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TITLE: Selective Inhibition of Pathological Mitochondrial Fission to Improve Mitochondrial Function and Inhibit Neurodegeneration and Neuroinflammation in ALS

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CONTRACTING ORGANIZATION: Leland Stanford Junior University, Stanford, CA

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14. ABSTRACT

Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by the death of upper and lower neurons in the brain that control our muscles (called motor neurons). ALS is manifested clinically by progressive muscle wasting and paralysis, and individuals with ALS most commonly die of respiratory failure or pneumonia within 3–5 years of initial diagnosis. There is no cure for ALS but two drugs, riluzole and edaravone, are approved to treat ALS by modestly slowing the progress of disease. Given this, there is a strong need to develop new and improved treatment strategies for the ALS patient community. However, the primary cause of ALS remains unclear.

We rdecently demonstrated a critical role for mitochondrial dysfunction in general and excessive mitochondrial fragmentation as a major cause for the pathology; we showed that two proteins (Drp1 and Fis1) interact to initiate excessive mitochondrial fragmentation, and have designed a peptide inhibitor, P110 peptide, that blocks Drp1/Fis1 interaction in mitochondria and is effective in ALS patient-derived cells and in an ALS mouse model. In the first year of the proposal, we identified and evaluated novel small molecule mimetics of P110, SC9, as potential therapeutic candidates for ALS treatment. Since dysfunctional mitochondria have been observed in a broad range of ALS patients, we anticipate treatment with a fission inhibitor will provide some benefit to all patients, regardless of the mechanism underlying disease development. In the coming year the pharmacological properties of SC9 and analogues will be evaluated.

15. SUBJECT TERMS

Amyotrophic Lateral Sclerosis, Blood brain barrier, Drp1, Fis1, Mitochondrial fragmentation, Neurodegeneration, P110, Small molecule

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Introduction

Amyotrophic Lateral Sclerosis (ALS) is a progressive neurodegenerative disease, characterized by progressive weakening of the muscles, leading to paralysis, and is ultimately fatal. Mitochondrial dysfunction has been noted in ALS patients and animal models of ALS, and mitochondria, and thus have emerged as a potential target for ALS therapeutic development[1]. We have already found that a rationally designed inhibitor of pathologic mitochondrial fragmentation, a selective inhibitor Drp1/Fis1 – the **P110** heptapeptide, is a safe and effective inhibitor of the pathologies in ALS patient-derived cells, and in an ALS mouse model[2]. We have also identified several limitations for the use of P110 as a treatment in ALS patients. The purpose of this project is to overcome these limitations, by identifying a small molecule that mimics P110's action but has superior pharmacological characteristics. Our project addresses the following four **HYPOTHESES**: that: **(1) small molecule mimetics of P110 will overcome P110's pharmacological barriers** and can be identified using library-based drug discovery methodologies combined with *in silico* analyses; **(2) P110-SMs will provide similar therapeutic benefit to P110 peptide in culture**[2]; **(3) a validated, stable, safe and well-tolerated lead candidate, P110-SM, with spinal cord penetration can be identified;** and **(4) lead P110-SM will provide therapeutic benefit** in an ALS mouse model.

Keywords: Amyotrophic Lateral Sclerosis, Blood brain barrier, Drp1, Fis1, Inhibitor, Mitochondria, Mitochondrial fragmentation, Neurodegeneration, Peptide, Pharmacokinetics, P110, Safety, Small molecule

Accomplishments

What were the major goals of the project?

1. Identify P110 small molecule mimetics and select for therapeutic candidates**.**

2. Determine P110-SM's effect on mitochondrial dynamics and cell viability in cultured cells.

3. Stability, safety and PK studies (including BBB penetrability) of top P110-SM candidates, in vivo.

4. Evaluate P110-SM pharmacology, toxicology, and efficacy in the SOD1 G93A ALS mouse model.

What was accomplished under these goals?

Accomplishments under the above-mentioned goals during this first year of funding are as follows:

Goal 1: Identify P110 small molecule mimetics and select for therapeutic candidates.

Major activity 1: Using high-throughput screening, identify a small molecule therapeutic candidate that mimics the properties of the peptide P110.

We worked on the development of fluorescence polarization-based high throughput assay in a 384-well format to screen for small molecules that can bind to Drp1 with similar binding mode as P110. We used fluorescently labelled P110 and measured the fluorescence polarization in the presence of Drp1. We used

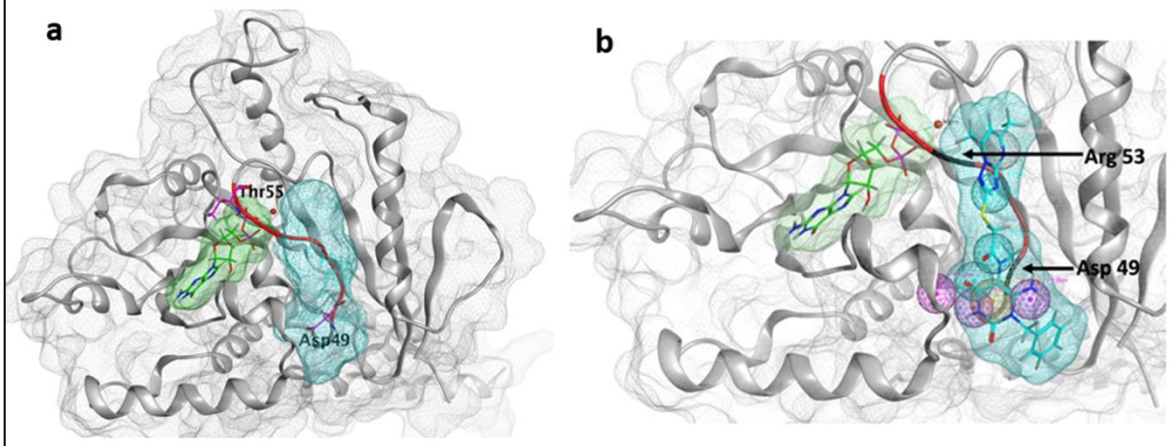
unlabeled P110 as a positive control that should displace fluorescently labelled P110, and we expected that true P110 mimetics will show similar results. The change in polarization signal was not large and therefore the signal to noise ratio was not acceptable [Expected: Polarization (Δ>200mP) and Signal to Noise ratio (Z' 0.5-1)]. Therefore, we could not use this assay further to screen the small molecule library.

We initially projected that this activity would be accomplished in 8 months. We worked on assay development for over 4 months and due to the lack of progress we deprioritized this activity and focused on alternative activity discussed next.

Major activity 2: Using in silico modeling, screen for P110-mimetic small molecules.

Using computational modelling with the drug discovery software platform MOE (Molecular Operating Environment)[3], we identified the likely P110-binding pocket (Switch I- adjacent groove; SWAG) in Drp1 (**Fig**. 1a). We used MOE's pharmacophore modelling tool, AutoPH4[4], and screened $7x10^6$ small molecules in the Commercial Compound Library (CoCoCo)[5] (**Fig**. 1a, b).

