Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** Drexel University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010-12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Anne Martella

4. **Grant Contact Person's Telephone Number:** (215) 895-6471

5. **Grant SAP Number:** 4100050893

6. **Project Number and Title of Research Project**: 7 - *Novel Oligomers Targeting AMPA Receptors as Therapeutics for ALS*

7. **Start and End Date of Research Project:** 1/1/2010 - 6/30/2011

8. **Name of Principal Investigator for the Research Project:** Gordon Lutz, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 153,500

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Lutz, Gordon	PI	5%	$9,833.75
Tallent, Melanie	Co-PI	2.5%	$2,644
Tang, Wei	Research Technician	100%	$31,638.79

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Heiman-Patterson	Co-PI	10%

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
NONE		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No___**X**_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____**X**_____ No_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
Oligonucleotides that Modulate AMPA Receptor Alternative Splicing as Drug Candidates for ALS	■NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	Dec 2010	$458,274	$458,274
Preclinical testing of the splice modulating oligonucleotide LSP-GR1	■NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)	April 2013	$1,500,000	$1,500,000

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____**X**_____ No_____

If yes, please describe your plans:

LifeSplice Pharma, LLC., was founded as a Drexel 'spinout' by the PI (Lutz) and Co-PI (Tallent), on the CURE grant (the subject of this report). They are no longer at Drexel University College of Medicine (DUCOM), and are full-time CEO and CSO respectively, of LifeSplice. The research from the CURE grant played a role in the success of LifeSplice securing the 2 SBIR grants listed above.

The future plan is to submit a follow-on Phase 2B SBIR grant in early 2015, to complete the GLP ADMET-CMC studies of their lead drug for ALS in non-human primates.

12. Future of Research Project. What are the future plans for this research project?

The future plan is for LifeSplice Pharma to submit an IND for their lead drug to treat ALS and to conduct clinical trials for this drug in ALS patients.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____ No____**X**_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				
Female				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				
Unknown				
Total				

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				
Other				
Unknown				
Total				

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____**X**_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes_____ No____**X**_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No___**X**____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes___**X**____ No_____

If yes, please describe commercial development activities that resulted from the research project:

The lead drug candidate at LifeSplice Pharma for treating ALS (LSP-GR1) is patented and under development as late stage pre-clinical research, prior to commercialization.

16(C) Did the research lead to new involvement with the community?

Yes_____ No___**X**____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Amyotrophic lateral sclerosis (ALS) is characterized by the progressive and selective loss of motor neurons (MNs) in the motor cortex, brainstem, and spinal cord, resulting in patient death typically 3-5 years after diagnosis. Glutamate-mediated excitotoxicity in the CNS has been firmly implicated as a major contributor to the manifestation of both sporadic and familial ALS. Excitotoxicity is the result of excessive Ca2+ influx into MNs, coupled with the diminished capacity of MNs to handle Ca2+ overload, which appears to result in excessive Ca2+ entering mitochondria and the release of harmful molecules from mitochondria, which appear to damage glutamate transporters on adjacent astrocytes. This sets up a disastrous feedback cycle in which synaptic glutamate levels continually rise, further increasing Ca2+ influx and exacerbating mitochondrial Ca2+ overload, ultimately leading to MN cell death. Therefore, drugs which effectively lower Ca2+ influx in the MNs during excitatory activity offer great promise as therapeutics for ALS. In this project we evaluated the efficacy of our lead SMO after continuous ICV delivery to reduce GluR3-flip isoform expression in relatively short-term 3 wk trials in ALS mice; focusing on the dose-response of GluR3-flip knockdown throughout the CNS. We then conducted longer-term ICV delivery trials of the efficacy of our lead SMO in ALS mice; focusing on delaying motor deficits and increasing lifespan.

The proposed experiments were all empirical in nature and were product-driven with the express intent to provide 'proof of principle' that our novel drug candidates are efficacious in improving motor function and lifespan in an animal model of ALS. There are no drugs in clinical usage that specifically decrease AMPA channel GluR-flip subunit expression or increase desensitization kinetics. The SMOs in this project may ultimately be used clinically as neuroprotective agents for treating ALS, by reducing Ca^{2+} influx in MNs and breaking the feedback cycle involved in glutamate-mediated excitotoxicity.

METHODS

Animals

Two strains of adult ALS mice were used. For the continuous ICV delivery experiments we used congenic G93A SOD1 ALS mice backcrossed to a C57Bl/J6 background strain for over 20 generations (B6.Cg-Tg(SOD1*G93A)1Gur/J; Jackson Labs stock 004435). These ALS/B6 mice carry 25 copies of human mutant (G93A) SOD1, which is the genetic basis for their MN

degenerative phenotype. ALS mice of this strain, referred to simply as SOD1/B6 ALS mice, were derived from in-house breedings between males hemizygous for the human mutant G93A SOD1 transgene and wild-type C57Bl6 females. Genotype was established post-weaning using a standard Real-time PCR protocol to detect the mutant SOD1 gene. For the bolus lumbar spinal infusion studies we switched to the G93A SOD1 ALS mice bred on a mixed SJL/B6 background (Jax; B6SJL-Tg(SOD1*G93A)1Gur/J). These ALS mice typically survive to about 128.9 (+/-9.1 d). ALS mice of this strain, referred to simply as ALS/B6-SJL mice, were purchased in sets of littermates from Jackson Labs at about 5-6 weeks of age.

ALS mouse experimental groups

Continuous ICV delivery using Alzet cannulae/minipumps. SOD1 ALS-B6 littermates were randomly assigned into two groups: (i) GR1 SMO, and (ii) saline.

Bolus spinal injections. SOD1 ALS-B6/SJL littermates were randomly assigned into two groups: (i) GR1 SMO, and (ii) saline.

