12 AAV9-RCas13d injected but in none of the naïve or AAV9_Scramble injected animals. Tacrolimus treatment did not prevent motor weakness.

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; Qiang Zhu, Cleveland lab; and Izumi Shunsuke, 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 and UCSD-wide meetings.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?
(1) Development of a new preclinical models of C9-ALS: We demonstrated, for the first time, that spinal and cerebral organoids can be used to model disease-relevant molecular phenotypes of C9ALS (presence of repeat RNA foci and poly-dipeptides). 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. We also established a robust and rapid mouse model of C9ALS that recapitulates all key pathological features of the disease. We anticipate that similarly, this mouse model with be of significant value for preclinical research and the development of targeted therapies.
(2) Identification of immunotoxicity as a significant challenge of Cas-protein based therapeutic applications. Our results delineate the limitations of delivery of Cas proteins (relevant to both DNA- and RNA-targeting strategies) as a treatment of C9ALS. In response, we are currently exploring the therapeutic potential of a human-protein based approach to target C9ALS repeat expansion RNAs (see below).

What was the impact on other disciplines?
Our results, which are also now supported by many other studies across other disease areas, delineate the limitations of long-term delivery of Cas proteins (relevant to both DNA- and RNA-targeting strategies) in treatment of CNS diseases.

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
As described above, upon consultation and with agreement of the DoD Grants Officer, we used (1) a Cas13d-based strategy, which circumvents the need for engineering size-reduced Cas9 constructs, and (2) our new rapid and robust mouse model that is based on AAV-mediated delivery of C_4G_2 RNA. In addition, we pioneered the use of stem-cell based cortical and spinal organoids as in vitro ALS disease models, with which we validated efficacy of our therapeutic AAV constructs. These changes are all within the scope of our goal of developing an RNA-directed therapy for C9ORF72-linked ALS.

Actual or anticipated problems or delays and actions or plans to resolve them
We experienced a delay in our in vivo studies due to the COVID-19 pandemic, specifically the restrictions imposed by USCD with respect to research personnel presence in the lab (at 25% of normal for over a year). However, with new relaxed density restrictions in place since March/April, we completed these studies within our originally proposed timeframe.

Critically, we observed significant motor neuron death (see **Fig. 6A**), accompanied by glial activation (inflammation), and motor weakness (see **Fig. 6B**), upon delivery of our therapeutic AAV construct at 10^{11} gc per injection site, but not using AAV vectors not carrying the Cas13d cargo. We believe that this is due to the bacterial nature of the Cas protein, a problem that is not unexpected and common to all Cas-based (including Cas9) therapeutic approaches, as recent studies across several fields have now shown. We down-titrated the Cas13d dose 10-fold to 10^{10} gc per injection site to minimize immunogenicity; this dose was below the lower bound of efficacy based on our prior experience. Indeed, with these lower doses, motor neuron death was still evident. We therefore believe that a Cas protein-based approach for treatment of C9ALS is not viable with native Cas sequences. A significant investment would be required to identify the immunogenic domains of the Cas protein, and to replace these with humanized sequences or with sequences otherwise designed to have reduced immunogenicity, and re-testing efficacy and immunogenicity.

In response, we re-directed our efforts toward developing an ideal RNA-targeting system would be non-immunogenic, degrade both sense and antisense toxic transcripts, and be encoded in AAV vectors allowing long-term continuous production of therapeutic materials *in situ* and take advantage of the growing variety of tissue-targeted AAV serotypes which can effectively be delivered into CNS and spinal cord. Excitingly, our team has generated compelling preliminary data supporting the feasibility of an innovative therapeutic strategy that capitalizes on novel RNA-targeting human zinc finger (ZNF) technology that can degrade both sense $(G_4C_2)^{exp}$ and antisense $(C_4G_2)^{exp}$ C9ORF72 transcripts simultaneously.