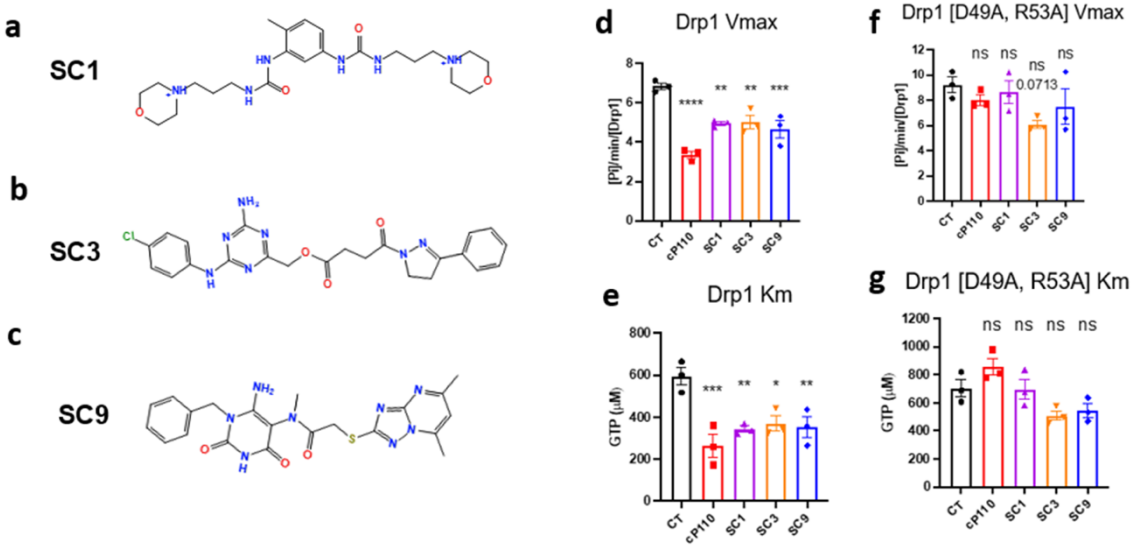
Fig. 1: *In silico* modeling, virtual screen for P110-mimetic small molecules



a, Relative position of SWAG (cyan) in the overall structure of Drp1. **b,** Relative positions of the nucleotide binding site (green), SWAG (cyan) and the endogenous P110 sequence DLLPRGT (red) in the GTPase domain of Drp1. Pharmacophore features of SWAG are shown as colored spheres. Magenta sphere indicates donor, cyan sphere indicates acceptor, orange sphere indicates aromatic, or pi ring and light orange sphere indicates donor or acceptor feature.

Top hits from the pharmacophore-based screening were tested in the Drp1 GTPase assay and we found that three molecules SC1, SC3 and SC9 (**Fig**. 2a, b, c) uncompetitively partially inhibited the GTPase activity of Drp1 like P110 (**Fig**. 2d, e). These three molecules decreased both Vmax and Km of Drp1 GTPase activity (**Fig**. 2d, e). The site directed mutants in the Switch I- adjacent groove (SWAG) made Drp1 insensitive to the inhibitory activity of P110 and the SC molecules, suggesting that these molecules work in the same way by engaging SWAG (**Fig**. 2f, g). The identity and purity of these top molecules were established using proton NMR, carbon NMR and high-resolution mass spectrometry (HRMS) (See **Appendix S2**). The molecules were also re-synthesised and tested to confirm their identity.

Fig. 2: Inhibition of Drp1 GTPase activity by SC compounds.



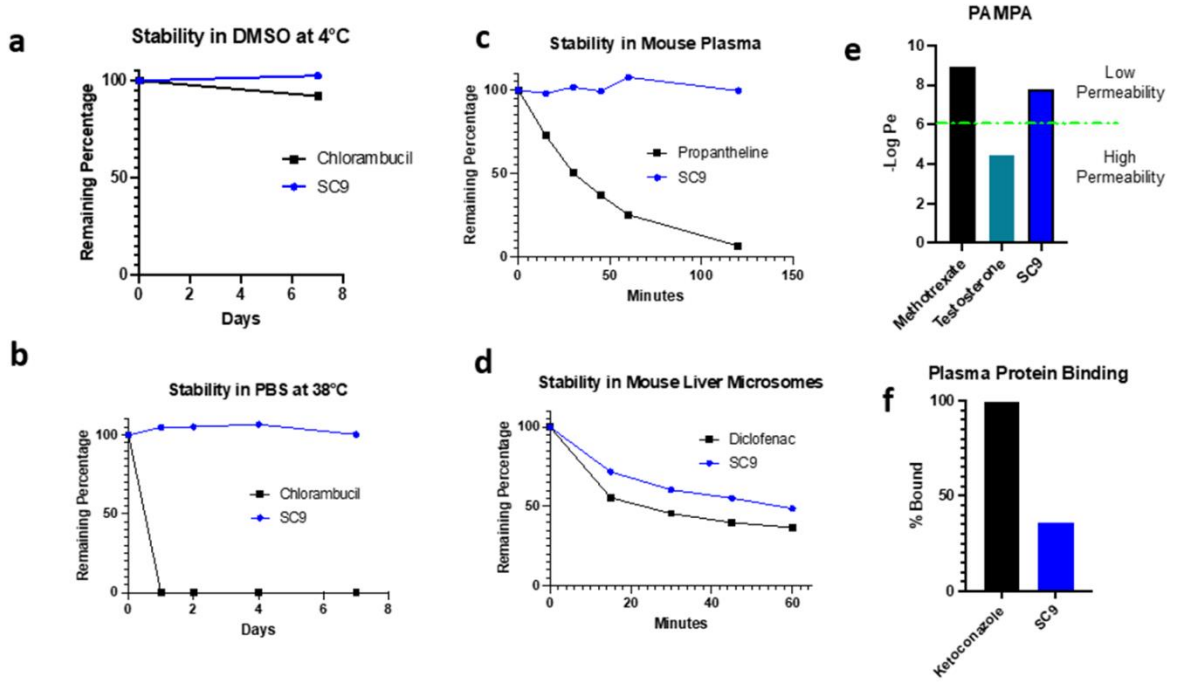
a, Structure of SC1, SC3 (**b**) and SC9 (**c**). **d,** Vmax and Km (**e**), of GTPase activity of Drp1 in the presence of cP110 and SC compounds. **f,** Vmax and Km (**g**) of GTPase activity of Drp1 with mutations in SWAG in the presence of cP110 and SC compounds.