GR1: This SMO targeted the 3' splice site and adjacent exonic splice enhancer motifs of the GluA1-flip pre-mRNA where its sequence is divergent from all other GluA isoforms.

Alzet cannulae/osmotic pump continuous ICV delivery of SMOs

Mice were implanted with a cannula in the lateral ventricle connected to an Alzet minipump for continuous delivery of SMOs to the CSF. Mice were anaesthetized with isoflurane and a cannula (Alzet, Brain Infusion Kit 3) was inserted stereotaxically into the right lateral ventricle with the cannula tubing connected to a sub-cu implanted Alzet minipump (1004), pre-loaded with SMO or saline. The entire system was internalized beneath the skin. Prior to implant, pumps were filled with SMO or saline and equilibrated in sterile saline at 37oC for 24 h. The Alzet pump delivered 2.4+ 0.2 μL per day with enough volume (100 μL) for at least 28 d of continuous infusion. In the ALS mouse trials, the first pump and cannulae were implanted at 62 d of age. The pump was replaced after 4 weeks (day 90) and 8 weeks (108) with a rapid minor surgery where the expiring pump was simply replaced with a new pump that was reconnected to the original cannula tubing. Then after an additional 4 wks (day 146), another minor surgery was performed where the pump was removed, and the cannulae tubing was cutoff close to the cannulae insertion point, and the cannulae port was sealed shut. Incisions were closed and mice quickly recovered after removal from anesthetic.

Bolus lumbar spinal infusions of SMOs

Mice were anaesthetized with isoflurane and a small 1 cm incision was made to expose the musculature above the L5-L6 region of the spinal cord. A 32 gauge needle mounted on a 25 μL Hamilton syringe was inserted slowly at a 45° angle into the L5-L6 gap until the needle tip punctured the dura (detected by a tail flick reflex) and then the angle was lowered and the needle was advanced another ~2-3 mm into the spinal column. A 6 μL solution of SMO or saline was delivered into the CSF of the spinal column slowly over a 3 min period and then the needle was held in place for 30 sec prior to removal. The small incision was closed with a single stitch and mice recovered to full ambulation within 5 min.

RESULTS

Efficacy of GR1 SMO throughout the CNS of normal adult mice after continuous ICV delivery

Because SMOs do not cross the blood brain barrier, they must be delivered directly to the CSF. Implantation of cannulae in the spinal cords of mice for continuous long-term delivery of drugs is technically difficult and damaging to the spinal cord. Thus, we elected to begin our studies of SMO efficacy using continuous ICV delivery. Using a stereotaxic system, normal C57Bl6 adult (90 d old) mice were implanted with cannulae in their right lateral ventricle, which was connected through tubing to a sub-cu embedded osmotic minipump (see Methods), which was pre-loaded with either the GR1 SMO or saline. The dose-response profile of GR1 after 4 weeks of continuous ICV delivery system was determined by measuring GluA1-flip and GluA1-flop transcript levels throughout the CNS. Dosing rates of GR1 from 1-20 µg per day for 4 weeks all produced significant decreases in GluA1-flip in all regions of brain and spinal cord compared to saline-treated controls (Fig. 1a). Given that our ICV dosing in ALS mice will be continuous for 12 weeks, we used these 4 week dosing data in normal mice to select 10 ug/d for the our efficacy studies of GR1 in ALS mice.

Influence of continuous ICV delivery of GR1 SMO on motor function and lifespan of ALS mice
The B6 congenic ALS mice were dosed with the GR1 SMO (10 µg per day) for 12 weeks, beginning at 62 days of age. Although the number of mice is clearly far too low for definitive conclusions (N = 4 mice per group), the GR1 SMO-treated mice still showed a significant increase in lifespan compared to saline-treated controls (Fig. 1b). The GR1 SMO-treated mice also showed a delay in disease onset, assessed by a delay in abnormal splay response (Fig. 1c; higher splay score indicates hindlimb motor weakening), and improved motor function for other tests including beam walk and ladder climbing ability (not shown).

Influence of bolus spinal delivery of GR1 SMO on motor function and lifespan of ALS mice
The fact that ALS pathology, including loss of MNs, progresses in a dorsal to proximal direction in MNs of ALS mice, provided strong validation that periodic bolus spinal injections of GR1 may produce a more potent reduction in ALS disease progression than continuous ICV delivery, and has many potential advantages for clinical application.

ALS-B6/SJL mice were given a spinal lumbar bolus injection of 50 µg of GR1 at age 60 (pre-symptomatic) and a follow up injection of 25 µg of GR1 at age 90 (post symptomatic). Littermate control mice were given saline injections at the same time point. A full battery of phenotypic and motor tests was performed 2 times per week on all mice from age 50 until endstage. ALS-B6/SJL mice that received spinal bolus injections of GR1 lived to 129.3 ± 2.6 d, which was 7.6 days longer than their saline-treated littermates (Fig. 2).
Bolus spinal injections of GR1 also significantly delayed the onset of both forelimb and hindlimb tremor in ALS mice (Fig. 3). Spinal bolus injections of GR1 also significantly delayed the progression of deficits in the splay reflex response when analyzed over the full time course from 50 days of age to endstage compared to their saline-injected littermates (Fig. 4a). In addition, analysis at specific stages showed that GR1 significantly delayed progression to a mean splay score of 1 as well a splay score of 2 (Fig. 4b).

Spinal administration of GR1 significantly delayed deficits in beam walk performance when analyzed over the full time course from 50 days of age to endstage, compared to their saline-injected littermates (Fig. 5a). In addition, GR1 significantly delayed progression to a mean beam scores of 1 and 2 (Fig. 5b). Similar to its effect on both splay and beam indicators of disease

progression, administration of GR1 also significantly delayed deficits in upward and downward ladder climbing ability (Fig. 6).

