

AWARD NUMBER: W81XWH-15-1-0480

TITLE: Development of Novel Local Analgesics for Management of Acute Tissue Injury Pain

PRINCIPAL INVESTIGATOR: Clifford Woolf, MB, BCh, Ph.D.

CONTRACTING ORGANIZATION: Children's Hospital Corporation, Boston, MA 02115

REPORT DATE: September 2017

TYPE OF REPORT: Annual

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

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

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. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE September 2017		2. REPORT TYPE Annual		3. DATES COVERED 09/01/2016-08/31/2017	
4. TITLE AND SUBTITLE Development of Novel Local Analgesics for Management of Acute Tissue Injury Pain				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-15-1-0480	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Clifford Woolf, MB, BCh, PhD (Co-PI) Nick Andrews, PhD (PD) E-Mail: Clifford.Woolf@childrens.harvard.edu; nick.andrews@childrens.harvard.edu				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Children's Hospital Corporation Office of Sponsored Programs 300 Longwood Avenue Boston, MA 02115-5724				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The goal of our project is to develop novel, non-addictive, treatments for acute soft tissue and skeletal injuries, such as seen in battlefield trauma, without the problems associated with opioid drugs. Our strategy is to target the delivery of small, membrane impermeant sodium channel blockers through large pore TRP channels, such as TRPV1. These channels act as the innate "trigger" of nociceptive and inflammatory pain, and are therefore open at the site of acute injury and during the subsequent phases of inflammatory pain that follow. We initiated a series of <i>in vitro</i> drug screens to identify new chemical entities that show promise as potential clinical candidates for non-opioid treatment of pain related to battlefield injury. Of the 31 compounds screened during the review period, we have identified 5 that show superior properties to our tool compound, QX-314.					
15. SUBJECT TERMS Nothing listed					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT Unclassified	18. NUMBER OF PAGES 22	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code)

Table of Contents

	<u>Page</u>
1. Introduction.....	4
2. Keywords.....	4
3. Accomplishments.....	4
4. Impact.....	6
5. Changes/Problems.....	7
6. Products.....	10
7. Participants & Other Collaborating Organizations.....	13
8. Special Reporting Requirements.....	16
9. Appendices.....	16

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

The goal of our project is to develop a new approach to the management of acute pain caused by battle wounds that can be applied quickly and safely and by untrained personnel, and which does not have the problems associated with morphine or similar opioid drugs. To do this we are conducting an *in vitro* and *in vivo* drug screening program to identify novel cationic sodium channel blockers capable of selectively blocking nociceptors for prolonged periods, with the aim to identify safe and effective drug candidates, suitable for progression

2. **KEYWORDS:**

pain, traumatic injury, battlefield wounds, analgesics, sodium-channel blockers, lidocaine, opioids, morphine, non-addictive analgesics, pain control

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

What were the major goals of the project?

The major goals of the project are:

1. Identify novel compounds (New Chemical Entities, NCEs) that are potent intracellular sodium channel blockers capable of permeation through TRPV1 channels. 75% complete
2. Obtain NCEs in volumes sufficient for *in vivo* testing (5g of each NCE) that show no extracellular CVS or CNS activity and that permeate through TRPV1 in DRG neurons. 70% complete
3. Identify 3 best NCEs ranked by analgesic profile and lack of motor impairment. 50% complete.
4. Determine analgesic IC₅₀, and assess biodistribution, tolerance and toxicity of candidate NCEs. 15% complete

What was accomplished under these goals?

1) Major Activities

The purpose of the studies funded in the present award is to identify potential clinical candidate molecules with properties superior to those of our tool compound, QX-314. QX-314 works differently than any other sodium-channel blocker currently on the market, in that it has little to no sodium channel blockade extracellularly and is able to be targeted only into pain-sensing nerves by virtue of membrane bound large pore ion channels (e.g., TRPV1, TRPA1) that are selectively expressed by pain sensing nerve fibers (nociceptors) but not by CNS neurons or motor nerve fibers. These TRP channels are active during inflammation, and largely inactive otherwise, permitting the selective silencing of only those nociceptors actively mediating pain signals. In other words, the compounds we are testing will define a completely new therapeutic class, and we are therefore relegated to comparing the properties of our compound to those of a tool compound, QX-314 (which is not an ideal candidate due to IP and other limitations).