SC9 showed promising activity and safety in cells and *in vivo* models (discussed later) and therefore we pursued this molecule further. We tested *in vitro* pharmacokinetic properties such as chemical stability (**Fig**. 3a, b), stability in plasma and liver microsomes (**Fig**. 3c, d), plasma protein binding and biological membrane permeability (**Fig**. 3e, f). We found that SC9 is chemically stable and did not have detectable degradation in mouse plasma in 2 hours and was also reasonably stable in mouse liver microsomes. SC9 showed low biological membrane permeability in the parallel artificial membrane permeability assay and showed low plasma protein binding.

To improve the membrane permeability and increase plasma protein binding, we are working on synthesizing hydrophobic analogues of SC9. We are making modifications on one polar region of the molecule (triazolo pyrimidine moiety) to yield analogues with lower topological polar surface area (TPSA). This work is ongoing, and we are planning to test activity and membrane permeability of these molecules using cell-based assay.

This activity was projected to be completed in10 months but this work is ongoing.

Fig. 3: *In vitro* pharmacokinetic properties of SC9.



a, Stability of SC9 in DMSO at 4°C, (**b**) in PBS at 38°C, (**c**) in mouse plasma and (**d**) in mouse liver microsomes. **e**, Parallel artificial membrane permeability assay and (**f**) plasma protein binding of SC9.

Major activity 3: Perform crystallographic structural analysis of Drp1 in complex with P110-mimetic small molecules.

We attempted to co-crystallize Drp1 with SC9/P110 but have not been successful so far. Alternately, we also tried soaking Drp1 crystals with SC9/P110 but have not been able to observe electron density for the ligands yet.

This activity was projected to be accomplished in 10 months, but this work is still ongoing.

Goal 2: Determine P110-SM's effect on mitochondrial dynamics and cell viability in cultured cells.

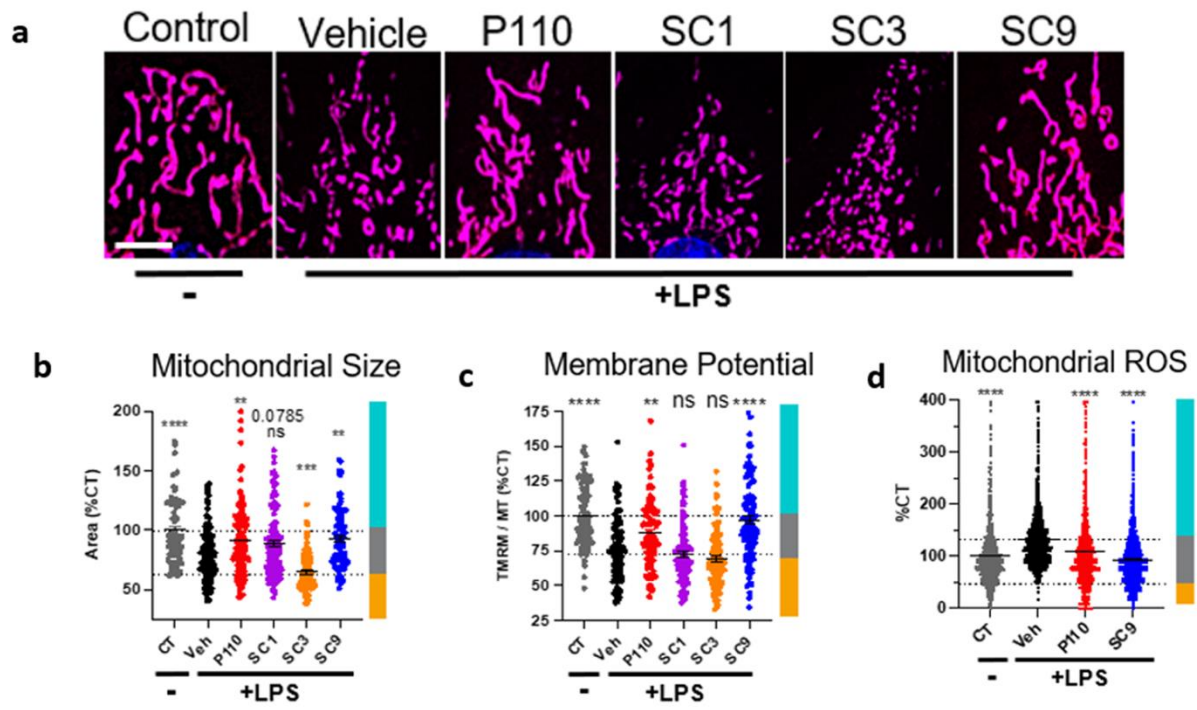
Major activity 1: Using a murine motor neuron-like cell expressing mutant human SOD1G93A determine whether P110-SM improves mitochondrial structure and function, and cell health.

We first performed the proof-of-concept studies of SC9 effect on mitochondrial dynamics in cultured cells using H9C2 cardiomyocytes under stress. In H9C2 cardiomyocytes, we observed lipopolysaccharide (LPS) treatment caused mitochondrial fragmentation and SC9 treatment prevented this fragmentation, similar to the effect of P110 (**Fig**. 4a, b). We found that SC9, like P110, reversed the mitochondrial membrane depolarization caused by LPS treatment (**Fig**. 4c) and also decreased the reactive oxygen species (ROS) production (**Fig**. 4d).

In the coming months, we will use murine motor neuron-like cells expressing mutant human SOD1 G93A to test the efficacy of SC9 in improving mitochondrial structure and function, and cell health.

This activity was projected to be accomplished in 14 months and this work is ongoing.

Fig. 4: Activity of P110 and SC compounds in cells in culture



a, Representative images of mitochondria staining with MitoTracker Deep Red FM and nuclei staining with blue Hoechst stain after LPS (2ug/ml) and 2µM compound co-treatment for 16hrs (white scale bar = 10µm). **b**, Manually segmentation single cell average mitochondrial size. **c,** Manually segmented single cell average TMRM signal (TMRM integrated density/MitoTracker 2D area). **d,** Auto-segmented single cell MitoSOX signal (MitoSOX median intensity/MitoTracker 2D area).

Major activity 2: Using primary human ALS-patient derived fibroblasts, determine whether P110 SM improves mitochondrial structure and function, and cell health.

We have obtained healthy control and ALS-patient-derived fibroblasts with different genetic forms of ALS, [e.g., carrying pathogenic mutations in SOD1 I113T (superoxide dismutase), in FUS1 R521G (fused in sarcoma) or in TDP-43 G289S (TAR DNA-binding protein 43)]. In the coming months, we plan to test the efficacy of SC9 in improving mitochondrial structure and function, and cell health in these fibroblasts.

This activity was projected to be accomplished in 16 months and this work is ongoing.

Goal 3: Stability, safety and PK studies (including BBB penetrability) of top P110-SM candidates, in vivo.

Major activity 1: Submit documents for ACURO approval.

ACURO approval was obtained. This activity was accomplished in the first 12 months as projected.

Major activity 2: Local IACUC approval for all in vivo components.