CONCLUSIONS

It has long been known that ionotropic AMPA GluA subunits are alternatively spliced at their flip/flop exon, and that AMPA channels comprised of different GluA flip and flop splice variants have substantially different kinetics. Given that AMPA channels clearly play a pivotal role in the process of excitotoxicity, which has been implicated in a wide range of neurological diseases, it is remarkable that there are no drugs in clinical usage that regulate GluA alternative splicing. Here we showed that a novel SMO, GR1, which potently and specifically reduced the expression of GluA1-flip, significantly improved motor function and increased the lifespan of ALS mice after just two bolus injections into the lumbar spinal cord. These data confirm previous work implicating AMPA-Rs as having a pivotal role in producing excessive Ca2+ influx in MNs, and triggering excitotoxicity. Our innovative approach to AMPA channel modulation provided the first opportunity to evaluate the impact of direct manipulation of GluA flip/flop alternative splicing on ALS disease progression. Our data, showing significant efficacy of SMOs after periodic intrathecal bolus injections, are the first of their kind in rodents, and pave the way toward clinical usage of SMOs for treating both familial (FALS) and sporadic (SALS) patients.

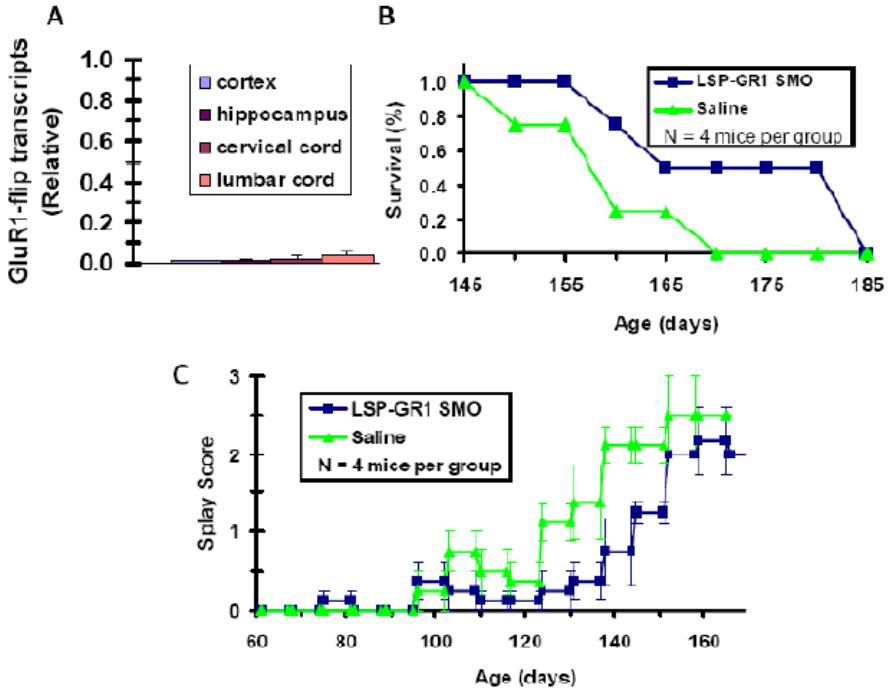

Figure 1. (A) GluR1-flip transcripts are nearly completely depleted in the CNS after 4 wk continuous ICV infusion of GR1 in normal mice (10 µg/d; N=3 mice). **(B-C)** Continuous 12 wk ICV infusion of GR1 beginning at 62 days of age in ALS mice significantly increased lifespan **(B)**, and delayed disease onset, based on the delay in abnormal splay response **(C)**.

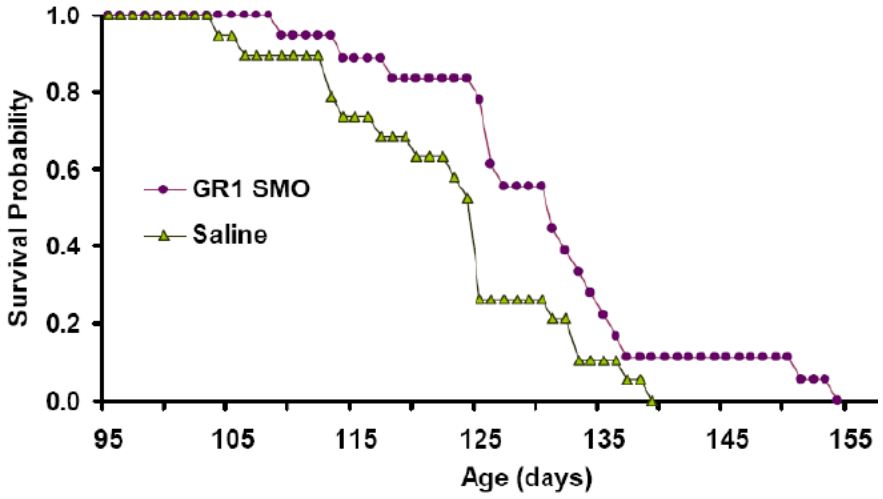

Figure 2. Kaplan-Meier survival plot of ALS mice treated with spinal bolus injections of GR1 increased longevity compared to their saline-injected littermates. At 60 days of age, mice were injected with 50 µg of SMO, followed at 90 days with a 25 µg injection. GR1 delayed death in ALS mice ($p < 0.05$, n = 19 for saline and n = 18 for GR1-treated). Same cohort of mice are shown for all of the following figures.