We continued *in vitro* testing of new chemical entities (NCEs) and exploration of properties of compounds from the designed and delivered NCEs to determine their internal, external and use-dependent Nav1.7 channel blocking properties in HEK cells. We continued performing assays to test DRG cell permeation of our cationic sodium channel blockers and began new assays to measure their effect on cardiomyocyte activity. We also performed additional *in vivo* assays to identify the ability of our lead candidates to reverse pain readouts in a rodent model of inflammatory pain.

2) Specific Objectives

We continued our studies comparing the effects of our tool compound, QX-314 (a.k.a., BW001), with the effects of new compounds from our delivered NCEs, looking at: 1) Use-dependent block in Nav1.7-expressing HEK cells, 2) internal block of Nav1.7 channels by 10 μ M compound in HEK cells and 3) extracellular block determination in Nav1.7⁺ HEK cells. We completed cardiotoxicity testing of six of our compounds that showed the greatest promise our *in vitro* screen and found that 5 of the 6 compounds do not produce evidence of cardiotoxicity in calcium imaging and thallium flux assays. Additionally we found one additional NCE, BW005, that produces significant reduction of inflammatory pain in a rat CFA (inflammatory pain) model.

3) Key Outcomes and Developments

Of the 6 most promising compounds identified through our *in vitro* screening program to date (BW004, BW005, BW031, BW035 and BW041), we have now identified 5 with Nav1.7 channel blocking profiles comparable or superior to those of our tool compound, QX-314 (BW001), and without evidence of cardiotoxicity (Table 1).

Table 1.

Lead candidate molecules with properties superior to those of our tool compound, QX-314.

Compound ID	<u>Nav1.7 use-dependent block</u> Filter: Sodium current block >70% at 100µM <10Hz stimulation	<u>Nav1.7 internal block</u> Filter: More block by 10 µM compound with 10 Hz stimulation than QX-314	<u>HEK extracellular block</u> Filter: ≤ 15% block		<u>DRG cell permeation</u> Filter: 60% sodium current block at <10x intracellular IC50.	
	%Block	%Block	Molarity	%Block	Molarity	%Perm
QX-314	81%	37%	100 µM	0%	100 µM	62%
BW-004	95%	79%	50 µM	2%	100 µM	86%
BW-005	91%	74%	50 µM	14%	100 µM	82%
BW-025	85%	68%	100 µM	23%	no data	
BW-031	97%	65%	100 µM	15%	100 µM	73%
BW-035	82%	48%	100 µM	2%	100 µM	54%
BW-041	88%	44%	100 µM	9%	100 µM	60%

Only one of these compounds, BW041, at 100µM increased beat amplitude while decreasing beating rate, suggesting potential cardiomyopathy, as shown in Figures 1-2, below. The remaining compounds (BW004, BW005, BW031 and BW035) did not produce changes in cardiomyocyte beating rate or beat amplitude at physiologically relevant doses.