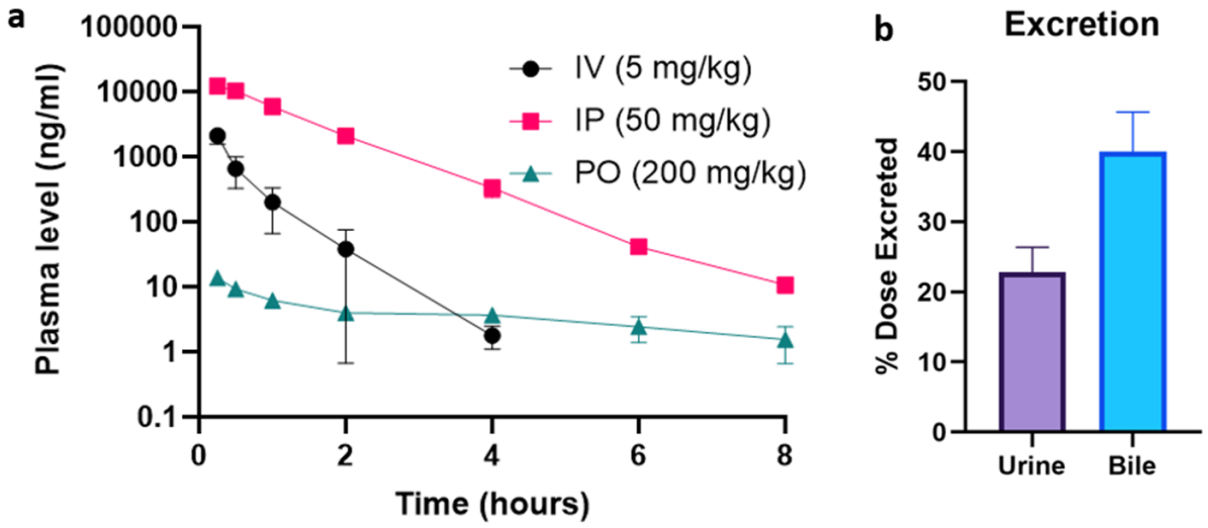
IACUC approval was obtained. This activity was accomplished in the first 12 months as projected.

Major activity 3: Perform stability testing of P110-SM in wildtype rats.

We measured the level of SC9 in plasma at different time points after oral, intraperitoneal and intravenous administration (**Fig**. 5a). We found that SC9 is not orally bioavailable and following intraperitoneal and intravenous administration, the plasma level of SC9 decreases rapidly and becomes almost undetectable after 4 hours. The drug is rapidly cleared from the body in urine and bile. Almost 25% of the drug was cleared in urine and 40% of the drug was cleared in bile in 24 hours (**Fig**. 5b).

This activity was projected to be accomplished in 16 months, but it was completed in the first 12 months.

Fig. 5: *In vivo* pharmacokinetic properties of SC9.

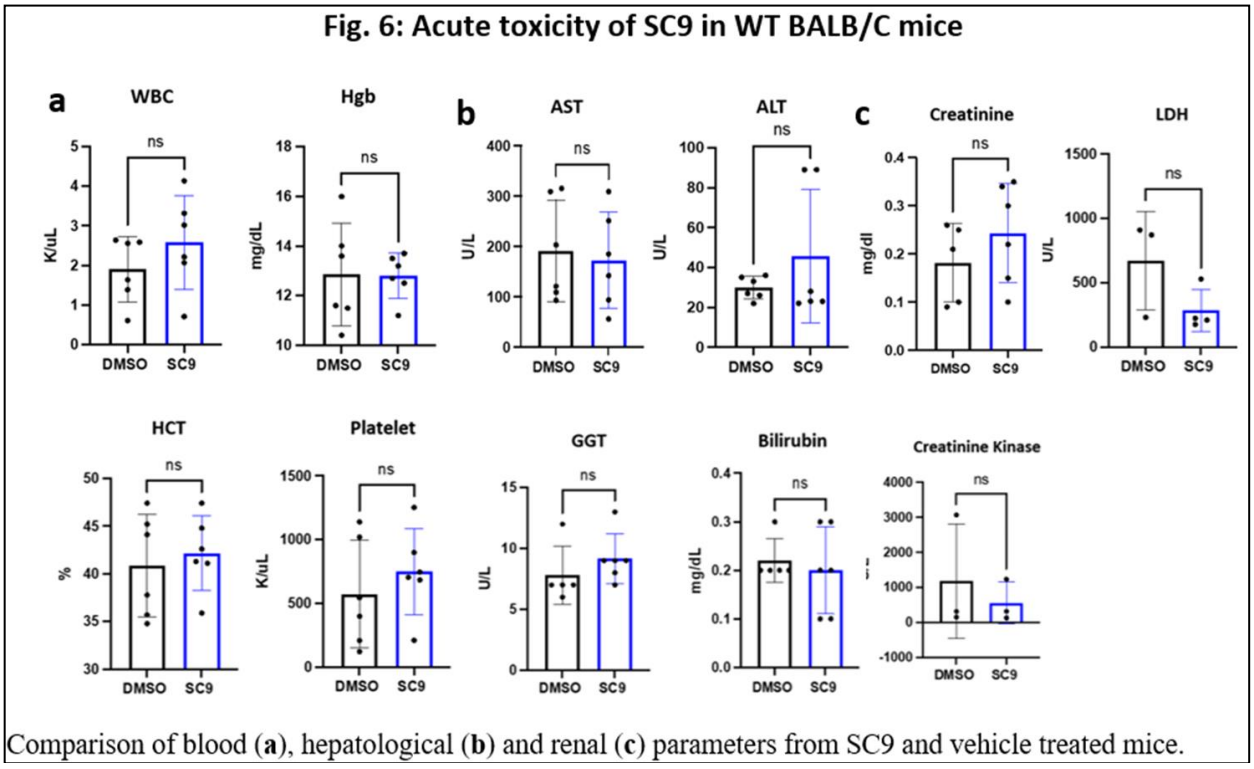


a, Plasma level of SC9 at different time point after administration. **b,** Excretion of SC9 in urine and bile.

Major activity 4: Assess the safety of P110-SM using single ascending doses and multiple ascending doses studies in wildtype mice and determine BBB and spinal cord penetrability.

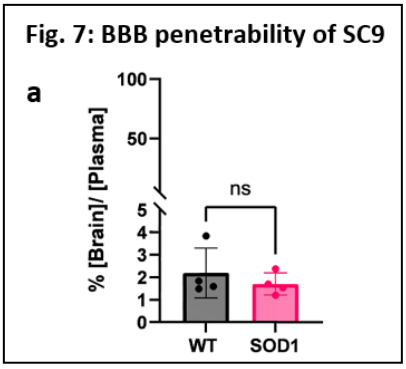
We determined the acute safety of SC9 at 50mg/kg dosage administered once intraperitoneally. We didn't observe death or other major adverse outcomes. In a study where the observer was blinded to the

experimental protocol, we measured the hematological, renal and hepatic parameters and observed no statistically significant difference between the vehicle treated and SC9 treated wildtype BALB/C mice (**Fig**. 6a, b, c).



