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AL180126

TITLE: RNA-directed Therapy for C9ORF72-Linked ALS

PRINCIPAL INVESTIGATOR: YEO, EUGENE

CONTRACTING ORGANIZATION: UNIVERSITY OF CALIFORNIA SAN DIEGO

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Fort Detrick, Maryland 21702-5012

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**5e. TASK NUMBER**
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**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
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**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

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## TABLE OF CONTENTS

## 1. INTRODUCTION

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.

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.

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). Lastly, 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 physiological relevant systems that monolayer models.

## 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 have 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).

**Successful generation of cerebral and spinal organoids.** In recent We have generated several lines of cerebral and spinal organoids, differentiated from C9-ALS patient iPSCs and control lines (**Fig. 2**). Samples of each line are currently being collected 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.

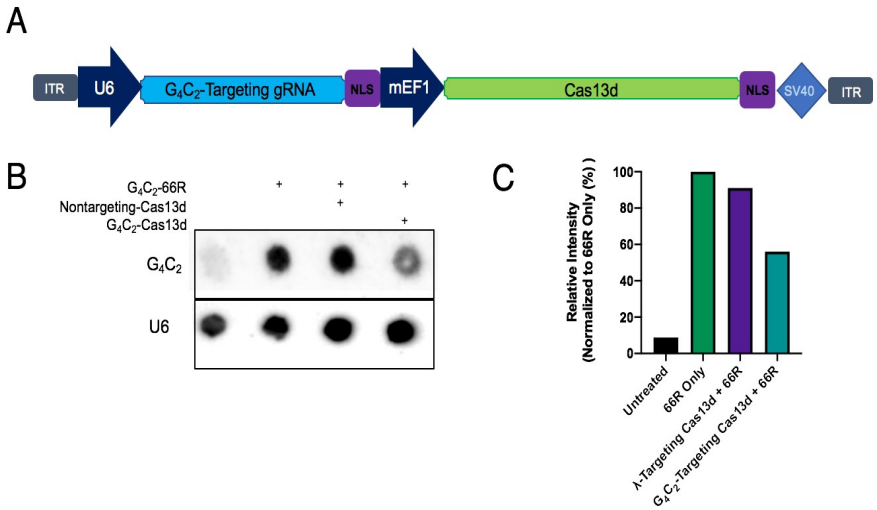


**Figure 1. Degradation of $G_4C_2$-expanded RNA with RNA-targeting Cas13d. (A)** Diagram of $G_4C_2$-targeting Cas13d adeno-associated viral vector (Cas13d- AAV). **(B)** RNA dot blot of expanded G4C2 RNA levels in COS-M6 cells transfected with a transcript containing 66 $G_4C_2$ repeats ($G_4C_2$-66R),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

**Successful demonstration of molecular hallmarks of C9ALS in iPSC-derived C9ALS cerebral organoids.**
We have 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.**
Using our high-titer Cas13d/gRNA AAV preparations, we demonstrate reduction (by ~50%) of $G_4C_2$ repeat foci in patient iPSC-derived C9ALS cortical organoids (**Fig. 4a**). We have also