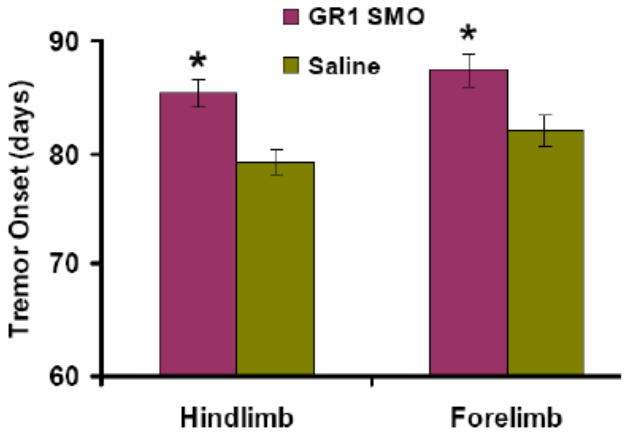

Figure 3. Both hindlimb and forelimb tremor onset was delayed after bolus spinal injections of GR1 in ALS mice compared to their saline-treated littermates. In GR1-treated ALS mice, mean age at which a tremor was first observed was significantly delayed in both hindlimb ($p < 0.001$) and forelimb. ($p < 0.01$). GR1-treated mice ($n = 18$) and saline-injected littermates ($n = 19$).

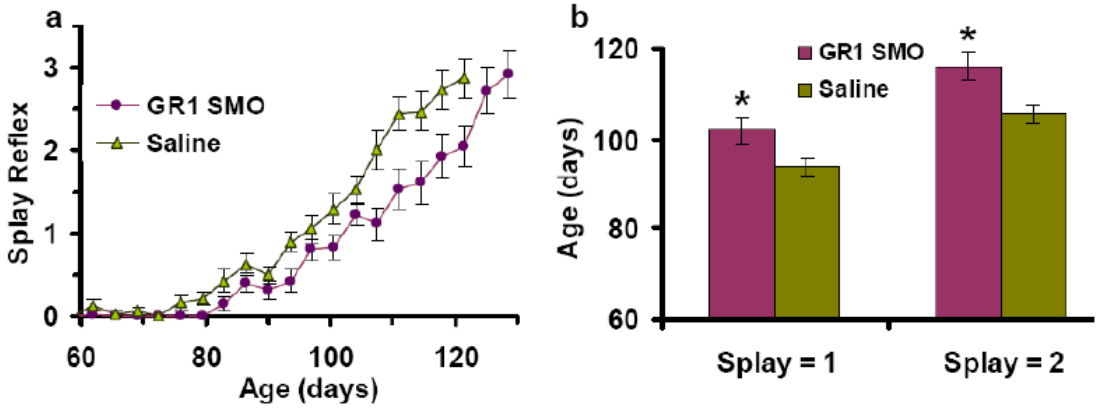

Figure 4. Deficits in splay reflex were delayed after bolus spinal injections of GR1 in ALS mice compared to their saline-treated littermates. **a.** Splay reflex was assessed across the lifespan of ALS mice from P60 to the time-point when 50% of each group had died. GR1-treated mice showed a significant delay in onset of splay deficit ($p < 0.005$). **b.** A significant delay in onset of splay scores of 1 ($p < 0.05$) and 2 ($p < 0.01$) were observed in GR1-treated mice. GR1-treated mice ($n = 18$) and saline-injected littermates ($n = 19$).

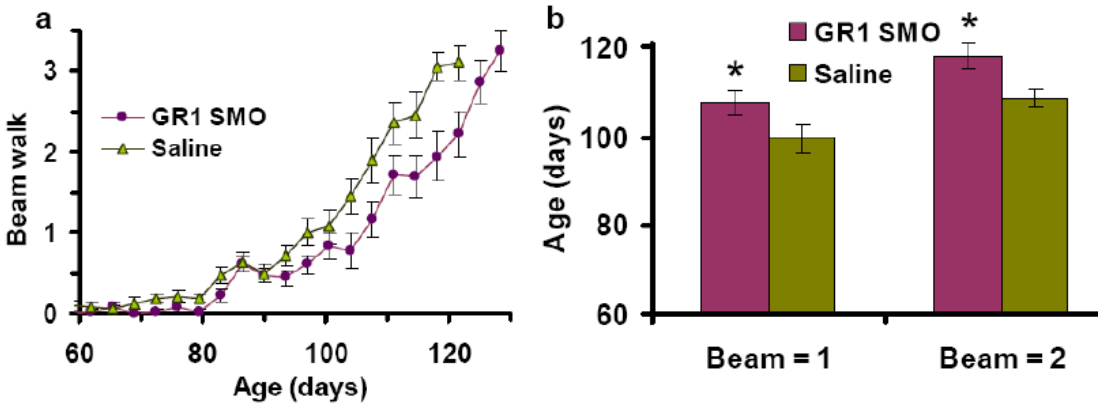

Figure 5. Deficits in beam walk performance were delayed after bolus spinal injections of GR1 ALS mice compared to their saline-treated littermates. **a.** Deficits in beam walking from P60 to time of death of 50% of each cohort showed a delay in progression in GR1-treated mice ($p < 0.01$). **b**. Mean time of onset of a beam score of 1 ($p = 0.049$) and 2 ($p = 0.009$) were significantly delayed in ALS mice treated with GR1 versus their saline-treated littermates. GR1-treated mice (n = 18) and saline-injected littermates (n = 19).