Comparison of blood (**a**), hepatological (**b**) and renal (**c**) parameters from SC9 and vehicle treated mice.

We also measured the level of SC9 in brain after 15min post intraperitoneal administration at 20mg/kg dosage. We observed that only 2% of the plasma SC9 level was present in the brain (**Fig**. 7a). The observation was consistent in both B6SJL Tg (SOD1 G93A)1Gur/J mice and WT littermates. This showed that SC9 doesn't penetrate the blood brain barrier.



This activity was projected to be accomplished in 16 months and it is still ongoing.

<u>Major activity 5: Determine availability of P110-SM following subcutaneous delivery using Alzet pump in wildtype B6SJL.</u>

All the solvents compatible with Alzet pump material were tested for solubility of SC9 at the concentration required for 20mg/kg daily dosage. Unfortunately, SC9 crashed out of solution overnight in all the solvents. We had to use 100% DMSO as a solvent. However, after a few weeks post pump implantation, some mice developed dermatitis and based on the recommendation of the veterinary care team, this study was discontinued.

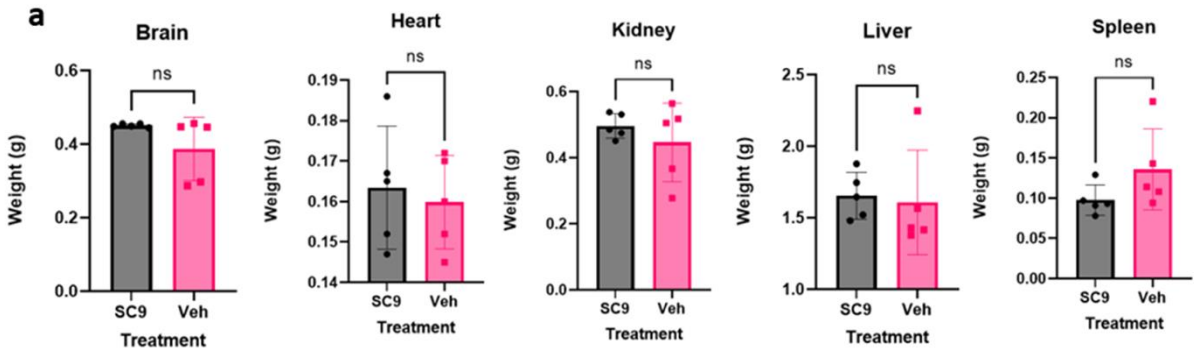
This activity was projected to be accomplished in 16 months, but it was completed in 12 months.

Major activity 6: Investigate safety of P110-SM after chronic delivery in which wildtype B6SJL compared to vehicle control.

We administered 20 mg/kg of SC9 intraperitoneally once daily for 3 months to wildtype B6SJL mice. The mice behavior was monitored by an observer blinded to the experimental conditions. Blood and organs were collected and analyzed at the end of the study. There were no observed abnormal behavior, death or any other adverse outcomes in either SC9- or vehicle-treated wildtype mice. We also weighed organs from the vehicle- and SC9-treated mice and did not observe any significant difference (**Fig**. 8a). Complete blood count, plasma metabolites, urine metabolites are currently being analyzed to assess potential toxicity of chronic SC9 administration relative to vehicle control. We are also performing histopathological analyses to measure damage in soft tissues due to SC9 administration.

This activity was projected to be accomplished in 18 months and it is still ongoing.

Fig. 8: Sub-chronic safety of SC9.



a, Comparison of organ weights between SC9 and vehicle treated WT B6SJL mice. These mice were treated for 3 months with daily IP injection.

Goal 4: Evaluate P110-SM pharmacology, toxicology, and efficacy in the SOD1G93A ALS mouse model.

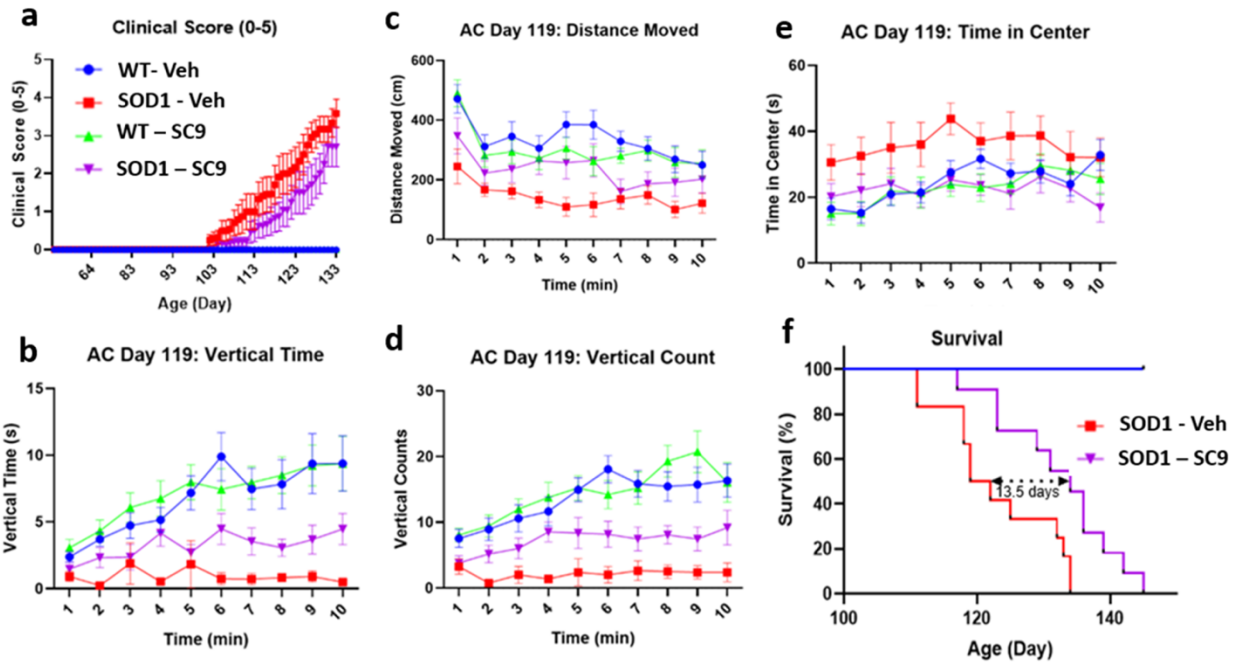
Major activity 1: Evaluate the efficacy of P110-SM in the SOD1G93A ALS mouse model.

We obtained 45 days old B6SJL Tg (SOD1 G93A)1Gur/J mice and WT littermates. We genotyped them and confirmed the presence or absence of SOD1 G93A mutations. We randomized the SOD1 and WT mice and divided them into two groups each. There were 15 animals in each of the four groups; vehicle-treated WT, SC9-treated WT, vehicle-treated SOD1 and SC9-treated SOD1. All the measurements were carried out by an observed blinded to the experimental protocol.

