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TITLE: **Improving Survival and Promoting Respiratory Motor Function After Cervical Spinal Cord Injury**

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CONTRACTING ORGANIZATION: **UNIVERSITY OF KENTUCKY**  
**LEXINGTON KY 40526-0001**

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<b>14. ABSTRACT</b> Most spinal cord injuries (SCI) occur near the neck. The cervical level of the spinal cord is located near this area. At this level are the connections to the cells which instruct the diaphragm, the major muscle used for breathing, to contract. Therefore, damage at the cervical level can lead to a paralyzed diaphragm and an inability to breathe and inspire air. Patients who survive this type of injury often need the use of a mechanical ventilator in order to survive. Use of the ventilator severely limits the quality of life of those injured and dramatically increases the demand for health care. However, despite these drastic interventions, the cervical injured patient is still susceptible to death due to respiratory complications. This application proposes to help improve survival, decrease early dependence on mechanical ventilation, and restore breathing after cervical spinal cord injury, as well as develop prognostic indicators for injury progression and recovery. Through these studies we endeavor to add to the standard of care for cervical SCI patients so as to lead to an improved quality of life, better-quality health care management, and improved functional outcomes.					
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## Table of Contents

	<u>Page</u>
<b>1. Introduction.....</b>	<b>4</b>
<b>2. Keywords.....</b>	<b>4</b>
<b>3. Accomplishments.....</b>	<b>4</b>
<b>4. Impact.....</b>	<b>5</b>
<b>5. Changes/Problems.....</b>	<b>6</b>
<b>6. Products.....</b>	<b>6</b>
<b>7. Participants &amp; Other Collaborating Organizations.....</b>	<b>6</b>
<b>8. Special Reporting Requirements.....</b>	<b>7</b>
<b>9. Appendices.....</b>	<b>7</b>
<b>Quad Chart.....</b>	<b>8</b>

## 1. Introduction

Most spinal cord injuries (SCI) occur near the neck. The cervical level of the spinal cord is located near this area. At this level are the connections to the cells which instruct the diaphragm, the major muscle used for breathing, to contract. Therefore, damage at the cervical level can lead to a paralyzed diaphragm and an inability to breathe and inspire air. Patients who survive this type of injury often need the use of a mechanical ventilator in order to survive. Use of the ventilator severely limits the quality of life of those injured and dramatically increases the demand for health care. However, despite these drastic interventions, the cervical injured patient is still susceptible to death due to respiratory complications. This application proposes to help improve survival, decrease early dependence on mechanical ventilation, and restore breathing after cervical spinal cord injury, as well as develop prognostic indicators for injury progression and recovery. Through these studies we endeavor to add to the standard of care for cervical SCI patients so as to lead to an improved quality of life, better-quality health care management, and improved functional outcomes.

## 2. Keywords

SCI, breathing, phrenic nucleus, variability, axon, regeneration, neurotrauma

## 3. Accomplishments

*What are the major goals of the project?*

The major goals of the project are to:

- 1) Obtain regulatory review and approval by USAMRMC Animal Care and Use Review Office (ACURO) and local Institutional Animal Care and Use Committee (IACUC).
- 2) Test the hypothesis that inhibiting PTEN through bpV will promote recovery of respiratory motor function after cervical SC contusion.
- 3) Test the hypothesis that increasing respiratory drive immediately after cervical contusion will promote survival and independence immediately after cervical SCI.
- 4) Test the hypothesis that respiratory motor patterns and variability are surrogate endpoints that accurately predict functional improvement after cervical SC contusion and intervention.

*What was accomplished under these goals?*

As the Science Officers, Drs. Quntian Wang and Jessica Tilghman, and Grants Specialist, Amber Stillrich, are aware, in the first year of the award I changed institutions and am now located in the Spinal Cord and Brain Injury Research Center at the University of Kentucky in Lexington, KY. This move was effective 10/1/15.