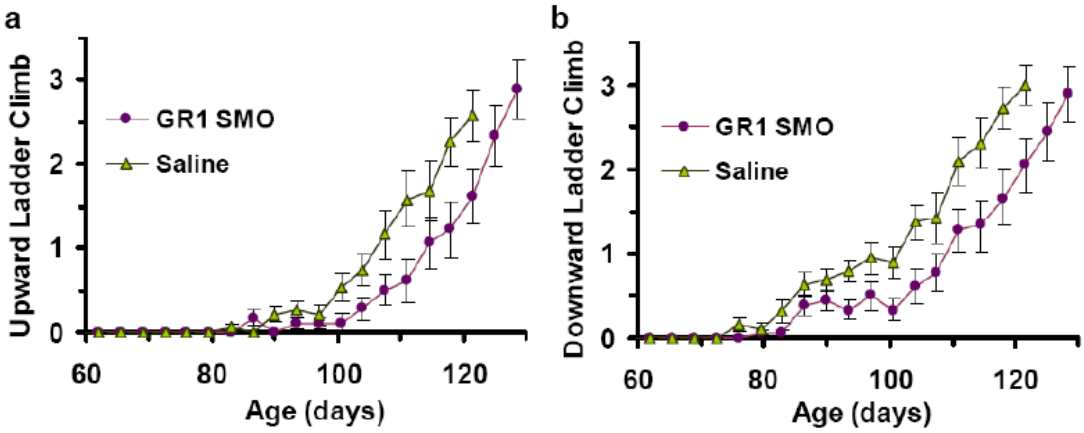

Figure 6. Deficits in both upward and downward ladder climbing capacity were delayed after bolus spinal injections of GR1 in ALS mice compared to their saline-treated littermates. **a.** Progression of deficits in upward ladder climbing was significantly delayed in mice administered GR1 ($p < 0.05$). **b.** ALS mice treated with GR1 also show improvement in downward ladder climbing compared to saline-treated mice ($p < 0.01$). GR1-treated mice (n = 18) and saline-injected littermates (n = 19).

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__**X**__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__**X**__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
__**X**__No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one

publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____**X**_____ No_____

If yes, please describe your plans:

We plan to submit a full-length article describing the effect of the oligonucleotide drug candidate developed in part by the CURE funds in improving motor function and lifespan of ALS mice.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention,

diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

The research project resulted in 'proof of concept' data that showed that the chemically-modified oligonucleotide we had previously developed was effective in improving the motor function and lifespan in a murine model of ALS.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes____x_____ No_____

If yes, please describe your plans:

The future plan is for LifeSplice Pharma to submit an IND for their lead drug to treat ALS and to conduct clinical trials for this drug in ALS patients.

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

NAME **Gordon J. Lutz**	POSITION TITLE CEO; LifeSplice Pharma, LLC
eRA COMMONS USER NAME **glutz2**	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
State University of New York, Plattsburgh	BS	1986	Biology
University of Pennsylvania, Philadelphia	Ph.D.	1994	Biology
University of Pennsylvania, Philadelphia	Postdoc	1994-1995	Muscle Physiology
University of California San Diego	Postdoc	1995-1998	Muscle Physiology

A. Personal Statement

My research is focused on the development of splice modulating oligomers (SMOs) as potential therapeutics for serious neurological disorders and non-CNS diseases. I first began working with SMOs in 2004 as therapeutics for Duchene Muscular Dystrophy. We developed nanopolymer and peptide carrier systems for improved delivery of SMOs to body musculature, and published several manuscripts in this area. More recently, in 2006 I led a study to use SMOs as therapeutics for treating Spinal Muscular Atrophy (SMA). Our group, in collaboration with Dr. Melanie Tallent, recently published the first paper showing that 'naked' SMOs (without a carrier) when delivered to the CSF of SMA mice, produced extraordinarily high levels of SMN expression and provided phenotypic improvement, including partial correction of motor deficits. This work was published as a J. Neuroscience article (highlighted paper of the week) and resulted in a featured article in SciBx, a Nature publication on seminal translational research papers. Further, this 2009 publication was mentioned in a perspective of the most promising translational findings in neurodegenerative diseases over the past four decades (J. Neurosci., 2009, 29:12722–12728), and has already been cited in 9 additional reviews.

Based on our findings that SMOs provided modulation of target genes throughout the CNS, I have focused my research efforts on utilizing advanced molecular engineering tools to optimize the specificity and potency of novel SMOs to modulate alternative and aberrant pre-mRNA splicing of target genes. Again working with Dr. Tallent, we successfully developed a panel of novel SMOs with extraordinary specificity and potency in modulating the alternative splicing pattern of the GluR subunits of AMPA receptors, to reduce the expression of high gain APMA receptors, therefore reducing neuronal network hyperexcitability and excitotoxicity, processes known to be involved in the etiology of epilepsy and ALS, respectively. We now have compelling evidence demonstrating the efficacy of these GluR SMOs in modulating splicing of their target genes throughout the CNS, and ameliorating pathology in mouse models of epilepsy and ALS (the subject of the present grant). To advance these promising drug candidates toward clinical usage for treating ALS, I have formed a very close collaboration with Terry-Heiman Patterson, Director of the MDA ALS Center of Hope at DrexelMed, and a long-standing leader in basic and clinical ALS research.

Very recently, Dr. Tallent and I co-founded **LifeSplice Pharma LLC**, a biotech company with a mission to develop novel, patent-protected, SMOs to treat serious neurological diseases.

B. Positions and Honors.

Positions and Employment

Teaching and Research Fellow, University of Pennsylvania 1989-1994

Postdoctoral Research Associate, University of Pennsylvania 1994-1995

Postdoctoral Research Associate, University of California San Diego 1995-1998

Assistant Research Scientist, University of California San Diego	1998-2000
Research Scientist, Veterans Medical Research Foundation	1998-2002
Assistant Adjunct Professor, University of California San Diego	2000-2002
Associate Professor, Drexel University College of Medicine	2002-06/30/2011
President and CEO, LifeSplice Phama, LLC	08/01/2010-present

Other Experience and Professional Memberships

Grant Reviews

NIH Challenge Grants 2009
Muscular Dystrophy Association, Reviewer 2005-2008; Standing Member 2009-present
Muscular Dystrophy Association, Member Steering Committee Pre-IND Application 2007-2009
MRC Development Pathway Funding Grants (2009)
Muscular Dystrophy Campaign, Ad Hoc. 2006-2009
The Association Française contre les Myopathies (AFM), 2006
NIH, NIA, Reviewer PPG Reverse Site Visit, October 2005