Treatment began when the mice were 60 days old, using 20mg/kg/day SC9 or vehicle control, as indicated above. Disease progression was assessed using 1. Body weight 2. Gait 3. Grip strength 4. Gross locomotor activity in activity chamber 5. Clinical score and 6. Neuro-score. We also collected time-matched urine from the animals to analyze urine biomarkers, such as hydroxy-2′-deoxyguanosine (8-OHdG)[6] and extracellular domain of p75NTR (p75ECD)[7]. We observed that SC9 treatment delayed disease progression (**Fig**. 9a), increased motor functions (**Fig**. 9b, c, d, e) and increased median survival by 13.5 days (**Fig**. 9f)

relative to vehicle-treated SOD1 mutant mice. We are currently performing QC checks and analysis on these data.

Fig. 9: Efficacy of SC9 in SOD1 G93A ALS mouse model



a, Clinical score. **b, c, d, e,** Motor functions and **f,** survival of vehicle and SC9 treated WT and SOD1 G93A mice.

Following these promising results, we repeated the study to make it more robust. We obtained 45 days old B6SJL Tg (SOD1 G93A)1Gur/J mice and divided them into 4 groups: 2 replicate groups of vehicle-treated and 2 replicate groups of SC9-treated with 10 SOD1 mice in each group. We observed that disease progression and survival was extremely variable between the two replicate groups within the same treatment group (See **Appendix S3**): One of the SC9-treated group and one of the vehicle-treated group shows increased survival, similar to the first cohort of SC9-treated mice. Conversely, one of the vehicle-treated group and one of the SC9-treated group shows short survival, similar to the first cohort of vehicle-treated mice. We are still investigating the cause of this variability. We plan to repeat this study again.

This activity was projected to be accomplished in 24 months and it is still ongoing.

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

Suman Pokhrel, my PhD student, was trained in pharmacology and drug discovery through this work.

How were the results disseminated to communities of interest?

1. Suman Pokhrel presented a poster on SC9 early discovery work in the Society for Neuroscience conference in San Diego in November 2022.
2. Manuscript on SC9 early discovery work is submitted for publication.

What do you plan to do during the next reporting period to accomplish the goals?

We plan to repeat the experiments for evaluation of efficacy of SC9 in the SOD1 G93A ALS mouse model to make the findings more robust (Major Activity 1 under Goal 4). We plan to synthesize and characterize SC9 analogues with greater biological membrane permeability and longer half-life in plasma. These will be tested using ALS-patient derived cells (Major Activity 2 under Goal 2). We plan to collate data generated in goals 1-4 and prepare manuscripts for publication.

Impact

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

1. We showed that inhibition of Drp1/Fis1 interaction on the surface of the mitochondria, which is seen in patients with ALS, is an effective strategy to treat ALS. Using a mouse model for the disease, we showed that at least some of the pathology associated with ALS is due to activation of excessive mitochondrial fission and fragmentation and inhibiting this excessive fission is beneficial in this animal model of ALS. We found that this treatment delays disease progression, improves motor function and increases survival in ALS mouse model. Therefore, our study may cause others to focus their work on improving mitochondrial functions for the treatment of ALS.
2. Our compound, SC9, does not penetrate blood brain barrier (BBB); yet it has a significant efficacy in the mouse model, suggesting that ALS has a systemic component and peripherally-restricted treatment may also be beneficial to treat ALS. Previously, researchers only focused on BBB penetrating molecules to treat ALS. Our findings may bring a change to this approach.

What was the impact on other disciplines?

1. The notion that protein/protein interaction (PPI) is 'not druggable', as it is flat and does not have a recognizable grove to target, is commonly repeated in the literature. We showed that a grove can be induced when one of the proteins undergoes a conformational change to become competent in binding the other. Here we show that Drp1/Fis1 interaction is induced by oxidative stress and that forms a druggable pocket in Drp1 into which SC9 docked. Other PPIs may have also an inducible pocket formation that can be used to develop PPIs.
2. *De novo in silico* searches for chemical entities that bind a target pocket are not often used. In silico searches are used more often once a hit has been identified through a biochemical- or cell-based screen. Our screen identified 600 potential hits, we tested only three: one had non-selective cell toxicity, the second affected also another large GTPase and the third – SC0 – was safe and effective inhibitor of Drp1/Fis1 interaction. we hope that our work will encourage others to use this approach more often.
3. Excessive mitochondrial fission and dysfunction is implicated in many other diseases, including other neurodegenerative, cardiovascular and inflammatory diseases[8,9,10,11]. Therefore, SC9 can be a valuable tool to study and treat these diseases.

What was the impact on technology transfer?

Patent for SC9 is licensed by Comorin Therapeutics Inc. to use this technology to develop treatments for mitochondrial diseases.

What was the impact on society beyond science and technology?

Nothing to report.

Changes/Problems

Changes in approach and reasons for change.

We used Sprague-Dawley rats instead of B6SJL mouse for pharmacokinetic studies.

> We wanted to measure the SC9 levels in plasma after 8 time points in a 24-hour period. Rats are larger in size and have larger blood volume than mice and therefore blood for 8 time periods could be collected from a single rat whereas we would need 8 mice to do the same study. Therefore, we decided to use Sprague-Dawley rats instead of B6SJL mouse for pharmacokinetic studies. The findings from the pharmacokinetic study on rats can be directly translated to B6SJL mouse.

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

ALS mouse model studies

> To repeat the efficacy studies, we obtained 45 days old B6SJL Tg (SOD1 G93A)1Gur/J mice and divided into 4 groups- 2 replicate groups of vehicle treated and 2 replicate groups of SC9 treated with 10 SOD1 mice each. We observed that disease progression and survival was extremely variable between the two replicate groups within the same treatment group (See **Appendix S3**). The findings were inconclusive, and we discontinued the experiment; we worry that the labels were switched and will try to use target occupancy to determine that. Other causes for this variability are also examined. We are planning to repeat this study again.

Changes that had a significant impact on expenditures

> Nothing to report.

Significant changes in use or care of human subjects.

> Nothing to report.

Significant changes in use or care of vertebrate animals.

> We used Sprague-Dawley rats instead of B6SJL mouse for pharmacokinetic studies.
>
> This study was approved by Stanford APLAC on 09/16/2022.

Significant changes in use of biohazards and/or select agents.

Nothing to report.

Product

Publications, conference papers, and presentations

Publications.

Rios, L.C.*, Pokhrel, S.*, Li, S., Heo, G., Haileselassie, B., and Mochly-Rosen, D. Targeting an allosteric site in dynamin related protein 1 to inhibit Fis1-mediated mitochondrial dysfunction. *Nature Communications*. Accepted. Acknowledgement of in part support by this DoD grant is included.

