

Award Number: W81XWH-11-1-0767

TITLE: "Rethinking Drug Treatment Approaches in ALS by Targeting ABC Efflux Transporters"

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REPORT DATE: August 2013

TYPE OF REPORT: Other

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

DISTRIBUTION STATEMENT: Approved for Public Release;  
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REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188		
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. <b>PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.</b>					
1. REPORT DATE U&f à\! 2013		2. REPORT TYPE Annual		3. DATES COVERED HEÀ\] c\ à\! ÆFGGÀ\] c\ à\! ÆFH	
4. TITLE AND SUBTITLE  Rethinking Drug Treatment Approaches in ALS by Targeting ABC Efflux Transporters				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER Y   FY Y P È F È È Ì Ì	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Piera Pasinelli, Ph.D- writing report Dena Jacob, Ph.D. –performing research  E-Mail: <a href="mailto:piera.pasinelli@jefferson.edu">piera.pasinelli@jefferson.edu</a>				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  Thomas Jefferson University Philadelphia, PA 19107				8. PERFORMING ORGANIZATION REPORT NUMBER	
				10. SPONSOR/MONITOR'S ACRONYM(S)	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
				12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited	
13. SUPPLEMENTARY NOTES					
14. ABSTRACT Despite multiple therapeutic efforts targeting a variety of underlying pathogenic mechanisms, approaches to cure the mouse the models amyotrophic lateral sclerosis (ALS) have failed. With the exception of Riluzole (the only drug approved by the FDA for treatment of ALS), we have been unsuccessful at translating promising results from pre-clinical mouse trials to effective pharmacotherapies for ALS patients. One of the problems in finding highly efficacious treatments in ALS may derive from the so far underestimated issue of disease-driven pharmacoresistance mediated by the multi-drug resistance (mdr) efflux transporter, P-glycoprotein (P-gp). These are proteins that are present at the blood and spinal cord brain barrier whose function is to protect the brain from xenobiotics including drugs. These proteins actively pump out from the nervous system (CNS) “foreign” substances. We have shown that in ALS, both in patients and in the ALS mice, there is an increased expression and activity of these efflux transporter P-gps and hypothesized that one of the problem in treating ALS derives from a disease-driven acquired pharmacoresistance due to increased P-gps. Riluzole, which only has a modest effect in patients, is a P-gp substrate. Thus, it is plausible that administration of Riluzole in combination with a P-gp inhibitor could improve its therapeutic outcome. With this proposal we test the hypothesis <i>that co-administration of Riluzole with a potent P-gp inhibitor (Elacridar) will enhance Riluzole bioavailability and therefore will improve its therapeutic efficacy the SOD1-G93A ALS mice.</i>					
15. SUBJECT TERMS Drug resistance, ALS, Therapy, Riluzole, Drug Efflux Transporters					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	7	19b. TELEPHONE NUMBER (include area code)

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- **Introduction (Narrative that Briefly (one paragraph) describes the subject, purpose and scope of the research):**

Research on Amyotrophic Lateral Sclerosis (ALS) has identified many potential therapeutics targeting pathogenic mechanisms in animal models, yet there has been limited progress in translating them into a successful pharmacotherapy in patients. Among other factors, problems identifying highly effective ALS treatments may result from an underestimated issue of drug bioavailability and disease-driven pharmacoresistance, mediated by the ATP-binding cassette (ABC) drug efflux transporters. ABC transporters are predominately localized to the lumen of endothelial cells of the blood-brain barrier (BBB) where they limit the entry into the Central Nervous System (CNS) of a wide range of neurotoxicants, as well as therapeutics. We reported a disease-driven increase in expression and function of P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) in ALS mouse spinal cord capillaries and found that expression also increases in spinal cord tissue of ALS patients (Jablonski et al.) ***underscoring the idea that acquired pharmacoresistance may negatively affect drug bioavailability (and efficacy) in ALS.*** Interestingly, Riluzole, the only FDA-approved medication currently available to treat ALS, is a substrate of P-gp and Bcrp. Riluzole consistently prolongs survival of ALS patients by 6 to 9 months. Perhaps because of this somewhat limited efficacy, the ALS community at large concentrated its efforts in finding new and better drugs rather than finding ways to improve Riluzole's therapeutic effects. However, our findings that in ALS there is an acquired pharmacoresistance mediated by P-gp and Bcrp, together with the realization that Riluzole is a substrate for these two transporters, ***led us to hypothesize that Riluzole bioavailability and, ultimately, efficacy are limited by the action of P-gp and Bcrp and that blocking P-gp and/or Bcrp function may significantly improve the therapeutic effects of Riluzole.***

Thus, the present grant is a **proof-of-principle study** that takes advantage of the ALS mouse model to reconsider the therapeutic potentials of Riluzole. The question is: *Can we make Riluzole better, by improving its bioavailability?* Our hypothesis is that ***we can***. By inhibiting pharmacoresistance in ALS and blocking P-gp and Bcrp that normally pump out Riluzole from the spinal cord (thus reducing its bioavailability and its efficacy), we can make Riluzole better and more efficacious.