However, with this move there was a delay in obtaining approval of an award transfer. This was accomplished 8/16/16. In effect, the first year of the award was in reality 1.5 months of actual time devoted to the project (8/15/15 (project start date)-10/1/15(day I transferred UK)).

We accomplished the first major goal of the project which was obtaining regulatory review and approval by the USAMRMC Animal Care and Use Review Office (ACURO) and the local Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky. IACUC approval was obtained 2/22/16. ACURO approval was later attained 3/24/16.

In the second year of the award, newly hired staff and incoming students were trained in the proper methods and approaches of performing cervical SCI's, which is a key component of this project. Much time was devoted to this training as cervical SCI is a complicated and difficult surgery and much practice is needed in order to produce consistent injury outcomes. Additionally, staff and students were trained to perform diaphragmatic EMG recordings, which will be used to measure key functional outcomes after injury and treatment. These project specific techniques were in addition to the acquisition of general animal handling skills, including post surgery care and drug administration.

After this long period of training, we initiated experiments as outlined in the proposal. Specifically, evaluation of the therapeutic agent, bisperoxovanadium (bpV), a potent PTEN inhibitor, to promote rescue of damaged bulbospinal pathways which mediates breathing. Results thus far have been encouraging. Alongside evaluating bpV as the lone treatment, we combined bpV with rehabilitative training in the form of repeated intermittent hypoxia (IH) exposures. This combined therapeutic approach appears to have yielded the best results in promoting the rescue of damaged respiratory pathways and breathing activity. Control treated animals (no bpV or IH) or IH alone did not yield repair of damaged pathways and promotion of breathing function.

We have also initiated Aim 2 experiment to improve survival rates acutely following cervical contusion injuries. We are only in the beginning stages of these experiments.

*What opportunities for training and professional development did the project provide?*

Staff and graduate students at the Spinal Cord and Brain Injury Research Center were provided specialized training in how to perform cervical spinal cord injuries and the necessary post-surgical care.

*How were the results disseminated to communities of interest?*

Preliminary results were disseminated primarily through invited seminars given by the P.I., Dr. Alilain. Moreover, we anticipate presenting some of our findings this year at appropriate meetings, e.g. the annual Society for Neuroscience meeting or the National Neurotrauma meeting.

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

We have initiated the experiments as outlined in the Statement of Work, specifically Aims 1 and 2. In the next reporting period, we will complete data analysis of experiments conducted in these Aims and initiate Aim 3 studies.

#### **4. Impact**

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

While experiments are still in the preliminary stages, it appears that bpV treatment alone can promote rescue of damaged pathways which mediate breathing. This reparative potential is augmented when combined with rehabilitative training in the form of IH. No beneficial effects on the repair of damaged pathways were observed in control groups or IH only groups.

Collectively, the impact of these findings is many:

First, our studies will be the first to suggest that damaged *respiratory* pathways can be made more efficacious to support breathing when left entirely on their own. We can make this

statement since previous studies have utilized the lateral C2 hemisection model of SCI to study respiratory motor insufficiency, but in this model recovery is dependent upon the *uninjured* crossed phrenic pathway which circumvents the lesion. Other studies utilizing cervical contusion injuries do not isolate any recovery observed solely to injured pathways and therefore recovery can still be partially attributed to uninjured pathways. Second, these studies can serve as the launch point to further examine the potential of bpV to inhibit PTEN and promote recovery in chronic SCI. Ultimately, if these studies indicate some beneficial effect of bpV administration, translation of these pre-clinical therapeutic approaches to the human SCI population can begin to be considered.

*What was the impact on technology transfer?*

Nothing to report.

*What was the impact on society beyond science and technology?*

Nothing to report.

## **5. Changes/Problems**

The only major change encountered so far has been the change in institution for the contract. This process took some time and was longer than anticipated leading to reduced time in the first year (essentially 1.5 months of actual project work).

Problems that were initially encountered include a high mortality rate acutely after injury. This could be partially attributed to the relative inexperience of the staff and students performing the surgeries and injuries.