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

Nothing to report.

Other publications, conference papers, and presentations.

Rios, L.C., Pokhrel, S., Haileselassie, B., and Mochly-Rosen, D., 2022. Targeting an allosteric site in dynamin related protein 1 to inhibit Fis1-mediated mitochondrial dysfunction. Presented at Society for Neuroscience Conference, San Diego, CA.

Rios, L.C. 2022. Targeting an Allosteric Site in Dynamin-Related Protein 1 to Inhibit Fis1-Mediated Mitochondrial Dysfunction. Stanford University Thesis and Dissertations.

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Mochly-Rosen, D., Pokhrel, S., Rios, L.C., and Haileselassie, B., 2022. Small Molecule Modulators of GTPase Enzymes and Uses Thereof. PCT/US2023/061879.

Other Products

Nothing to report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Daria Mochly-Rosen
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0002-6691-8733
Nearest person month worked:	0.96
Contribution to Project:	Dr. Mochly-Rosen has supervised all the work related to this project
Funding Support:	This award

Name:	Soichi Wakatsuki
Project Role:	Principal Investigator
Researcher Identifier (e.g. ORCID ID):	0000-0001-5896-7968
Nearest person month worked:	0.48
Contribution to Project:	Dr. Wakatsuki has supervised crystallography and structural biology effort.
Funding Support:	This award

Name:	Suman Pokhrel
Project Role:	Graduate student
Researcher Identifier (e.g. ORCID ID):	0000-0003-2258-8768
Nearest person month worked:	6.0
Contribution to Project:	Mr. Pokhrel performed experiments and analyzed results.
Funding Support:	Stanford Graduate Fellowship

Name:	Gwangbeom Heo
Project Role:	Post doctoral scholar
Researcher Identifier (e.g. ORCID ID):	-
Nearest person month worked:	3.0
Contribution to Project:	Dr. Heo performed experiments and analyzed results.
Funding Support:	This award.

Name:	Kate Samardzic
Project Role:	Post doctoral scholar
Researcher Identifier (e.g. ORCID ID):	-
Nearest person month worked:	4.0
Contribution to Project:	Dr. Samardzic performed experiments and analyzed results.
Funding Support:	This award.

Name:	Luis C. Rios
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	-
Nearest person month worked:	3.0
Contribution to Project:	Mr. Rios performed experiments and analyzed results.
Funding Support:	Stanford Graduate Fellowship (graduated)

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

Daria Mochly-Rosen

COMPLETED AWARDS:
NIH R01 HD084422
NIH R01 HL52141
Booz-Allen Award
NIH HL144388

PENDING NOW ACTIVE FUNDING:

NARLABS (National Applied Resarch Labs/Taiwan)

NEW FUNDING:

Identifying ALDH4A1 Activators to Treat Pediatric Hyperprolinemia Type II
Name of PD/PI: Mochly-Rosen, D.
Source of Support: Stanford (Internal) SPARK/Weston Havens funding
Project/Proposal Start and End Date: 08/01/2022-07/31/2023
Total Award Amount (including Indirect Costs):
Effort Person Months: 0.0 calendar months (0%)

Reducing Transplant Graft Dysfunction Through Targeted Immunosuppression.
Name of PD/PI: Sallam K, Hollander S
Mochly-Rosen Role: Co-Investigator
Source of Support: American Heart Association
Project/Proposal Start and End Date: 07/01/2022-06/30/2025
Total Award Amount (including Indirect Costs):
Effort Person Months: 0.12 calendar months (1%)

Development of outpatient antiviral cocktails against SAR-CoV-2 and other potential pandemic RNA viruses
Project Number: U19 AI171421
Name of PD/PI: Glenn, J.
Mochly-Rosen Role: Co-Director
Source of Support: National Institutes of Health
Project/Proposal Start and End Date: 05/16/2022 – 04/30/2025
Total Award Amount (including Indirect Costs):
Effort Person Months: 0.60 calendar months (5%)

Molecular Strategies to Widen the Therapeutic Index of Radiotherapy
Project Number: P01 CA257907
Name of PD/PI: Le, Quynh-Thu
Mochly-Rosen Role: Co-Investigator
Source of Support: National Institutes of Health
Project/Proposal Start and End Date: 09/21/2022 - 08/31/2027
Total Award Amount (including Indirect Costs):
Effort Person Months: 0.24 calendar months (2%)

Soichi Wakatsuki

COMPLETED AWARDS:
Pediatric Cancer Research Foundation
DOE BER FWP 100651
NIH R01 HD084422

NEW FUNDING:
A Synchrotron Radiation Structural Biology Resource
Status of Support: Active
Project Number: 5P30-GM133894-04
Name of PD/PI: Hodgson, Keith O.
Source of Support: National Institutes of Health
Project/Proposal Start and End Date: 06/2020 – 02/2025
Total Award Amount (including Indirect Costs):
Effort Person Months: 0.60 calendar months (5%)

Fluorescent Lifetime Imaging Microscopy of Mitochondria-Rich Extracellular Vesicles for Direct Augmentation of Myocardial Bioenergetics
Project Number: N/A
Name of PD/PI: Wakatsuki, Soichi
Source of Support: (Internal Award) Stanford Beckman Center
Project/Proposal Start and End Date: 01/2021 – 11/2023
Total Award Amount (including Indirect Costs):
Effort Person Months: 0.60 calendar months (5%)

Development of multi-color wide-field high speed 3D fluorescence lifetime imaging for investigations of metabolic pathways in plants and microorganisms
Project Number: 100721
Name of PD/PI: Wakatsuki, Soichi
Source of Support: Department of Energy, BER
Project/Proposal Start and End Date: 09/2021 – 08/2024
Total Award Amount (including Indirect Costs):
Effort Person Months: 0.60 calendar months (5%)

Optical and X-ray multimodal-hybrid microscope systems for live imaging of plant stress responses and microbial interactions
Project Number: 100878
Name of PD/PI: Wakatsuki, Soichi
Source of Support: Department of Energy, BER
Project/Proposal Start and End Date: 09/2022 – 08/2025
Total Award Amount (including Indirect Costs):
Effort Person Months: 1.20 calendar months (10%)

SSRL BER Structural Biology Support
Project Number: DE-AC02-76SF00515
Name of PD/PI: Hodgson, Keith O.

Source of Support: Department of Energy, BER
Project/Proposal Start and End Date: 10/2022 – 09/2023
Total Award Amount (including Indirect Costs):
Effort Person Months: 2.04 calendar months (17%)

SSRL Operations and Research BES
Project Number: DE-AC02-76SF00515
Name of PD/PI: McIntyre, Paul
Source of Support: Department of Energy, BES
Project/Proposal Start and End Date: 10/2022 – 09/2023
Total Award Amount (including Indirect Costs):
Effort Person Months: 2.40 calendar months (20%)

Development of a quantum-optimal bioimaging system for plant-microbiome interactions
Project Number: 100882
Name of PD/PI: Kasevich, Mark; Wakatsuki, Soichi
Source of Support: Department of Energy, BER
Project/Proposal Start and End Date: 09/2022 – 08/2025
Total Award Amount (including Indirect Costs):
Effort Person Months: 0.60 calendar months (5%)

John Day

Nothing to Report

What other organizations were involved as partners?