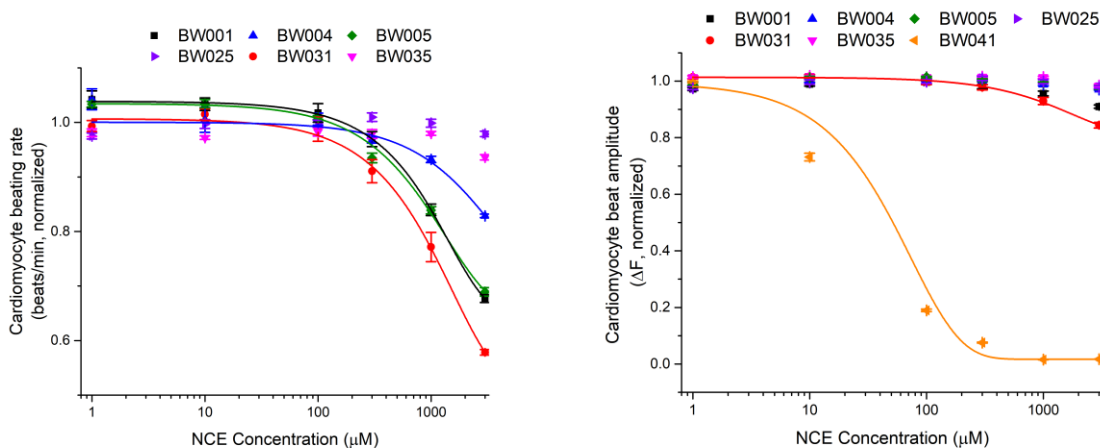


Figure 1. NCE Cardiomyocyte Beating Rate **Figure 2.** NCE Cardiomyocyte Beat Amplitude

3) Key Outcomes (cont.)

Additionally, we continued testing compounds for their ability to reverse pain measures in a rat model of inflammatory pain, and found that compound BW005 significantly reduces pain-related rodent behaviors (Figure 3), as we previously showed with BW004. However BW005 appears to be less effective at reversing pain-related behaviors than QX-314, whereas compound BW004 produced better analgesic efficacy than QX-314 (BW001) in a rat model of inflammatory pain.