The project has one specific aim: ***“To investigate if co-treatment of Riluzole and elacridar, a potent and selective P-gp inhibitor, will improve Riluzole's therapeutic benefit in the ALS mice”***, and it is based on the hypothesis and rationale that improving the bioavailability of Riluzole by inhibiting P-gp efflux transporter(s) will improve and prolong its therapeutic effect in the ALS mice.

- **Body (describe the research accomplishments associated with each task outlined in the approved Statement of Work):**

The project has one specific aim: ***“To investigate if co-treatment of Riluzole and elacridar, a potent and selective P-gp (and Bcrp) inhibitor, will improve Riluzole's therapeutic benefit in the ALS mice”***, and it is based on the hypothesis and rationale that improving the bioavailability of Riluzole by inhibiting P-gp efflux transporter(s) will improve and prolong its therapeutic effect in the ALS mice.

As reported in the original SOW, the project entails 4 STEPS:

#### 1) **CHRONIC ELACRIDAR TOXICITY**

The Elacridar Safety Trial in the SOD1-G93A mice has been completed and detailed results have been presented in Year 1 Progress Report. At the doses tested, ELACRIDAR

2) **METHODS OF RILUZOLE ADMINISTRATION AND REPLICATION OF PREVIOUSLY PUBLISHED STUDIES**

Both studies, optimization of Riluzole route of administration and pre-clinical study of effect of Riluzole in the SOD1-G93A have been reported in details in Year 1 Progress Report. We found that RILUZOLE GIVEN IN THE CHOW IS DELIVERED EFFICIENTLY TO THE MICE (Year 1 Progress Report) and that DEPENDING ON THE TIME OF ADMINISTRATION, THE EFFECT OF RILUZOLE ON DISEASE PROGRESSION IS AS REPORTED IN THE LITERATURE. Hence, when given PRE-SYMPTOMATICALLY, RILUZOLE SLOWS DOWN DISEASE PROGRESSION (Year 1 Progress Report), when GIVEN AT SYMPTOMS IT HAS MARGINAL BUT NO SIGNIFICANT EFFECT (See below Survival Curve-Figure 1)

3) **MEASURE SPINAL CORD AND PLASMA RILUZOLE LEVELS**

We first optimized a Mass spectrometry-based method for the sensitive, reliable and quantifiable detection of Riluzole in the CNS (Brain and spinal cord) and blood. The methods for tissue preparation and Riluzole extraction were optimized and presented in Year 1 Progress Report. At the time of Year 1 Progress Report, analysis of Riluzole concentrations in the experimental groups (Riluzole treated animals vs Riluzole+Elacridar treated animals) was ongoing. Most of the data have been now collected and SHOW THAT IN THE ELACRIDAR TREATED MICE (RILUZOLE+ELACRIDAR COHORT) RILUZOLE BLOOD CONCENTRATION/BIOAVAILABILITY IS ELEVATED AS COMPARED TO THE RILUZOLE ONLY TREATED MICE. **We are performing further analysis in brain and spinal cord where we detect a trend in increased Riluzole concentrations but, due to issues with Riluzole extraction in tissues other than blood, results are variable.**

4) **EVALUATE PRE-CLINICAL EFFICACY OF ELACRIDAR IN TREATING SOD1-G93A ALS MICE WITH RILUZOLE**

With the first 3 steps completed (except the second part of Step 3 in which we need to re-evaluate the ability of Elacridar to consistently increase tissue [brain and spinal cord] Riluzole concentrations in a measurable manner), we embarked starting Year 2 in the pre-clinical study of Riluzole+Elacridar. Ultimately, this study tests our main hypothesis that ***co-administration of Riluzole with the P-gp inhibitor Elacridar will enhance Riluzole bioavailability and, ultimately, therapeutic efficacy in the SOD1-G93A ALS mice.***

As outlined in the original proposal, this part requires a lot of mice [**45 mice in total for survival and disease progression analyses** if we assume three cohorts (Elacridar alone, Riluzole alone and Riluzole+Elacridar) of 15 mice each and **15 additional mice for the pathological analysis** (5 mice for each cohort)]. The project is going well and overall, we are on schedule gathering survival and histopathology analysis data right now. Our data, so far, support our original hypothesis showing that:

1) MICE TREATED WITH Elacridar+Riluzole LIVE LONGER THAN MICE TREATED Riluzole alone (Figure 1). **In this respect, though, we need additional months of work.** We added additional mice to each cohort to increase the power of our statistical analysis on the survival data and now need to wait for the different cohorts of mice (treated with Riluzole alone or with Riluzole+Elacridar) to run their course of life and

monitor their survival. Since mice are treated at disease onset (100 days), we had breed and age the additional mice, which began treatment recently. Additionally, if our treatment is successful, as the data shown in Figure 1 would indicate, mice live longer and we cannot stop their life earlier. **We are therefore waiting for these mice and the work is ongoing.**