C. Selected peer-reviewed publications (limit 15; in chronological order)

Most relevant to current application (listed chronologically)

1. Sirsi, S. R., Williams J. H., and **G. J. Lutz**. (2005) Poly(ethylene imine)-Polyethylene Glycol Copolymers Facilitate Efficient Delivery of Antisense Oligonucleotides to Nuclei of Mature Muscle Cells of mdx Mice. *Hum. Gene Therapy*. 16:1307-1317

2. Glodde, M., Sirsi S.R., and **G. J. Lutz**. (2006) Physiochemical Properties of Low and High Molecular Weight PEG-Grafted Poly(ethylene imine) Copolymers and their Complexes with Oligonucleotides. *Biomacromolecules* 7(1):347-356.

3. Williams J. H., Sirsi, S. R., and **G. J. Lutz**. (2006) Induction of dystrophin expression by exon skipping in mdx mice following intramuscular injection of antisense oligonucleotides complexed with PEG-PEI copolymers. *Molecular Therapy* 14(1):88-96.

4. Sirsi, S. R., Schray, R. C., Williams J. H., Agisim M. E., and **G. J. Lutz.** (2008). Functionalized PEG-PEI copolymers complexed to exon skipping oligonucleotides improve dystrophin expression in mdx mice. *Human Gene Therapy*; 19(8):795-806.

5. Williams J. H., Sirsi, S. R., and **G. J. Lutz**. (2008). Nanopolymers improve delivery of exon skipping oligonucleotides and concomitant dystrophin expression in skeletal muscle of mdx mice. *BMC Biotechnology*; 2;8:35.

6. Kim Y, Tewari M, Pajerowski JD, Cai S, Sen S, Williams J, Sirsi S, **Lutz GJ**, Discher DE. (2009). Polymersome delivery of siRNA and antisense oligonucleotides. *J. Controlled Release*; 134(2):132-40.

7. Sirsi, S. R., Schray, R. C., Guan, X., Wheatley M. A., and **G. J. Lutz**. (2009) Formulation of polylactide-co-glycolic acid nanospheres for encapsulation and sustained release of poly(ethylene imine)-poly(ethylene glycol) copolymers complexed to oligonucleotides. *J Nanobiotechnology*; 7:1.

8. Williams, J. H., Schray, R. C., Patterson, C. A., Ayitey, S. O., Tallent, M. K., and **G. J. Lutz**. (2009) Oligonucleotide-Mediated SMN Expression in CNS Improves Phenotype in a Mouse Model of Spinal Muscular Atrophy. *J. Neuroscience*, 29(24):7633-8.

Additional publications (listed chronologically)

10. Rome, L.C., R.P. Funke, R.M. Alexander, **G.J. Lutz**, H. Aldridge, F. Scott, and M. Friedman. (1988). Why animals have different muscle fiber types. *Nature*. 335: 824-827.

BIOGRAPHICAL SKETCH

NAME Melanie K. Tallent, Ph.D.	POSITION TITLE Chief Scientific Officer LifeSplice Pharma LLC
eRA COMMONS USER NAME Tallent1	

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Tennessee Tech. University, Cookeville, TN	BS	1982-86	Chemistry
University of Tennessee, Knoxville, TN		1987-1989	Physiology
University of Pennsylvania, Philadelphia, PA	Ph.D.	1989-1995	Neuroscience
The Scripps Research Institute, La Jolla, CA		1995-1999	Neuropharm

A. Personal Statement

My background is in neuroscience, with the majority of my training in electrophysiology and behavior. At LifeSplice, I am Chief Scientific Officer, thus my main responsibilities are directing and managing the on-site research. Since LifeSplice is a small company, I have many other responsibilities as well, and as part of the board of managers am involved in all executive-level decisions.

Dr. Lutz and I have in a short time established a successful collaboration in terms of acquiring funding and completing collaborative projects. We published a well-received J. Neuroscience article using an SMO to ameliorate disease symptomology in a mouse model of SMA. This was a highlighted paper of the week and resulted in a featured article in *SciBx*, a *Nature* publication that evaluates the most important translation research papers. Further, this 2009 publication was mentioned in a perspective discussing the most promising translational findings in neurodegenerative diseases in the past 4 decades (J. Neurosci., 2009, 29:12722–12728). A manuscript describing the use of LSP-GR1 in neonatal epilepsy is in revision for Nature Neuroscience.

In 2010 Dr. Lutz and I, along with Mike Christini, co-founded LifeSplice Pharma, a biotech company focused on developing splice modulating oligonucleotides to treat neurological and other serious diseases. We obtained two Phase 1 SBIRs from NINDs to do further preclinical testing of LSP-GR1 in epilepsy and ALS mouse models. LifeSplice's Scientific Advisory board included Dr. Gregory Holmes, a renowned pediatric neurologist who is chair of the Department of Neurological Sciences at University of Vermont. Dr. Holmes has extensive experience in clinical trials for childhood epilepsies, and will be a valuable resource in clinical development of the SMOs designed in this grant proposal.

B. Positions and Honors

Positions:

1/90-5/95: Graduate Research Fellow, Institute of Neurological Sciences, Univ. of Pennsylvania.

6/95-11/95: Postdoctoral Fellow, Dept. of Pharmacology, Univ. of Pennsylvania.

11/95-4/98: Research Associate, Dept. of Neuropharmacology, The Scripps Research Institute.

5/98-9/99: Senior Research Associate, Dept. of Neuropharmacology, The Scripps Research Institute.

9/99-7/03: Assistant Professor, Dept. of Neuropharmacology, The Scripps Research Institute.