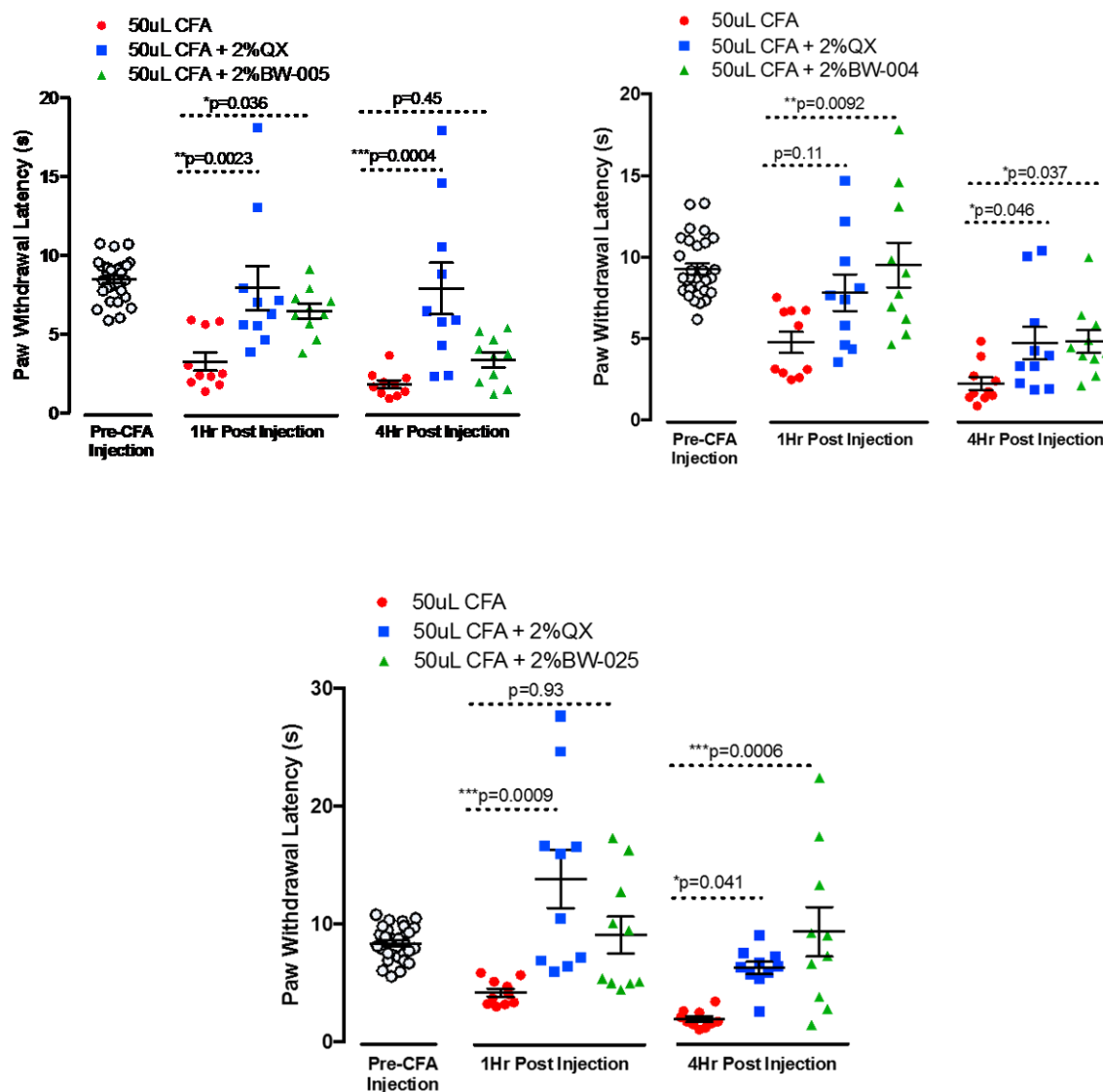


Figure 3. Paw Withdrawal Latency following treatment with QX-314 (labeled “QX”, a.k.a. BW001), BW005, BW004 & BW025 and vehicle.

We also conducted PK studies that showed the levels of both QX-314 (the parent compound) and lidocaine (a possible metabolite of QX-314) following intraplantar injection of QX-314 were undetectable in plasma. When the intraplantar bolus of QX-314 was injected intravenously, QX-314 was detectible in the first 5 minutes and afterwards rapidly eliminated.

In summary, we have now identified 5 compounds that demonstrate potent sodium channel blocking activity in vitro, without apparent cardiotoxicity. We revealed that two of these compounds (BW004 and BW005) show analgesic activity in a rat model of inflammatory pain, and we showed that our tool compound, QX-314, does not redistribute appreciably beyond the site of peripheral administration, as predicted.

4) Other Achievements

We performed no assays using funding from this award beyond the scope of SOW.

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

Nothing to Report.

How were the results disseminated to communities of interest?

Nothing to Report.

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

In Award Year 3 we will continue assessing new compounds as part of our overall screening process. We will assess the action of existing and any newly identified compounds on cardiomyocytes to assess safety, and then demonstrate their ability to block pain-related behavior in preclinical inflammatory and tissue injury models for extended periods of time without impacting motor function. For remaining lead candidate molecules, we will determine the analgesic IC₅₀ for induced pain, determine analgesic in vivo biodistribution (PK studies) and tolerance, and study local and systemic tolerability (toxicity studies), with a view to identifying clinical candidates.

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

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

We have initiated a series of efficacy and safety drug discovery screens (screening funnel) to identify new chemical entities that show promise as potential clinical candidates for treatment of pain, such as the immediate pain of wounded soldiers while they are being evacuated to a hospital setting. Such a candidate, if shown to be safe in phase 1 and effective in phase 2 studies, may be able to provide prolonged block of acute pain caused by battle wounds, without the problems associated with opioid compounds.

What was the impact on other disciplines?

In addition to blocking “pain signals,” the population of nerves that we are silencing are also expressed by lung sensory neurons, where they detect pain, cough and other forms of lung irritation. With this in mind, we began testing the lead candidates identified through our screening funnel in a Guinea pig cough model. We found that both our tool compound and our lead candidate molecules can silence chemical lung irritation and could therefore be potential candidates for treatment of or prophylaxis for chemical warfare agents and other lung irritants that could be encountered in the battlefield.

What was the impact on technology transfer?

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

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

Our end goal is to develop a new therapy capable of relieving the acute pain generated by battlefield injury. This same strategy could also be applied to the management of postoperative pain for elective or emergency surgery. To arrive at one or more of these clinical end goals, we will partner with industry and/or private investment firms to ensure that this technology is transferred into the clinical development pipeline for subsequent clinical trials.

What was the impact on society beyond science and technology?

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

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

Nothing to Report.

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

Changes in approach and reasons for change

Nothing to report.

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

Nothing to Report.

Changes that had a significant impact on expenditures

Nothing to Report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

Nothing to Report.