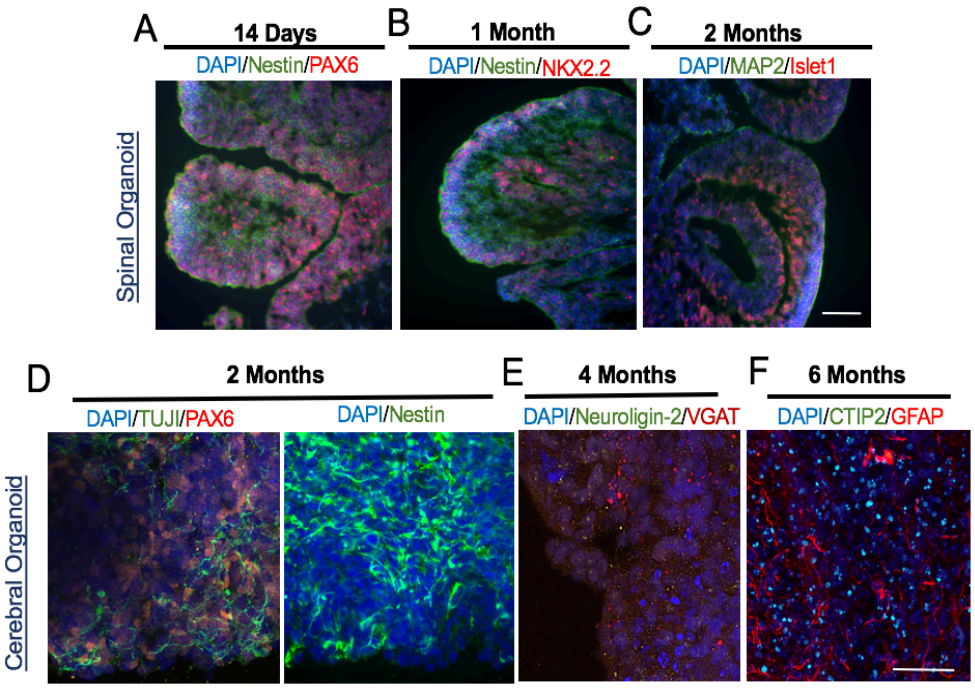


**Figure 2. Spinal & Cerebral Organoid Differentiation. (A)** Neural progenitor (PAX6+, Nestin+ cells) are observed within the developing spinal organoids as early as 14 days post-differentiation. **(B)** Over time, neural progenitors start to differentiate into interneuron & oligodendrocyte precursors (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+).

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.

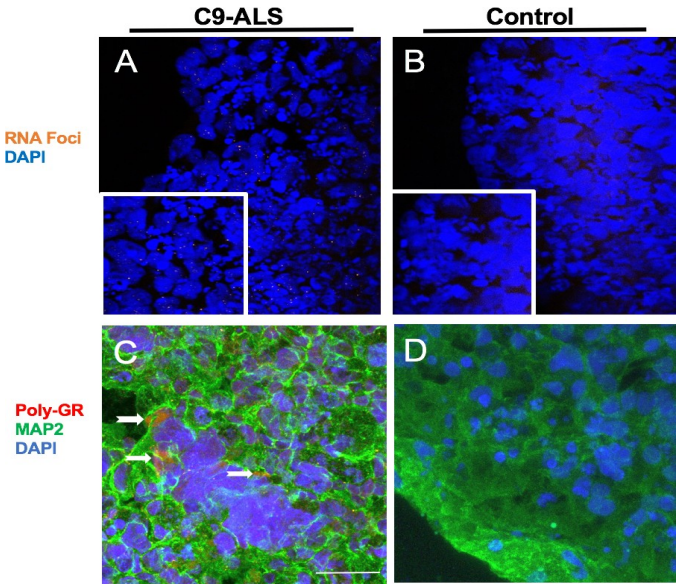


**Figure 3. Cerebral organoids display molecular hallmarks of C9-ALS. (A-B)** C9-ALS cerebral organoids display $G_4C_2$-mediated RNA foci detected through RNA fluorescent in situ hybridization as early as 1 month post-differentiation, a time-point when cerebral organoids mainly consist of early neural 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).

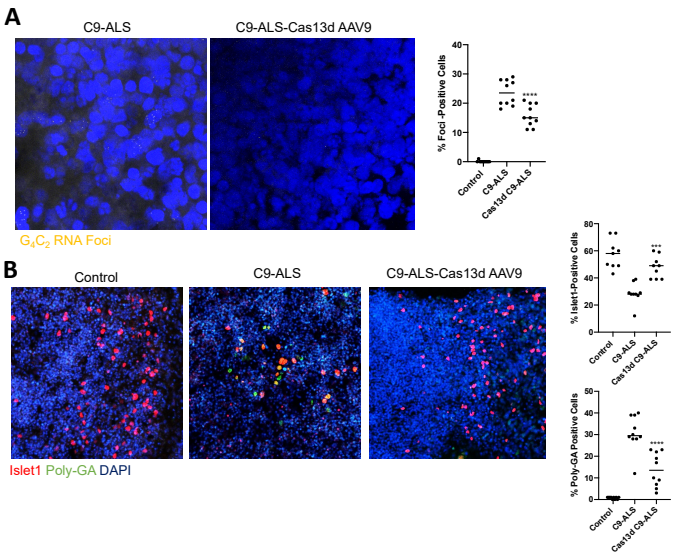


**Figure 4. Reduction of molecular hallmarks of C9ALS in iPSC-derived spinal organoid models of C9ALS. (A)** RNA-FISH targeting $G_4C_2$ repeats in C9ALS patient iPSC-derived spinal organoids without (left) and with (middle) transduction of AAV9 viral particles delivering out $G_4C_2$ targeting Cas13d 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.

**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 have 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 have also begun to use our AAV9-packaged Cas13d system for subpial delivery into naïve mice. As shown in **Fig. 6a**, 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 Fig. 5, the AAV dose used leads to significant cell death, and we have also observed a significant degree of immune response (glial activation). We are currently optimizing the AAV dose to achieve maximum transduction while minimizing cellular toxicity and immune response, and are also evaluating behavioral effects. These critical experiments are ongoing but have been delayed due to the COVID-19 pandemic, specifically the restrictions imposed by USCD with respect to research personnel presence in the lab (currently at 25% of normal). We anticipate to complete these studies within our originally proposed timeframe.