8/01/02-6/30/10: Assistant Professor, Dept. of Pharmacology and Physiology, Drexel University College of Medicine

7/1/10-present: Chief Scientific Officer, LifeSplice Pharma LLC, Malvern, PA

Honors:

1989: Science Alliance Award, Univ. of Tennessee and Oak Ridge National Laboratories, Graduate Group in Physiology.

3/00: NINDS Travel Award for "Curing Epilepsy: Focus on the Future", a White House-initiated Conference, Washington, D.C.

NIH study section member

1999—Brain Disorders and Clinical Neurosciences, ad hoc

2002—2006 Special Emphasis Panel F03B, Molecular, Cellular, and Developmental Neurosciences, NRSA review panel, ad hoc (no permanent members because it is not a standing committee).

2004, 2006, 2009— Neurotransporters, receptors, channels and calcium signaling (NTRC), ad hoc.

2005-2006—Brain Disorders and Clinical Neuroscience (BDCN) 11: Pharmacology and Diagnostics for Neuropsychiatric Disorders Small Business, ad hoc.

2007. NCCR COBRE Special Emphasis Panel, ad hoc.

Foundation Grant Reviews

2007—Citizens United for Research in Epilepsy, research grant review, ad hoc.

2008—Wellcome Trust, United Kingdom, Project grants, ad hoc.

2009-2010—Alzheimer's Association, research grants, ad hoc.

C. Selected Peer-reviewed Publications (out of 28)

Most relevant to current application (listed chronologically)

1. **Tallent, M. K.**, Fabre, V., Qiu, C., Baratta, M. V., Lamp, T., Sánchez-Alavez, M., Suzuki, C., Calbet, M., Criado, J. R., Siggins, G. R., Henriksen, S. J., Roberts, A., and de Lecea, L. (2005) Cortistatin overexpression in transgenic mice produces deficits in synaptic plasticity and learning, Mol. Cell. Neurosci 30:465-475. PMID: 16182561, PMCID Journal-in process.
2. Qiu, C., Johnson, B. N., and **Tallent, M. K.** (2007) K+ M-current regulates the transition to seizures in immature and adult hippocampus. Epilepsia. 48 (11): 2047-2058.
3. **Tallent, M. K.** (2008) Presynaptic inhibition of glutamate release by neuropeptides: use-dependent synaptic modification. *Results Probl Cell Differ*. 44:177-200. PMID: 17554500, PMCID Journal-in process.
4. Qiu, C., Zeyda, T., Johnson, B. N., Hochgenshwender, U., de Lecea, L., and **Tallent, M. K.** (2008) Somatostatin receptor subtype 4 couples to the K+ M-current to regulate seizures and hippocampal excitability. *J. Neurosci*. 28 (14): 3567-3576 (*highlighted paper of the week*). PMID: 18385315, PMCID Journal-in process.
5. Williams, J. H., Schray, R. C., Patterson, C. A., Ayitey, S., **Tallent**, **M. K.** and Lutz, G. J. Oligonucleotide-Mediated SMN Expression and Improved Phenotype in a Mouse Model of Spinal Muscular Atrophy (2009), *J. Neurosci*. 29(24):7633–7638 *(highlighted paper of the week)*. PMID: 19535574, PMCID Journal-in process.
6. **Tallent, M. K**., Varghis, N., Skorobogatko, Y., Hernandez-Cuebas , L., Whelan , K., Vocadlo, D. J., and Vosseller, K. (2009) O-GlcNAc is a novel synaptic signaling component of hippocampal neuronal plasticity, *J. Biol. Chem.* 284(1): 174-181. PMID: 19004831, PMCID Journal-in process.
7. Einstein, E. B., Patterson, C.A., Hon, B. J., Mateer, M. J., Johnson, B. N., and **Tallent, M. K.** Somatostatin signaling in neuronal cilia is critical for object recognition memory, (2010) *J. Neurosci.3*0 (12): 4306-4314. PMCID Journal-in process.

BIOGRAPHICAL SKETCH

NAME: Terry D. Heiman-Patterson **TITLE:** Professor of Neurology

EDUCATION/TRAINING:

1969-1975	Rensselaer Polytechnic Institute	B.S.	Troy, New York
1971-1975	Albany Medical College	M.D.	Albany, New York
1975-1978	Resident in Neurology	Albany Medical College	Albany, New York
1978-1979	MDA Clinical Postdoctoral Fellow	University of Pennsylvania	Philadelphia, Pa
1979-1982	MDA Research Postdoctoral Fellow	University of Pennsylvania	Philadelphia, Pa

A. Personal Statement

My research is focused on the clinical and basic aspects of motor neuron diseases and specifically Amyotrophic Lateral Sclerosis.

My basic research is directed at identifying genetic modifiers of disease in the G93A SOD1 Transgenic mouse model. To this end we have bred the transgene on different backgrounds (B6, SJL, and the standard B6SJL mixed background) and demonstrated differences in survival. We have used our congenic B6 and SJL lines to create an F2 that was used to link severity of disease to a QTL on Chromosome 17 of the mouse and are actively involved in identification of the responsible gene in collaboration with Dr. Greg Cox at Jackson Laboratories. In addition, we have active colonies of G93A SOD1 mice on the B6SJL and the B6 backgrounds that we have characterized clinically and pathologically. We have used these colonies to examine inflammatory markers (SPLA2) in collaboration with Dr. Timothy Cunningham and also to evaluate possible therapies (CHEC 9 an inhibitor of SPLA2 and more recently the splice modulating oligonucleotides directed at GluR subunits of the AMPA receptor with Dr. Gordon Lutz).