Significant changes in use or care of vertebrate animals.

Amendments through the year

- 11/15/16: Novel chemical entities coming through from the in vitro screening as being equal or improved potential efficacy relative to QX314 (BW001) were added as new compounds for testing in the CFA model of inflammation, the intra-plantar, incisional model, and the blood sampling experiment; as such the number of animals was also adjusted authorized for use under this protocol.
- 06/10/16: modify the existing procedure for the plantar incisional model to include lifting of the plantaris muscle and closing of the incision with sutures; add a procedure for sampling blood from the jugular vein.

Annual Renewal of IACUC protocol - 20th June 2017

The goal of our project is to develop novel, non-addictive, treatments for acute soft tissue and skeletal injuries, such as seen in battlefield trauma, without the problems associated with opioid drugs. Our strategy is to target the delivery of small, membrane impermeant sodium channel blockers through large pore TRP channels, such as TRPV1. These channels act as the innate “trigger” of nociceptive and inflammatory pain, and are therefore open at the site of acute injury and during the subsequent phases of inflammatory pain that follow. We initiated a series of in vitro drug screens to identify new chemical entities that show promise as potential clinical candidates for non-opioid treatment of pain related to battlefield injury. Of the 31 compounds screened thus far, we have identified 5 that show superior in vitro properties to our tool compound, QX-314 and these 5 were then prioritised to enter in vivo testing. BW-004 (ED-01), BW-005 (ED-02), BW-008 (ED-05), BW-025 (N-ethyl-etidocaine), BW-031 (ACS8180-6B) were the compounds chosen. So far we have found that BW004 has superior efficacy to QX314 and that BW005 has comparable efficacy to QX314 in the intra-plantar CFA-induced inflammation model. We carried out an experiment to measure the extent of systemic distribution of compounds following local (intra-plantar) and systemic (i.v.) administration and this study revealed no detectible systemic redistribution following plantar injection of our tool compound which is important as this shows that we should not expect safety concerns to arise from local administration of our charged inhibitors. Once we have completed testing of the other compounds we will select 1 or 2 of the best and perform the same analysis of systemic distribution following local administration in order to assess whether there are any safety issues to be expected.

Significant changes in use of biohazards and/or select agents

Nothing to Report.

6. PRODUCTS:

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

Journal publications.

Nothing to Report.

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

Nothing to Report.

Other publications, conference papers, and presentations.

Nothing to Report.

- **Website(s) or other Internet site(s)**

Nothing to Report.

- **Technologies or techniques**

Nothing to Report.

- **Inventions, patent applications, and/or licenses**

Nothing to Report.

- **Other Products**

Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment, and/or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:

- *data or databases;*
- *biospecimen collections;*
- *audio or video products;*
- *software;*
- *models;*
- *educational aids or curricula;*

- *instruments or equipment;*
- *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
- *clinical interventions;*
- *new business creation; and*
- *other.*

Nothing to Report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name: Clifford Woolf, MB, BCh, PhD
Project Role: Co-PI
Researcher Identifier (e.g. ORCID ID): 0000-0002-6636-3897
Nearest person month worked: 1
Contribution to Project: Dr. Woolf assisted in the determination of identity of new chemical entities and supervised the establishment of in vitro and in vivo assays.

Name: Bruce Bean, PhD
Project Role: Co-PI
Researcher Identifier (e.g. ORCID ID): 0000-0002-5093-3576
Nearest person month worked: 1
Contribution to Project: Dr. Bean helped identify of new chemical entities and supervised the in vitro testing of new compounds.

Name: Nick Andrews, PhD
Project Role: PD
Researcher Identifier (e.g. ORCID ID): 0000-0001-9966-5093
Nearest person month worked: 12
Contribution to Project: Dr. Andrews oversaw in vitro and in vivo assay development and testing, coordinated data recording and submission protocols, and prepared quarterly report.

Name: Sebastien Talbot, PhD
Project Role: Research Fellow
Researcher Identifier (e.g. ORCID ID): 0000-0001-9932-7174
Nearest person month worked: 2
Contribution to Project: Dr. Talbot developed in vitro assays and performed key in vitro experiments and in vivo pilot experiments.