Our current lead therapeutic agent is a novel RNA-targeting ZNF fusion protein fused to the PIN RNA endonuclease domain, termed "Z1" (**Fig. 7A**). With RNA-targeting ZNF fusion proteins as a surrogate RNA-binding motif, we can target and degrade disease-causing RNA transcripts that contain expanded G_4C_2/C_4G_2 hexanucleotide repeats. Preliminary studies show Z1 fused to an RNA endonuclease domain efficiently eliminates $(G_4C_2)^{exp}$ and $(C_4G_2)^{exp}$ RNAs *in vitro* determined with plasmid overexpression studies and subsequent semi-quantitative RNA dot blot (**Fig. 7B,C**). Further plasmid overexpression studies using either CUG or CAG repeats and subsequent semi-quantitative RNA dot blots were performed to determine if Z1 specifically eliminates RNA transcripts that contain expanded G_4C_2/C_4G_2 hexanucleotide repeats without degrading other GC-rich transcripts (**Fig. 7D,E**). Results show that the Z1 fusion proteins specifically targets and degrades G4C2 and C4C2-expanded transcripts while leaving other GC-rich transcripts intact.

Future directions of our study include preclinical studies that will determine efficacy of Z1 to reverse or halt the progression of motor neuron disease in a mouse model of C9-ALS without adverse effects. Our zinc-finger fusion proteins will be the first compact all-human protein-based agents to target and modify human RNA transcripts

that does not contain foreign peptide sequences. The unique combination of targeted delivery and efficient, specific elimination of both sense and antisense toxic RNAs simultaneously with no predicted immunogenicity positions us to generate an entirely new class of therapeutics targeting repeat expansion diseases, with a first application focused on C9-ALS.

