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TITLE: Evaluation of PPAR-Delta/Gamma Agonist Therapy as a Novel Treatment Paradigm for ALS

PRINCIPAL INVESTIGATOR: Albert R. La Spada, MD, PhD

CONTRACTING ORGANIZATION: University of California, Irvine

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Albert La Spada, MD, PhD
Distinguished Professor

E-Mail: alaspada@uci.edu

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N/A

14. ABSTRACT

The nuclear receptor PPAR-delta is a highly expressed transcription factor in neurons and non-neuronal cells, and is required for neural function. PPAR-delta turns on genes that promote mitochondrial function and favor energy production, and genes that help neurons get rid of misfolded proteins and damaged organelles. PPAR-delta and a related transcription factor, PPAR-gamma, reduce inflammation. Drug compounds that activate PPAR-delta, known as 'agonists', can boost the function of processes that decline as we age and contribute to the development of neurodegenerative diseases, such as ALS. Here we will test if the PPAR delta/gamma agonist T3D-959 can prevent cellular and molecular abnormalities in ALS, and if T3D-959 can improve the quality of life of mice that recapitulate key features of ALS. In the first Aim, we will evaluate T3D-959 in cultures of neurons expressing ALS-causing disease proteins. In the second Aim, we will test if T3D-959 drug treatment of mice that develop ALS is an effective therapy by tracking disease progression in T3D-959-treated ALS mice and in vehicle (placebo)-treated ALS mice, comparing outcomes of the two different cohorts. We will employ two different mouse models of ALS, one featuring expression of a disease protein (TDP-43) implicated in ALS, and another based upon expressing the disease mutation underlying C9orf72 ALS, a common form of familial ALS.

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			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified	22	19b. TELEPHONE NUMBER *(include area code)*

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1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

In this project, we are testing if the PPAR-delta/gamma agonist drug T3D-959 can prevent ALS disease phenotypes in cultured cortical neurons expressing ALS disease proteins. We are also performing preclinical trials of T3D-959 in two different ALS mouse models, one featuring the expression of a disease protein (TDP-43) implicated in ALS, and the other expressing a gene (C9orf72) known to cause the most common form of familial ALS. At the conclusion of this two-year project, we will know if T3D-959 offers promise as a treatment for human ALS patients.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

PPAR-delta; PPAR-gamma; agonist; nuclear receptor; transcription factor; TDP-43; C9orf72 gene; dipeptide; mouse model; cognitive function; histopathology; molecular pathology; read-outs

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?
List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

This is a two-year project. Goals for end of Year 1 were as follows:
Major task 1 = determine if T3D-959 can rescue disease phenotypes in cortical neurons expressing TDP-43 (40% completed)
Major task 2 = determine if T3D-959 can rescue disease phenotypes in cortical neurons expressing dipeptide repeat constructs (100% completed)
Major task 3 = perform a pharmacodynamics study to identify the optimal dose of T3D-959 to be used in the preclinical trials (100% completed)

Tasks to be initiated in Year 1, but not completed until the end of Year 2:
Major task 4 = Preclinical trial of T3D-959 in TDP-43 transgenic mice (20% completed)
Major task 5 = Preclinical trial of T3D-959 in G4C2-149R C9orf72 transgenic mice (20% completed)

What was accomplished under these goals?
For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

1) Major activities

We spent the first year of this project performing Major Tasks 1 and 2, which comprise Aim #1 of this project, which is to determine if **PPAR δ/γ activation can prevent ALS neurotoxicity phenotypes in primary neuron models of ALS.** We also focused on Major Task 3, the first step of Aim #2, the goal of which is to complete a **pharmacodynamics study to establish the maximal safe dose and the delivery regimen of T3D-959 to achieve engagement with target in the CNS of treated mice** as a prelude to initiating two preclinical trials of T3D-959 in two different ALS mouse models. We also initiated Major Tasks 4 and 5, which comprise the remainder of Aim #2, which is to organize and then perform two preclinical trials of T3D-959 in two different ALS mouse models.