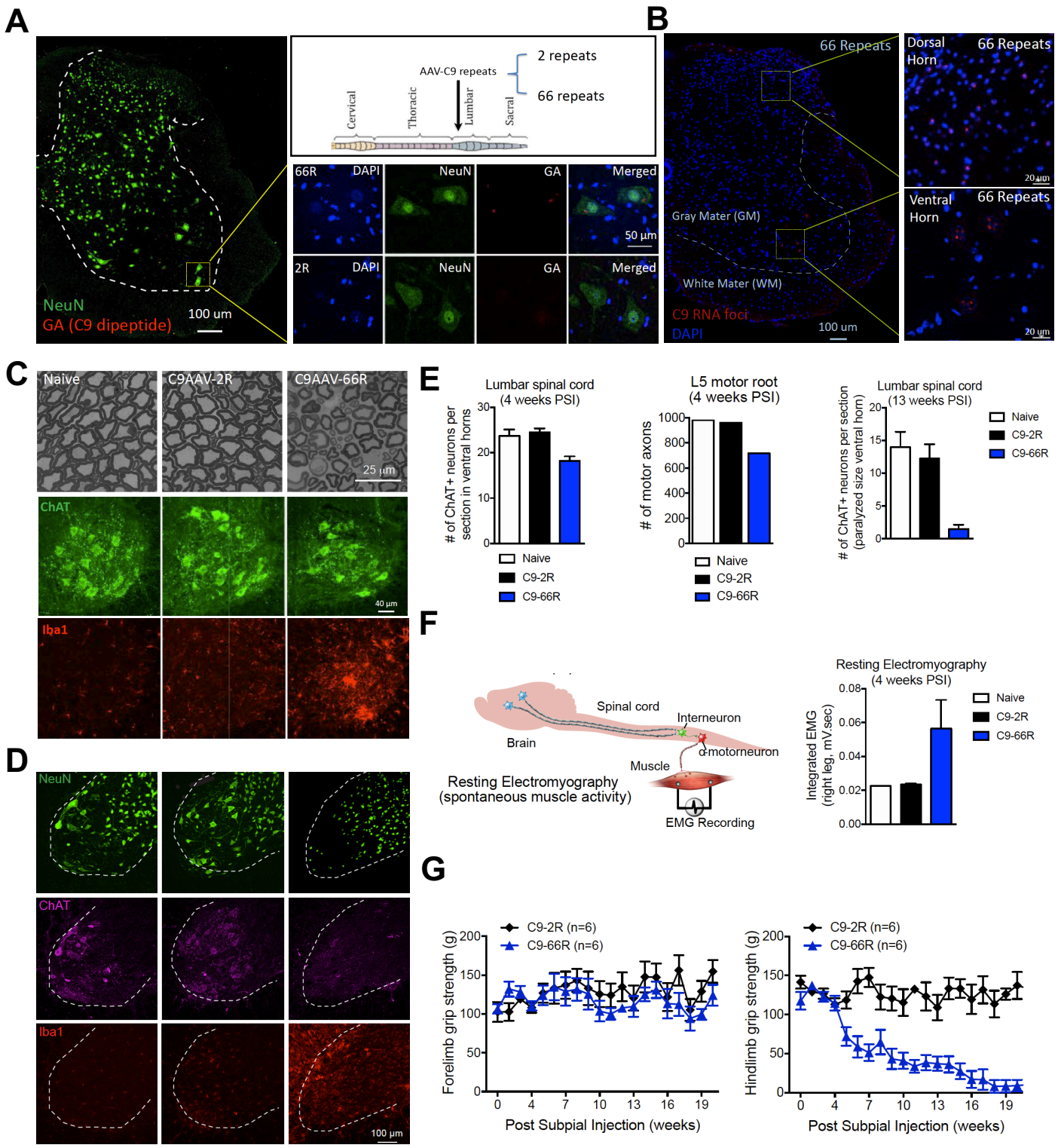


**Figure 5. Hallmark C9ALS molecular phenotypes and neuromuscular dysfunction induced by lumbar subpial injection of AAV vectors expressing 66 repeats of $C_4G_2$ RNA.** Top inset shows experimental design. **(A)** Production of poly(GA) dipeptide repeats 4 weeks post-injection (disease onset). **(B)** 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.