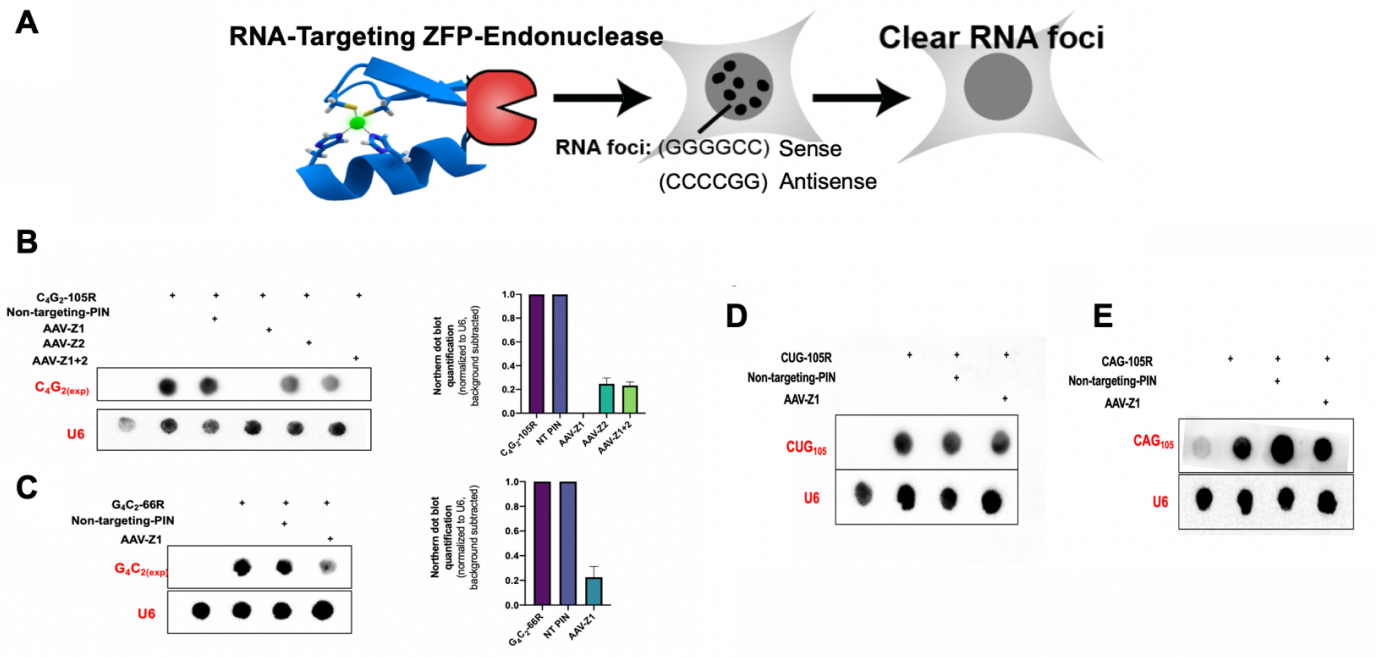


Figure 7. Human zinc finger protein based therapeutic strategy for C9ALS. (A) Fusion of an $(G_4C_2/C_4G_2)^{exp}$ RNA-targeting ZNF fusion protein to a nonspecific RNA endonuclease and delivery to diseased cells supports elimination of hexanucleotide RNA foci and reversal of downstream molecular phenotypes of disease such as translation of toxic repetitive protein products, dysfunctional nuclear-cytoplasmic transport, or RNA splicing alterations. (B) RNA dot blot of (C_4G_2) expression levels in COS-M6 cells transfected with $(C_4G_2)_{105}$, G_4C_2 +C_2G_4-targeting ZFP AAVs (AAVZ1, AAV-Z2, or AAV-Z1+2) or a non-targeting AAV construct expressing PIN nuclease (Non-targeting PIN). U6 snRNA served as a loading control. **(B-C)** RNA dot blot of (G_4C_2) expression levels in COS-M6 cells transfected with $(G_4C_2)_{66}$, G_4C_2 +C_2G_4-targeting ZFP AAV, AAVZ1, or a non-targeting AAV construct expressing PIN nuclease (Non-targeting PIN). U6 snRNA served as a loading control. **(D-E)** RNA dot blot of (CUG) expression levels in COS-M6 cells transfected with $(CUG)_{105}$, G_4C_2 +C_2G_4-targeting ZFP AAVs (AAVZ1, AAV-Z2, or AAV-Z1+2) or a non-targeting AAV construct expressing PIN nuclease (Non-targeting PIN). U6 snRNA served as a loading control. (**B**) RNA dot blot of (CAG) expression levels in COS-M6 cells transfected with $(CAG)_{105}$, G_4C_2 +C_2G_4-targeting ZFP AAV, AAVZ1, or a non-targeting AAV construct expressing PIN nuclease (Non-targeting PIN). U6 snRNA served as a loading control.

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.

6. PRODUCTS

Publications, conference papers, and presentations
Dr. Morelli is currently preparing a manuscript for submission that describes the spinal and cortical organoid preclinical paradigms. 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 established and fully characterized a new mouse model of C9ALS. We have also validated stem cell models of cortical and spinal development as new tools for preclinical research in ALS.

Inventions, patent applications, and/or licenses
Nothing to report. 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	Don Cleveland
Project Role	Co-Investigator
Researcher identifier	0000-0002-1934-3682
Nearest person month worked	1
Contribution to project	Lead of development and characterization of AAV-based mouse model
Funding support	This grant

Name	Martin Marsala
Project Role	Co-Investigator
Researcher identifier	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	N/A
Nearest person month worked	9
Contribution to project	Design/generation of AAV constructs, in vitro stem-cell based experiments
Funding support	This grant

Name	Qian Zhu
Project Role	Postdoc
Researcher identifier	N/A
Nearest person month worked	6
Contribution to project	Development and characterization of AAV-based mouse model
Funding support	NIH grant (Cleveland)

Name	Takahiro Tadokoro
Project Role	Postdoc
Researcher identifier	0000-0003-4884-0247
Nearest person month worked	6
Contribution to project	Subpial injections
Funding support	NIH grant (Marsala)

Name	Izumi Shunsuke
Project Role	Postdoc

Researcher identifier	N/A
Nearest person month worked	6
Contribution to project	Subpial injections
Funding support	NIH grant (Marsala)

Name	Izumi Shunsuke
Project Role	Postdoc
Researcher identifier	N/A
Nearest person month worked	6
Contribution to project	Subpial injections
Funding support	NIH grant (Marsala)

Name	Mariana Bravo-Hernandez
Project Role	Postdoc
Researcher identifier	0000-0001-8762-0357
Nearest person month worked	3
Contribution to project	Animal husbandry
Funding support	NIH grant (Marsala)

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Yes, see below (this list covers changes in active other support over the entire project period):

YEO

Ended:

Larry L. Hillblom Foundation Grant 2018-A-003-NET (Yeo, PI) **01/01/2019-12/31/2019**
Stress granule components as potential modulators of protein aggregation in ALS

Target ALS Foundation Industry-led consortium (Yeo, PI) **01/01/2017-12/31/2019**
Targeting stress granule dynamics for familial and sporadic ALS