2) Specific Objectives

We tested the hypothesis that T3D-959 can prevent ALS disease-relevant phenotypes in primary cortical neuron models. **We employed two different ALS neurotoxicity systems, which recapitulate key features of ALS disease pathology in SALS and in the most common form of FALS.** As TDP-43 histopathology is a defining feature of SALS and most forms of FALS, the first model system was based upon expression of full-length TDP-43 or C-terminal truncated TDP-43. As C9orf72 ALS due to expansion of a G4C2 repeat is the most common form of FALS, the second model system was based upon expression of synthetic dipeptide repeat constructs [(GR)30 and (GA)30] to model C9orf72 gain-of-function neurotoxicity due to RAN translation. We sought to the test the hypothesis that **PPAR δ/γ activation can prevent ALS neurotoxicity phenotypes in transgenic mice**, and so initiated the preclinical trial work by performing a pharmacodynamics study to establish the maximal safe dose and the delivery regimen of T3D-959 to achieve engagement with target in the CNS of treated mice. We then established the requisite cohorts for the two different preclinical trials and successfully initiated dosing of mice with either drug or vehicle.

3) Significant results / key outcomes

Major task 1: We were able to confirm that treating primary cortical neurons with T3D-959 increases the expression of key PPAR-delta target genes at doses of 100 nM, 1 uM, and 10 uM, but has no effect at 10 nM (**Figure 1**). Based upon these data, subsequent experiments were performed using T3D-959 at the three effective doses.

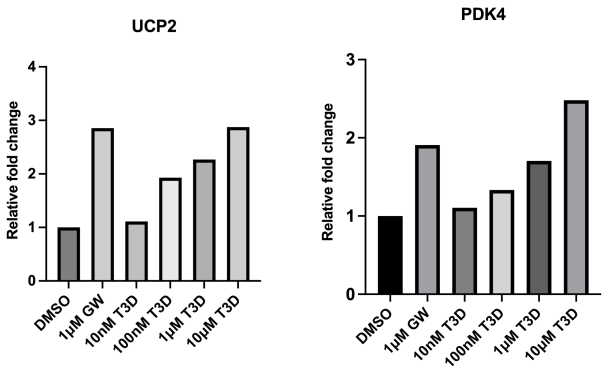


Figure 1. qRT-PCR analysis of PPAR-deta target genes in primary cortical neurons treated with T3D-959

Primary cortical neurons (PCNs) were cultured from postnatal day 1 mice and treated at DIV9 with the indicated dose of T3D-959 or the PPAR-delta agonist GW501516 (positive control). Neurons were cultured until DIV16 and RNA was isolated for downstream analyses.

However, we were unable to transfect primary cortical neurons with the GFP-only and TDP-t3 expression constructs at a high enough level of efficiency. Hence, we decided that we needed to switch to a system of transduction via infection with lentivirus. We therefore cloned each expression construct into a lentivirus vector, and provided the lentivirus vectors to a core service for production of high titer lentivirus for these experiments. We received the lentivirus vectors, and we were able to efficiently transduce primary cortical neurons with GFP-only and GFP-TDP-43 expression constructs (**Figure 2**), thereby completing Subtask 1. **However, when we assayed for cell death, we did not detect a significant increase in cell death in neurons expressing TDP-43 or C-terminal TDP-43**. For this reason, we are testing additional stressors (e.g. H_2O_2, which is an oxidative stress) in subsequent experiments with TDP-43 transduction, and *we will not pursue Subtask 2-4 until we achieve significant TDP-43 toxicity in our TDP-43 cortical neuron model of ALS*.

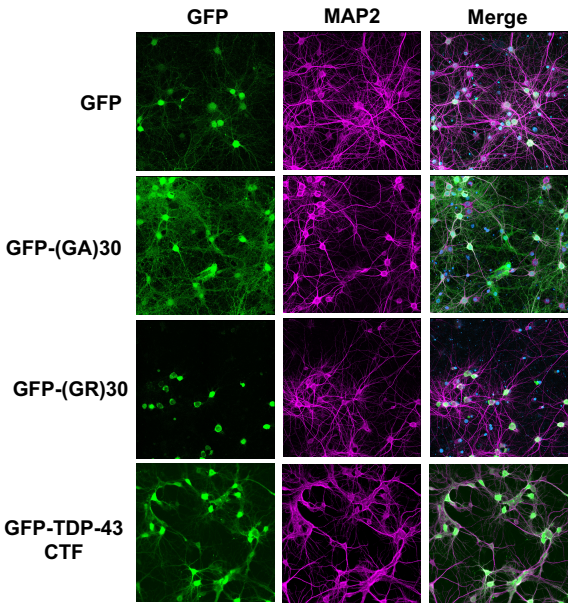


Figure 2. Efficient transduction of mouse primary cortical neurons with GFP, (GA)30, (GR)30, and TDP-43 C-terminal fragment