2) **CO-TREATMENT WITH Elacridar+Riluzole IMPROVES OUTCOME MEASURES (compound muscle potential [cMAP-Figure 2 B&C] muscle strength [Figure 2-D]) IN ALS MICE.** We need, however, **to perform a full characterization** of the improved effect of Riluzole in combination with Elacridar at the molecular, pathological and phenotypic (survival) **level before all the data are analyzed and written.** Indeed, we added a third and additional cohort of mice that we decided to treat with Riluzole+Elacridar and to sacrifice at three symptomatic stages to perform a thorough analysis (behavioral, electrophysiological, biochemical and histological) to determine how enhancing the Riluzole bioavailability affects the disease at the molecular and functional level. The goal is to correlate-while the mice are still alive and in the midst of the disease-increased concentrations of Riluzole in brain and spinal cord with improved functional and pathological outcomes in diseased mice.

3)

- **Key Research Accomplishments (Bulleted list of key research accomplishments emanating from this research):**
  1. We have proven our hypothesis that pharmacological inhibition of Pg-p transporters enhances Riluzole bioavailability
  2. In ongoing studies, we are confirming our initial hypothesis that that co-administration of Riluzole and Elacridar, improves efficacy of Riluzole in the SOD1-G93A ALS mice
  3. Ongoing promising results demonstrate that enhancing Riluzole bioavailability improves muscle strengths, motor performance (quality of life) in symptomatic mice

- **Reportable Outcomes**

A manuscript is in preparation with all the data already acquired through our analyses outlines in Steps 1-4 (Years 1 and 2). For the final manuscript we are waiting for the additional cohorts of mice for both survival analysis, pathology and outcome measurements over disease progression.

- **Conclusion (Summarize the results to include the importance and/or implications of the completed research and when necessary, recommend changes on future work to better address the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included in the conclusion of the report):**

As summarized above in "key Research Accomplishments", the experiments performed throughout the duration of this grant, support our hypothesis that Blocking Pg-p drug efflux transporters (1) enhances Riluzole bioavailability and (2) improves Riluzole therapeutic efficacy *in vivo* in the SOD1-G93A ALS mouse model. Over the course of Year 2, we elected to add additional mice to our study and to perform a more detailed characterization of the combined effect of Riluzole+Elacridar at the molecular and pathological level. Specifically:

- ✓ We added additional mice in our survival analysis to increase the power of our statistical analysis on the survival data. These mice are now being treated with Riluzole+Elacridar and we are waiting for the final results to add to the encouraging results presented in Figure 1.

- ✓ We added a third and additional cohort of mice that we decided to treat with Riluzole+Elacridar and to sacrifice at various symptomatic stages to perform a thorough analysis (behavioral, electrophysiological, biochemical and histological) to determine how enhancing Riluzole bioavailability affects the disease at the molecular and functional level. The goal is to correlate- while the mice are still alive and in the midst of the disease-increased concentrations of Riluzole in brain and spinal cord with improved functional and pathological outcomes in diseased mice. As shown in Figure 2, we have encouraging preliminary results with improved muscle strengths.
- ✓ We have requested, and were granted, a no-cost extension to complete these studies. We are positively encouraged by the results we obtained in Years 1 and 2 that prove our original hypothesis. Because of the potential immediate translation into the clinic of our results, we feel that we need to completely follow up on our additional cohort of mice performing a rigorous PK, bioavailability analysis of Riluzole *in vivo* and correlate that with “quality of life” outcome measures.

• **References**

M. R. Jablonski, D. A. Jacob, C. Campos, D. S. Miller, N. J. Maragakis, **P. Pasinelli** and D. Trotti. . Selective Increase of Two ABC Drug Efflux Transporters at the Blood-Spinal Cord Barrier Suggests Induced Pharmacoresistance in ALS. (2012) **Neurobiol. Disease 47(2): 194-200**

• **FIGURES**

**Co-treatment with Riluzole and Elacridar improves survival in SOD1-G93A mice**

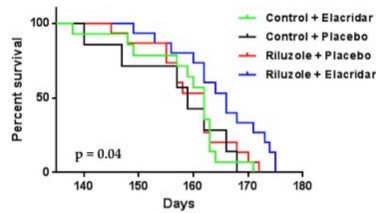


FIGURE 1

**Co-treatment with Riluzole and Elacridar increases compound muscle action potentials (cMAP) and improves behavior in symptomatic ALS mice**

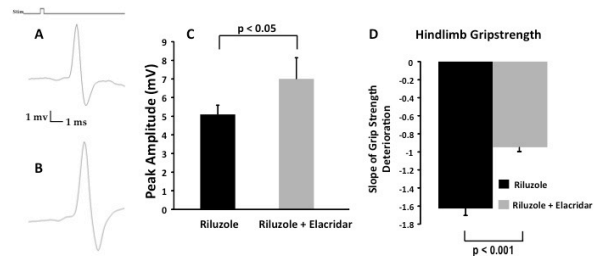


FIGURE 2