CIRM GC1R-06673-A: CRP **05/01/2016-12/31/2019**
Large-scale assessment of RNA localization and single-cell alternative splicing in neuronal stem cell models of disease

NIH/NGHRI U54HG007005 (Gravely, contact PI; Yeo, PI) **09/21/2012-03/31/2019**
Comprehensive analysis of functional RNA elements encoded in the human genome

Awarded:

NIH/NICHD R01 HD101534-01A1 (Barrett, PI; Yeo Co-I) **09/17/2020-08/31/2024**
Dissecting the role of FMRP in RNA processing using human stem cell models

NIH/NIEHS P42 ES010337-19S1 (Tukey, PI; Yeo Co-I) **09/01/2020-08/31/2022**
Harnessing Technological Innovation and Community-Engaged Implementation Science to Optimize COVID-19 Testing for Women and Children in Underserved Communities

NIH/NIA R56 AG1069098 (Yeo, PI) **07/01/2020-06/30/2021**
Evaluating and Targeting RNA granules in neurodegenerative diseases

Admin Supplement to U01 MH115747 (Krogan, PI; Yeo Co-I) **07/01/2020-06/30/2022**
Proteomics Integration and Expansion of Downstream Analysis Capabilities into the CReD Portal

Simons Foundation Autism Research Initiative Grant # 668241 (Yeo PI) **02/01/2020-01/31/2022**
Inhibition of UBAP2L as a treatment of fragile X syndrome

Grant # 2020-217276 (Coufal, PI; Yeo, Co-I) **03/01/2020-02/28/2022**
Deciphering the Microglial Inflammatory Response in 3D

CDC Contract (Andersen, PI; Yeo, subcontractor) **08/01/2020-07/31/2022**
Genomic sequencing of SARS-CoV-2 to investigate local and cross-border emergence and spread

IARPA Contract (Lo, PI; Yeo, subcontractor) **09/01/2020-08/31/2021**
High throughput, sensitive, rapid detection of viral infection and spread with an innovative isothermal lateral flow assay

U.S. DoD AL200196 (Yeo, PI) **01/01/2021-12/31/2022**
RNA-Directed Therapy for C9ORF72-Linked ALS Using Engineered Zinc Finger Nucleases

CLEVELAND

Ended:
NIH/NINDS R01 NS088578 (Ravits, PI; Cleveland Co-I) **07/01/2014 – 06/30/2019**
Developing ASO Therapy for Repeat Expanded C9orf72 ALS-FTD

UCSD Alzheimer's Disease Research Center (Brewer, PI, Cleveland co-I) **04/01/2014 – 03/31/2019**
Disease Mechanisms in Frontotemporal Dementia Linked to C9orf72 expansions

ALSA 11001 GE230 (Cleveland, PI) **04/01/2017 – 06/30/2019**
Oligonucleotide Therapy Development and Decoding Disease Mechanism

Awarded:
ALSA 1124 (Cleveland, PI) **05/01/2019 – 4/30/2021**
Antisense oligonucleotide therapy in ALS by restoring expression of stathmin-2

NIH/NINDS R01 NS112503-01A1 (Cleveland, contact PI) **04/01/2020– 03/31/2025**
Determining stathmin-2 function and potential as a therapeutic target in ALS/FTD

MARSALA

Ended:
SANPORC "Porcine Center" (Marsala, PI) **04/01/2014 - 03/31/2020**

Sanford Stem Cell Clinical Center "Alpha Clinic" (Marsala, PI) **02/01/2015 - 01/31/2020**

NIH/OD R01OD018272-01A1 (Marsala, PI) **07/01/2015 - 04/30/2019**
Modulation of spinal neurodegenerative diseases in swine by stem cell grafting

ALS Foundation (Marsala, PI) **04/01/2017 - 06/30/2019**
A combined Ludwig/UCSD/Children's Hospital team will undertake development of two therapeutic approaches for treatment of some forms of inherited and sporadic ALS

Awarded:
NIH/NINDS R01 NS112503-01A1 (Cleveland, contact PI; Marsala PI) **04/01/2020– 03/31/2025**
Determining stathmin-2 function and potential as a therapeutic target in ALS/FTD

What other organizations were involved as partners?
No change in partner organizations. Ludwig Institute for Cancer Research is the home institution of Dr. Cleveland.

8. SPECIAL REPORTING REQUIREMENTS
None

9. APPENDICES
None