Primary cortical neurons (PCNs) were cultured from postnatal day 1 mice and transduced at DIV8 with lentivirus encoding GFP, GFP-(GA)30, GFP-(GR)30, or TDP-43 C-terminal fragment (CTF) for 24 hrs. PCNs were then cultured until DIV16 then fixed and immunostained.

Major task 2: We were able to efficiently transduce primary cortical neurons with GFP, (GA)30 [ggacga], and (GR)30 [ggaaga] vectors (**Figure 2**), thereby completing Subtask 1. We performed three biological replicates to assess nucleolar morphology (Subtask 2) by quantifying the mislocalization of NPM1 from the nucleolus to the nucleoplasm. Treatment with T3D-959 did not consistently alter nucleolar morphology in any of the conditions tested (**Figure 3**). Progress has also been made on Subtask 3. Primary cortical neurons were transduced with an NLS-NES-tdTomato construct along with GFP, (GA)30, or (GR)30, and fluorescence recovery after photobleaching (FRAP) was performed of the nucleus to assess nucleo-cytoplasmic transport. While transduction with GR(30) had little effect on fluorescent recovery, (GA)30 expressing neurons did exhibit impaired nuclear

transport (**Figure 4**). Treatment with T3D-959, however, did not significantly improve fluorescent recovery in either condition (**Figure 4**).

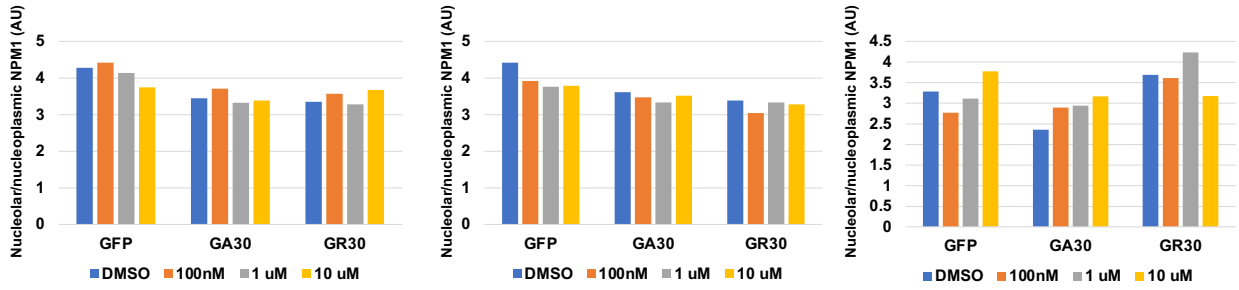


Figure 3. Analysis of nucleolar morphology in primary cortical neurons transduced with GFP, (GA)30, or (GR)30 and treated with T3D-959.

Primary cortical neurons (PCNs) were cultured from postnatal day 1 mice and infected on DIV8 with lentivirus encoding GFP, (GA)30, or (GR)30 for 24 hrs. PCNs were treated as indicated on DIV9, and fixed and immunostained on DIV16. Nucleolar morphology was determined by quantifying the relative fluorescence intensity of NPM1 in the nucleolus vs the nucleoplasm. Biological replicates 1 (left) and 2 (center) were analyzed using automated image analysis software. Biological replicate 3 (right) was analyzed manually using ImageJ. 50-300 neurons were quantified per condition.