No other organizations.

SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS

Nothing to report.

QUAD CHARTS

Nothing to report.

APPENDICES

Appendix S1

LITERATURE CITED:

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Appendix S2

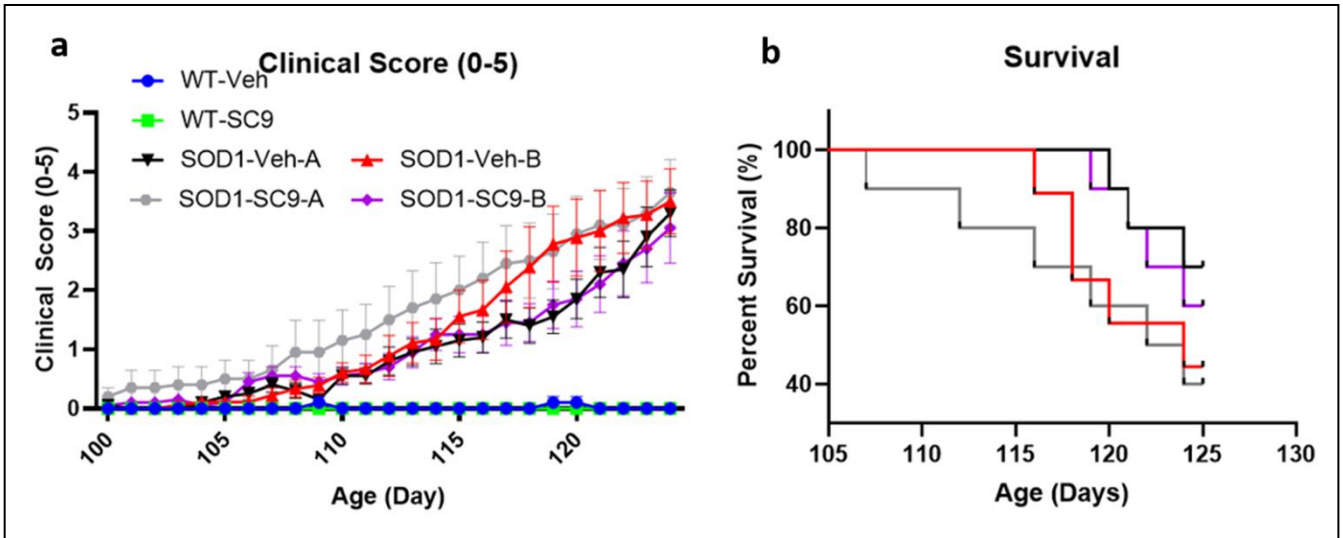
Characterization of SC1, SC3 and SC9.

4,4'-(((((4-methyl-1,3-phenylene)bis(azanediyl))bis(carbonyl))bis(azanediyl))bis(propane-3,1-diyl))bis(morpholin-4-ium) (SC1). ^{1}H NMR (600 MHz, dmso) δ 8.31 (s, 1H), 7.68 (d, *J* = 2.3 Hz, 1H), 7.49 (s, 1H), 7.09 (dd, *J* = 8.2, 2.2 Hz, 1H), 6.90 (d, *J* = 8.3 Hz, 1H), 6.49 (t, *J* = 5.7, 5.7 Hz, 1H), 5.99 (t, *J* = 5.8, 5.8 Hz, 1H), 3.55 (t, *J* = 4.7, 4.7 Hz, 8H), 3.11 – 3.04 (m, 4H), 2.35 – 2.30 (m, 6H), 2.30 – 2.24 (m, 6H), 2.06 (s, 3H), 1.59 – 1.52 (m, 4H). ^{13}C NMR (151 MHz, dmso) δ 155.79, 155.70, 139.07, 138.63, 130.32, 119.73, 112.07, 110.72, 66.65, 56.33, 56.31, 53.83, 37.86, 37.81, 27.14, 17.65. HRMS (m/z): calc. for C23 H38 N6 O4 (M+) 462.2955, obs. 462.2951.

(4-amino-6-((4-chlorophenyl)amino)-1,3,5-triazin-2-yl)methyl 4-oxo-4-(3-phenyl-4,5-dihydro-1H-pyrazol-1-yl)butanoate (SC3). ^{1}H NMR (600 MHz, dmso) δ 9.66 (s, 1H), 7.78 (d, *J* = 8.8 Hz, 2H), 7.75 – 7.70 (m, 2H), 7.46 – 7.42 (m, 3H), 7.29 (d, *J* = 8.9 Hz, 2H), 7.26 – 6.96 (m, 2H), 4.82 (s, 2H), 3.89 (t, *J* = 10.1, 10.1 Hz, 2H), 3.26 (t, *J* = 10.1, 10.1 Hz, 2H), 2.98 (t, *J* = 6.9, 6.9 Hz, 2H), 2.73 (t, *J* = 6.9, 6.9 Hz, 2H). ^{13}C NMR (151 MHz, dmso) δ 173.03, 172.67, 169.21, 166.99, 164.41, 156.84, 139.22, 131.73, 130.66, 129.19, 128.65, 126.96, 126.14, 121.73, 65.10, 44.49, 31.80, 28.80. HRMS (m/z): calc. for C23 H22 Cl N7 O3 (M+) 479.1473, obs. 479.1464.

N-(6-amino-1-benzyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)-2-((5,7-dimethyl-[1,2,4]triazolo[1,5-a]pyrimidin-2-yl)thio)-N-methylacetamide (SC9). ^{1}H NMR (500 MHz, dmso) δ 10.97 (s, 1H), 7.33 (td, *J* = 8.0, 7.8, 1.8 Hz, 2H), 7.24 – 7.18 (m, 3H), 7.16 (d, *J* = 7.8 Hz, 2H), 7.09 (s, 1H), 5.15 – 5.02 (m, 2H), 4.12 – 4.00 (m, 2H), 2.90 (s, 3H), 2.62 (s, 3H), 2.54 (s, 3H). ^{13}C NMR (151 MHz, dmso) δ 170.04, 165.89, 164.57, 159.86, 155.45, 153.39, 150.52, 146.68, 136.61, 128.98, 127.47, 126.33, 110.79, 94.37, 44.82, 35.37, 35.02, 24.85, 16.88. HRMS (m/z): calc. for C21H22N8O3S (M+) 466.1536, obs. 466.1528.

Appendix S3



a, Clinical scores and survival (**b**) of WT and SOD1 G93A mice with and without SC9 treatment in duplicates (A and B).