## **6. Products**

No products were produced in the previous year. We anticipate submitting a manuscript for publication in the next year, as well as present our findings in appropriate scientific meetings, e.g. Society for Neuroscience annual meeting, National Neurotrauma meeting.

## **7. Participants & Other Collaborating Organizations**

Warren J. Alilain, Ph.D.

Principal Investigator

Nearest person month worked: 3

Dr. Alilain is the Principal Investigator of this project and therefore keeps updated and knowledgeable of the applicable fields (SCI, respiratory plasticity, experimental interventions, etc.). He is also the primary author of the Animal Protocols submitted to ACURO and the University of Kentucky IACUC. Dr. Alilain has also trained all the personnel in the appropriate and necessary skills to conduct the experiments and complete the goals of this experiment.

Frank J. Jacono, M.D.

Optional Qualified Collaborator

Nearest person month worked: 1

Dr. Jacono is the Optional Qualified Collaborator of this project and helps oversee the quantification, analysis, and interpretation of the various quantitative measures, as well as the ventilatory pattern variability evaluations.

Chris Calulot

Research Technician/Analyst

Nearest person month worked: 4

Mr. Calulot learned the necessary animal procedures necessary for the completion of this project.

**8. Special Reporting Requirements**

please see attached Quad Chart in Appendix

**9. Appendices – please see quad chart**

# Improving Survival and Promoting Respiratory Motor Function After Cervical Spinal Cord Injury

W81XWH-15-1-0378, SC140243

PI: Warren J. Alilain, Ph.D.



## Study/Product Aim(s)

The major goals of the project are to:

- 1) Obtain regulatory review and approval by USAMRMC Animal Care and Use Review Office (ACURO) and local Institutional Animal Care and Use Committee (IACUC).
- 2) Test the hypothesis that inhibiting PTEN through bpV will promote recovery of respiratory motor function after cervical SC contusion.
- 3) Test the hypothesis that increasing respiratory drive immediately after cervical contusion will promote survival and independence immediately after cervical SCI.
- 4) Test the hypothesis that respiratory motor patterns and variability are surrogate endpoints that accurately predict functional improvement after cervical SC contusion and intervention.



Our work seeks to improve survival and restore breathing function after cervical spinal cord injury. Left image is of Matt Hampson, an individual with a cervical SCI, on a ventilator which helps enable him to breathe. We want to remove the need for mechanical ventilation. Photo from: [www.spinal-research.org](http://www.spinal-research.org). <http://www.spinal-research.org/news/spinal-research-supporter-matt-hampson-speaks-about-spinal-cord-injury-on-channel-4%E2%80%99s-embarrassing-bodies/>

## Schedule

Activities	16	17	18	19
1) Get IACUC and ACURO approval	■			
2) Inhibit PTEN after injury		■		
3) Enhance respiratory drive after injury		■		
4) Record and evaluate respiratory motor patterns		■		

Created: 11/29/2016; Modified: 9/21/2017

## Goals/Milestones:

**Milestone:** Local IRB/IACUC Approval

**Milestone:** HRPO/ACURO Approval

**Milestone 1:** Respiratory motor outcomes following treatments will be quantified.

**Milestone 2:** Optimal bpV dosage for improved respiratory motor determined mediated by damaged pathways will be determined.

**Milestone 3:** The effects of bpV treatment on tissue sparing, sprouting of 5-HT fibers, and regeneration associated signaling molecules will be quantified.

**Milestone 4:** Respiratory motor outcomes following bpV and rehabilitation will be quantified.

**Milestone 5:** The effects of bpV treatment and IH rehabilitation on SC tissue will be quantified.

**Milestone 1:** The effect of increasing respiratory drive with theophylline administration on improving survival and independence on mechanical ventilation immediately after cervical SCI will be quantified.

**Milestone 2:** Lesion volume will be characterized and quantified.

**Milestone 1:** Correlate patterns of altered ventilatory pattern variability with the severity of SCI, survival and response to pharmacological intervention (bpV and theophylline).

Green: in progress