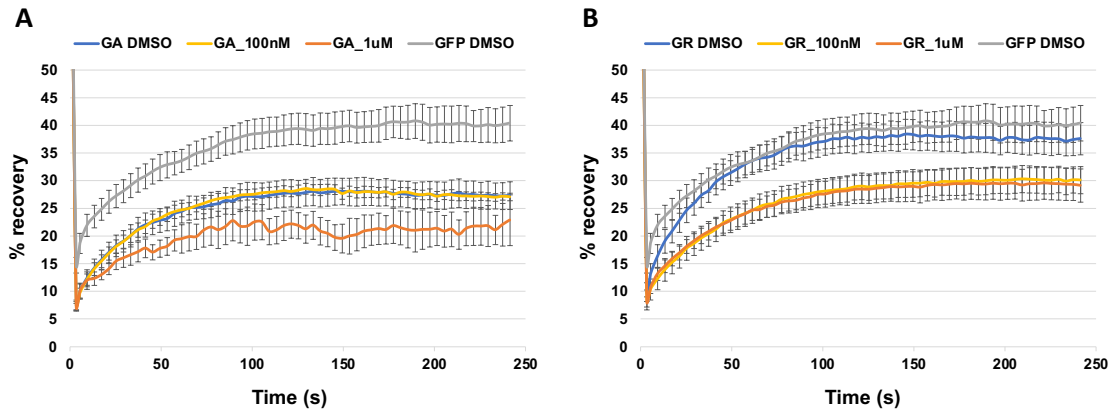


Figure 4. Nucleo-cytoplasmic transport in primary cortical neurons transduced with GFP, (GA)30, or (GR)30

Primary cortical neurons (PCNs) were cultured from postnatal day 1 mice and infected on DIV8 with lentivirus encoding NLS-NES-tdTomato and GFP, (GA)30, or (GR)30 for 24 hrs. PCNs were treated as indicated on DIV9 and fluorescence recovery after photobleaching (FRAP) of nuclear NLS-NES-tdTomato was performed on DIV16-17. Upon photobleaching, nuclear NLS-NES-tdTomato, fluorescent recovery of tdTomato was measured at 4 sec intervals for 4 min. For clarity, separate plots are shown comparing FRAP analysis of GFP PCNs with (GA)30 transduced neurons (**A**) and with (GR)30 transduced neurons (**B**) treated with DMSO, 100 nM T3D-959, or 1 uM T3D-959 as indicated. GFP (n=12), (GA)30 DMSO (n=12), (GA)30 100nM T3D-959 (n=13), (GA)30 1uM T3D-959 (n=4), (GR)30 DMSO (n=12), (GR)30 100nM T3D-959 (n=10), (GR)30 1uM T3D-959 (n=8). Error bars = S.E.M.

Pertaining to Subtask 4, we compared overall mitochondria content between neurons transduced with GFP, (GA)30, (GR)30 by measuring fluorescence intensity of the mitochondrial protein TOM20. We observed a significant increase in TOM20 intensity upon transduction of primary neurons with GA(30) and GR(30); however, treatment with T3D-959 did not affect this phenotype in a consistent manner (**Figure 5**). Hence, we have completed Major task 2, and found that T3D-959 treatment of GA(30)-expressing primary cortical neurons can rescue the altered mitochondrial phenotype caused by (GA)30.

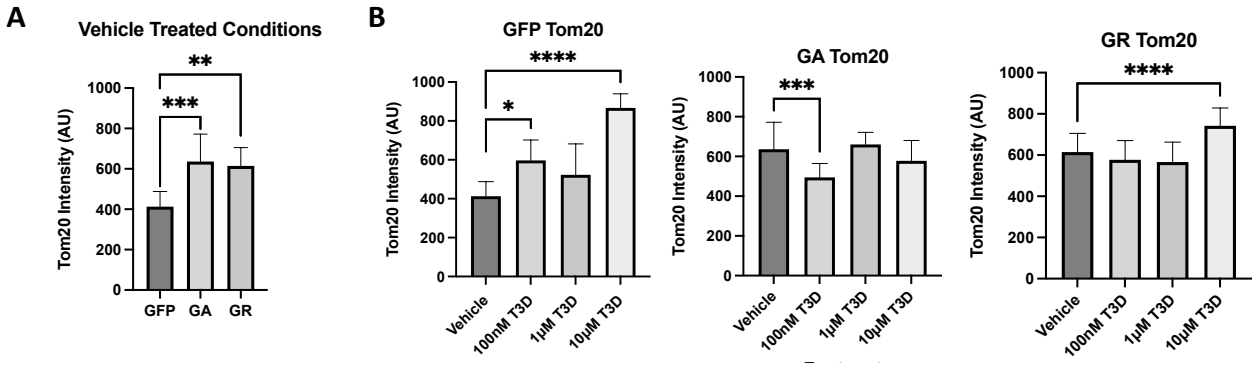
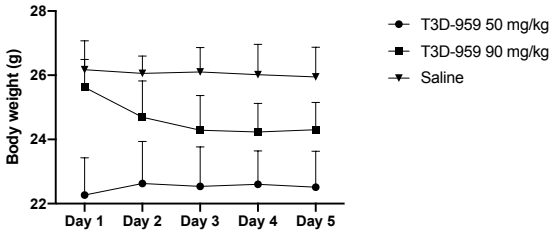