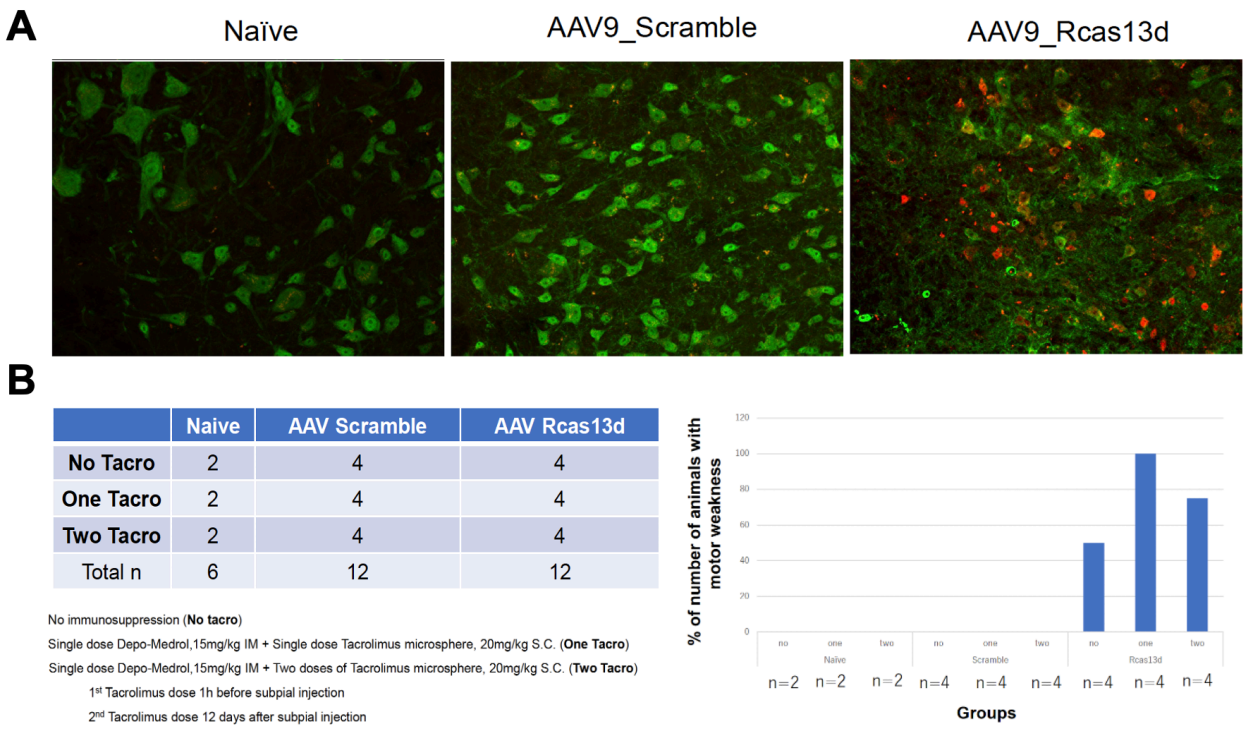


**Figure 6. Subpial injection of AAV9-packaged Cas13d causes widespread motor neuron death and motor weakness. (A)** Immunofluorescence study. Naïve C57BL/6 mice were injected (bilateral lumbal subpial) with 10 µl per side of AAV9-scramble or AAV9-RCas13d ($10^{11}$ gc), or remained uninjected. Twenty-four 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 skillsets, 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.

**What do you plan to do during the next reporting period to accomplish the goals?**
In the next year, we plan to continue optimizing the delivery regimen (timing and dose of “disease-driving” and “therapeutic” AAV constructs) to achieve relevant molecular and neuromuscular disease phenotypes and their reduction by repeat-targeting Cas13d AAV vectors. We will also perform a detailed analysis of in vivo safety, inflammatory and cytotoxic effects of the “therapeutic” AAV constructs, as described in Major Tasks 3 and 4.

## 4. IMPACT

**What was the impact on the development of the principal discipline(s) of the project?**
We have 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 have 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.

**What was the impact on other disciplines?**
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**
As described above, upon consultation and with agreement of the DoD Grants Officer, we are (1) using 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 have 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 have 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 have now completed these studies within our originally proposed timeframe.

Critically, we have 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 have now down-titrated the Cas13d dose 10-fold to $10^{10}$ gc per injection site to minimize immunogenicity; this dose is below the lower bound of efficacy based on our prior experience. However, at 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, replace these with humanized sequences or sequences designed to have reduced immunogenicity, and re-testing efficacy and immunogenicity.

**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**
Nothing to report. We plan to submit our results for publication at the end of the project period.

**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. Our inventions have been disclosed to our Technology Transfer Office at UCSD but no patent applications have been filed yet.

**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:

**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*

**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