My clinical focus has included the conduct of multiple clinical trials in ALS including Phase 1, 2, and 3 along with examination of clinically relevant topics directed at improving care for people living with ALS in the present. Most recently we have completed the clinical trial of KNS-760704 , Lithium, Zenvia, and Arimoclomol and are presently enrolling for Dexpramiprexole, CK-2017357, and Ceftriaxone. We are actively involved in collecting tissues through the development of our own tissue bank and clinical database as well as contributing samples to the NIH funded Biomarkers study in collaboration with Mass General. In addition to these studies, I am interested in several clinical aspects of ALS including respiratory management, autonomic involvement with attention to gastrointestinal motility, and enhancement of communication and independence through the use of the Brain Computer Interface technology. This interest extends to the use of technology to improve diagnosis and care as well as quality of life for the disabled, especially people living with ALS. I am committed to collaboration and acceleration of research in the area of ALS. As an additional step towards this goal I am on the Executive Board of the Northeast ALS Consortium, work with the International Alliance of MND Associations, am an active member of ALS RG, and am President of the ALS Hope Foundation.

B. Positions and Honors

1997-Present	Professor and Vice Chairman, Department of Neurology Director: Division of Neuromuscular Disease Director MDA/ALS Center of Hope Drexel University (formerly MCP Hahnemann University)	Philadelphia, PA
1996-1997	Associate Professor of Neurology Director: Division of Neuromuscular Disease Director MCP-Hahnemann ALS Clinic Allegheny University of the Health Sciences	Philadelphia, PA
1988-1996	Associate Professor of Neurology Thomas Jefferson University Hospital	Philadelphia, PA
1987-1988	Associate Professor of Neurology	

	Director: Division of Neuromuscular Disease Hahnemann University	Philadelphia, PA
1982-1987	Assistant Professor of Neurology Director: Division of Neuromuscular Disease Co-Director ALS Clinic (1984-1988) Hahnemann University	Philadelphia, PA
1982-present	MDA Clinic Director Good Shepherd Rehabilitation Center	Allentown, PA

C. Selected peer-reviewed publications (Limit 15; in chronological order)

1. Alexander GM, Erwin K, Byers N, Deitch J, Blankenhorn E, and **Heiman-Patterson TD**: Effect of gene copy number on survival in the G93A Mouse Model of ALS. Brain Molecular Research 2004 Nov 4;130 (1-2):7-15.
2. Shefner JM, Cudkowicz ME, Schoenfeld D, Conrad T, Taft J, Chilton M, Urbinelli L, Zhang, H Pestronk A, Caress J, Sorenson E , Bradley W, Lomen-Hoerth C, Pioro E, Rezania K, Ross M, Pascuzzi R, **Heiman-Patterson T**, Tandan R, Mitsumoto H, Rothstein J, and the NEALS Consortium. A Clinical Trial of Creatine in Amyotrophic Lateral Sclerosis Neurology. 63(9):1656-61, 2004
3. H**eiman-Patterson TD**, Deitch JS, Blankenhorn EB, et al:Background and Gender Effects on Survival in the TgN(SOD1-G93A)1Gur mouse model of ALS J Neuro Sci 236:1-7; 2005
4. Cudkowicz ME, Shefner JM, Schoenfeld et al: Trial of Celecoxib in Amyotrophic Lateral Sclerosis. Annals of Neurology. 60(1):22-31, 2006
5. **Heiman-Patterson TD** and Miller RW: NIPPV: A Treatment for ALS whose time has come. Neurology 67(5):736-7, 2006
6. Lange DJ, Lechtzin N, Davey C, David WD, **Heiman-Patterson TD**, and the HFCWO Study Group: A Randomized Controlled Trial of High Frequency Chest Wall Oscillation in ALS. Neurology. 67(6):991-7, 2006
7. Amin MR. Harris D. Cassel SG. Grimes E. **Heiman-Patterson T.** Sensory testing in the assessment of laryngeal sensation in patients with amyotrophic lateral sclerosis. [Comparative Study. Journal Article] Annals of Otology, Rhinology & Laryngology. 115(7):528-34, 2006 Jul.
8. Cudkowicz, ME, Shefner, JM, Simpson E, et al. Arimoclomol at Dosages up to 300 mg/day is Well Tolerated and Safe in Amyotrophic Lateral Sclerosis. Muscle & Nerve, (2008) 38:837-844.
9. Bedlack RS, Maragakis N, **Heiman-Patterson T**. Lithium may slow progression of amyotrophic lateral sclerosis, but further study is needed.Proc Natl Acad Sci U S A. 2008 Apr 22;105(16):E17;
10. Aggarwal S, Zinman, L, Simpson E, et al: Clinical Trial Testing Lithium in ALS Terminates Early for Futility. Lancet Neurology 2010: May;9(5):481-8.
11. Buchsbaum R, Kaufmann P, Barsdorf AI, and the QALS Study Group. Web-based data management for a phase II clinical trial in ALS. Amyotroph Lateral Scler. 2009 Oct-Dec;10(5-6):374-7.
12. Kaufmann P, Thompson JL, Levy G, et al: QALS Study Group. Phase II Trial of CoQ10 in ALS finds insufficient evidence to justify Phase III Ann Neurol. 2009 Aug;66(2):235-44.
13. Kasarskis E, Mendiondo M, Wells S et al: The ALS/NIPPV Study: Design, feasibility, and initial results. Amyotrophic Lateral Sclerosis 12:17-25 2011
14. Pioro EP, Brooks BR, Cummings J et al: Dextromethorphan Plus Ultra Low-Dose Quinidine Reduces Pseudobulbar Affect Ann Neurol 68: 693-702 2010
15. **Heiman-Patterson TD**, Sher R, Blankenhorn EA et al: Effect of Genetic Background on Phenotype Variability in Transgenic Mouse Models of Amyotrophic Lateral Sclerosis: A window of opportunity in the search for genetic modifiers. Amyotrophic Lateral Sclerosis.12:79-86 2011.