Figure 5. Analysis of TOM20 Intensity in primary cortical neurons transduced with GFP, (GA)30, or (GR)30 and treated with T3D-959.

Primary cortical neurons (PCNs) were cultured from postnatal day 1 mice and infected on DIV8 with lentivirus encoding GFP, (GA)30, or (GR)30 for 24 hrs. PCNs were treated as indicated on DIV9 and were fixed and immunostained on DIV16. Total mitochondria content was determined by quantifying the fluorescence intensity of TOM20 by confocal microscopy. (A) Comparison of GFP, (GA)30, or (GR)30 transduced PCNs treated with vehicle (DMSO). (B) PCNs were treated in duplicate with the following drug doses: Vehicle (DMSO), 100 nM T3D-959, 1 uM T3D-959 or 10 uM T3D-959. DIV16 PCNs were stained with MAP2 and TOM20 antibodies. To quantify the results, images were taken on a Nikon A1R confocal microscope at 60x and the mean intensity of TOM20 was analyzed in PCNs by generating a mask over MAP2-positive areas. 50-75 neurons were quantified per condition. Statistical significance was determined by one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001.

Major task 3: We did intraperitoneal injections in wild-type C57BL/6J control mice with different doses of T3D-959 dissolved in saline (30 mg/kg, 50 mg/kg, 70 mg/kg and 90 mg/kg) for one week to optimize the final dose for the preclinical trial. We monitored the body weight of these WT mice every day and assessed the general health of the T3D-959-treated WT mice in comparison to vehicle-treated WT mice (**Figure 6**). The expression levels of three PPAR-delta targets (ucp2, pdk4 and angptl4) in both cortex and spinal cord were measured by qRT-PCR (**Figure 7 & 8**). Based on the data of the induction of all three genes, along with absence of signs of disease, stress, or toxicity, we decided that 50 mg/kg is the best option for the dosage to be used in the preclinical trial and because of toxicity noted at higher doses, we chose to modify the dosing regimen to a Mon-Wed-Fri frequency.

- 10 mg/ml T3D-959 in saline
- Four groups of mice: WT w/o injection (3 mice), WT w/ saline (3 mice), WT w/ T3D-959 at 50 mg/kg (3 mice), WT w/ T3D-959 at 90 mg/kg (3 mice)
- IP injection for 5 days



- 20 mg/ml T3D-959 in saline
- Four groups of mice: WT w/o injection (3 mice), WT w/ saline (3 mice), WT w/ T3D-959 at 50 mg/kg (3 mice), WT w/ T3D-959 at 70 mg/kg (3 mice)
- IP injection for 5 days

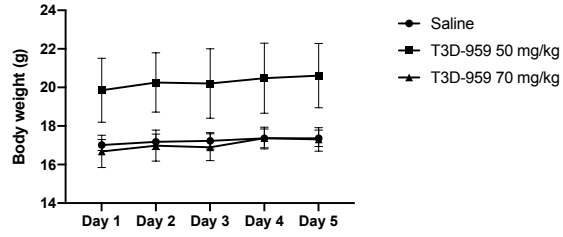


Figure 6. Effect of different dosages of T3D-959 on body weight in drug-treated control mice.

We performed intraperitoneal injection of T3D-959 at the doses indicated for one week, and tracked the body weight of drug-treated and vehicle (saline)-treated mice. Note that mice receiving a dose of T3D-959 at 90 mg/kg exhibited a significant degree of weight loss, revealing toxicity stemming from higher dosing of drug.

Major task 4: We bred PrP-TDP-43Q331K mice for the preclinical trial of T3D-959, and the baseline behavioral tests were finished at the age of 5 weeks. Based on the performance of the baseline of motor function, we balanced the genders and assigned these mice into T3D-959-treatment group and vehicle-treatment group. Due to concerns over toxicity noted in Major task 3, we initiated Mon-Wed-Fri intraperitoneal injections of 50 mg/kg/day T3D-959 at 6 weeks of age. The body weights of these PrP-TDP-43Q331K mice are being monitored on a weekly basis. Hence, Subtask 1 of Major task 4 has been completed within the first year of the project.

Major task 5: The (G4C2)-149R transgenic mice from the Petrucelli lab were transferred to our animal facility when they were 8 weeks of age to insure their survival. Due to the requirement of two-week quarantine, the baseline of rotarod and grip strength of these mice were not started until 10 weeks of age and required 2 weeks to complete including comprehensive analysis of the data. Based on the performance of the baseline of motor function, we balanced the genders and assigned these mice into the T3D-959-treatment group and vehicle-treatment group. We initiated Mon-Wed-Fri intraperitoneal injections of 50 mg/kg/day T3D-959 at 13 weeks of age. *As (G4C2)-149R transgenic mice do not develop appreciable disease phenotypes until 6 months of age, initiating the drug dosing at 13 weeks of age is an acceptable change to the protocol in our view*. The body weights of the (G4C2)-149R transgenic mice are being monitored every Monday. Hence, Subtask 1 of Major task 5 has been completed within the first year of the project.

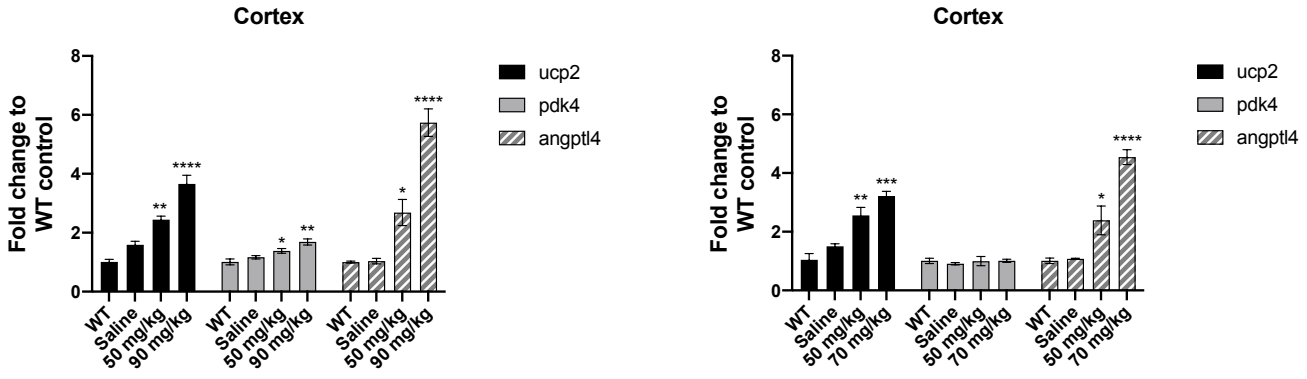


Figure 7. Pharmacodynamics study of PPAR-delta target engagement in the cortex of T3D-959-treated mice.

We performed intraperitoneal injection of T3D-959 into WT C57BL/6J control mice at the doses indicated for one week, and then euthanized the mice, dissected out the cortex, isolated RNA, and performed qRT-PCR analysis for three PPAR-delta target genes. Note significant induction of PPAR-delta target gene expression at the two tested doses of T3D-959 compared to untreated mice (WT) and mice subjected to intraperitoneal injection of diluent (Saline). Statistical significance was determined by one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Error bars = s.e.m.

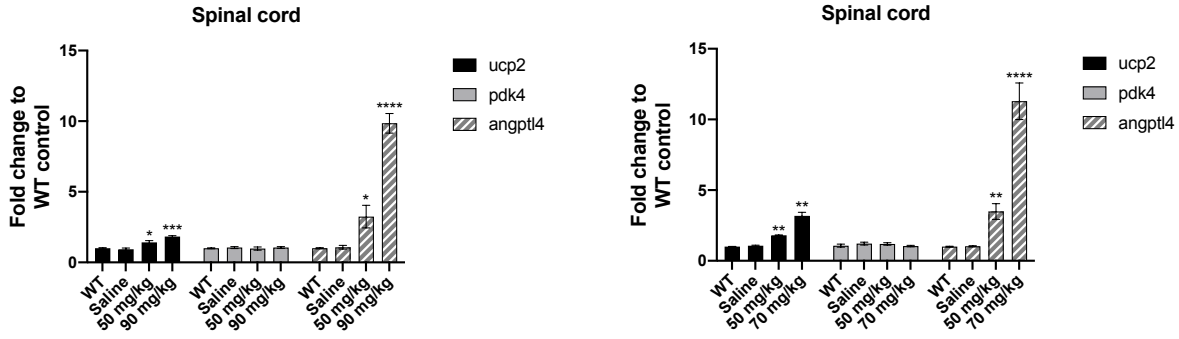


Figure 8. Pharmacodynamics study of PPAR-delta target engagement in spinal cord of T3D-959-treated mice.

We performed intraperitoneal injection of T3D-959 into WT C57BL/6J control mice at the doses indicated for one week, and then euthanized the mice, dissected out the spinal cord, isolated RNA, and performed qRT-PCR analysis for three PPAR-delta target genes. Note significant induction of PPAR-delta target gene expression at the two tested doses of T3D-959 compared to untreated mice (WT) and mice subjected to intraperitoneal injection of diluent (Saline). Statistical significance was determined by one-way ANOVA. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001. Error bars = s.e.m.

4) Other achievements
Nothing to report

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

Nothing to report

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

I presented the initial results of this study to an ALS patient group in Dec 2021.

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

We are proceeding with two preclinical trials of the drug T3D-959 in two different ALS mouse models, and we intend to complete these two preclinical trials by the end of calendar year 2022. We will also work on developing a TDP-43 cortical neuron model system that exhibits significant cell death and toxicity by exposing TDP-43 transduced neurons to various stressors to identify a treatment that elicits TDP-43 dependent neurotoxicity, and then will test if T3D-959 can rescue such neurotoxicity.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report (yet)

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

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report

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

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change

As noted above, we needed to switch to lentivirus infection to achieve successful transduction of primary cortical neurons with the TDP-43 expression constructs. This was an anticipated 'Possible Complication' as noted in our original proposal.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Despite successful transduction of primary cortical neurons with lentivirus TDP-43 expression constructs (-see **Figure 2**), we are yet to observe TDP-43 dependent neuron toxicity, as has been reported by other research groups in the published literature. Hence, we are simply going to expose cultured neurons to different stressors, which have been pathogenically linked to ALS disease, in order to identify a treatment paradigm that we can use to elicit TDP-43 dependent primary cortical neuron toxicity. Stressors to be tested include: MG132 (proteostasis inhibition), glutamate (excitotoxicity), oligomycin + antimycin (metabolic challenge), or hydrogen peroxide (oxidative stress).

Changes that had a significant impact on expenditures
Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

Not applicable (no human subjects)

Significant changes in use or care of vertebrate animals

Nothing to report

Significant changes in use of biohazards and/or select agents

Not applicable (no biohazards or select agents)

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

Nothing to report

Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

Nothing to report

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

Nothing to report

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

Nothing to report

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

Nothing to report

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

No significant participation to report from PIs at collaborating institutions

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

The PI is now supported by R35 NS122410 and an award from the Alzheimer's Association. Furthermore, the PI is no longer supported by R01 NS111588, R01 NS100023, or by the Michael J. Fox Foundation award 17914.

ACTIVE

R35 NS122140 (La Spada, A.R.) 4/1/21 – 3/31/29 6.0 cal. months
NIH/NINDS
"La Spada Outstanding Investigator Award"
This award mechanism will provide the PI with funds to continue his NINDS-funded research on the cellular and molecular basis of polyglutamine neurodegeneration, with a focus on mechanisms of skeletal muscle mediated lower motor neuron degeneration in SBMA and the role of altered transcription regulation in HD.

5R01 NS065874-11 (La Spada, A.R.) 7/12/18 – 1/31/22 0.1 cal. month
NIH/NINDS
"PPAR-delta pathway in neural function and Huntington's disease neuropathology"
In this project, we will determine how PPARδ promotes neuroprotection by examining bioenergetics and quality control, by defining the cistrome and active regulome of PPARδ in normal and HD neurons, by performing transcriptome analysis of HD mice treated with PPARδ agonist, and by validating identified PPARδ target genes and pathways.

5R01 EY014061-15 (La Spada, A.R.) 9/1/19-6/30/23 1.0 cal. month
NIH/NEI
"SCA7 neurodegeneration: Molecular epigenetic basis and therapy"
In this project, we will determine if epigenetic dysregulation, mitochondrial dysfunction, DNA damage, PARP1 hyperactivation, or excess NAD+ utilization promote SCA7 disease pathogenesis, and we will test the hypothesis that shared pathogenic processes, occurring in SCA7 and related, inherited cerebellar or retinal degenerations, offer an opportunity to develop treatments with potential for broad application.

3R01 AG033082-10 (La Spada, A.R.) 9/1/15-5/31/22 0.1 cal. month
NIH/NIA
“Molecular genetic regulation of autophagy in health and neurodegenerative disease”
The goal of this study is to define the genetic regulatory basis of neuronal autophagy and to examine the role of post-translational regulation of TFEB in modulating autophagy. This study also will test the hypothesis that master regulators in the amino acid sensing pathway are key nodes in the integration of nutrient sensing and proteostasis activation.

7RF1 AG057264-03 (MPI: Cortes & La Spada) 8/15/18 – 6/30/22 0.2 cal. month
NIH/NIA Subaward:
"Enhanced skeletal muscle proteostasis as a determinant of CNS protein quality control and neural function in the aging brain"
In this project, we will investigate the role of skeletal muscle-expressed Transcription factor E-B (TFEB), a master transcription factor regulator of autophagy and cellular clearance, in promoting CNS proteostasis during aging.

AA678129 (La Spada, A.R.) 9/1/20 – 8/31/22
1.0 cal. month
Alzheimer’s Association / Tau Consortium
“Evaluation of PPAR-delta agonist therapy as a treatment for tauopathy”
The goal of this project is to test if the PPAR-delta agonist KD3010 can prevent AD-relevant phenotypes in in a tauopathy mouse models of AD and in cortical neurons derived from AD / tauopathy patients.

2018-191999 (Eroglu, C.) 12/1/18 – 11/30/21 0.2 cal. month
Chan Zuckerberg Initiative Subaward:
“Role of Dysfunction Astrocyte-Neuron Signaling in Parkinson’s Disease”
The goal of this project is to study the roles of astrocytes, the most abundant non-neuronal cells of the brain, as important players in PD pathogenesis.
Role: Co-PI

1R01 AG065387-01 (Tautz, L.) 7/1/20 – 6/30/23 0.2 cal. month
NIH/NIA Subaward:
“Development of STEP allosteric inhibitors as novel therapeutics for Alzheimer’s Disease”
This project proposes to discover inhibitors of Striatal-Enriched Tyrosine Phosphatase as a therapeutic strategy to treat AD by high throughput screening, followed validation of efficacy and safety in both cellular and animal models.
Role: Co-I

AL190071 (La Spada, A.R.) 12/15/20 – 12/14/22 2.4 cal. months
Department of Defense
“Evaluation of PPARδ/γ agonist therapy as a novel treatment paradigm for ALS”
The goal of this project is to test if T3D-959 can prevent ALS disease-relevant phenotypes in primary cortical neuron models of ALS, and in mouse models of ALS.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ebrap.org/eBRAP/public/index.htm*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil/Pages/Resources.aspx) should be updated and submitted with attachments.*

9. **APPENDICES:** *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*