Name: Benjamin Doyle, MS
Project Role: Research Assistant
Researcher Identifier (e.g. ORCID ID): 0000-0002-9228-4121
Nearest person month worked: 12
Contribution to Project: Mr. Doyle performed in vitro and in vivo assays.

Name: Ivan Tochitsky, PhD
Project Role: Research Fellow
Researcher Identifier (e.g. ORCID ID): 0000-0003-0650-9193
Nearest person month worked: 6
Contribution to Project: Dr. Tochitsky developed in vitro assays and performed in vitro screening experiments.

Name: Sooyeon Jo, PhD
Project Role: Research Fellow
Researcher Identifier (e.g. ORCID ID): 0000-0001-5555-6514
Nearest person month worked: 9
Contribution to Project: Dr. Jo developed in vitro assays and performed in vitro experiments.

Name: Gui-Lan Yao, MD, PhD
Project Role: Research Associate
Researcher Identifier (e.g. ORCID ID): 0000-0003-2940-9443
Nearest person month worked: 1
Contribution to Project: Dr. Yao has supported the electrophysiology experiments by maintaining tissue culture equipment and supplies.

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

NIH grant R01DE022912 “Sleep Disturbance as a risk factor for Chronic Pain” ended 9/30/17”.

NIH grant P30HD018655 “Intellectual and Developmental Disabilities Research Center” ended 9/22/16 and was replaced with U54HD090255 “Intellectual and Developmental Disabilities Research Center, for the period 9/23/16-5/31/21.

Mass Life Sciences Consortium grant “Proof of Concept Use of iPSC-derived motor neurons in personalized medicine” was extended through 12/31/17.

Glaxosmithkline Wellcome grant “Discovering new ion channel modulator drugs for ALS” ended 4/29/17.

Harvard Brain Initiative grant “Matching gene transcription and disease phenotype in single motor neurons derived from ALS patients to determine underlying pathophysiological mechanism” ended 6/30/17.

Amgen “State-dependent interactions between peptide and small molecule inhibitors in binding to Nav1.7 sodium channel” ended 3/22/2017

HBI “Pharmacological modulation of motor neuron excitability in the SOD1-G93A mouse model of ALS” ended 6/30/2017

A new grant from Target ALS Foundation, Inc. began 5/1/17:

- a) Title: Mechanisms of Cortical Hyperexcitability in ALS
- b) Principal Investigator: Clifford Woolf, MB, BCh, Ph.D.
- c) Goals: the goal of this project is to explore if hyperexcitability is a disease biomarker for upper and motor neurons in patients with familial ALS due to C9ORF72 mutations and the mechanisms responsible.
- d) Specific aims/tasks 1) Determine if the presence of hyperexcitability a biomarker of motor neuron disease risk in C9orf72 expansion carriers, (2) Determine whether changes in excitability cause neurodegeneration, (3) Identify common molecular mechanisms underlying the changes in excitability using patient-derived iPSCs, purified populations of relevant cortical cell types from human postmortem samples and mouse models of C9orf72 disease to generate new candidate targets for therapeutic intervention.
- e) Start and end date: 5/1/17-4/30/19
- f) Level of effort: 0.12 calendar months

g) Point of Contact: Manish Raisinghani, President
PO Box 1598
Radio City Station
New York, NY 10101-1598
646-592-2541

H) overlap There is no scientific or budgetary overlap with this grant.

A new grant from NIH began 8/1/17:

a) Title: Identification of Susceptibility to chemotherapy induced peripheral neuropathy using patient stem cell derived sensory neurons
b) Principal Investigator: Clifford Woolf, MB, BCh, Ph.D.
c) Goals: to help identify patients at risk for CIPN prior to their treatment and understanding the factors which contribute to CIPN susceptibility.
d) Specific aims/tasks (1) Identify chemotherapy induced phenotypic changes in iPSC derived sensory neurons that correlate with patient neuropathy status; (2) Examine whether patient derived sensory neurons display differential sensitivity to different chemotherapeutic agents

a) Start and end date: 8/1/17-7/31/19
b) Level of effort: 0.24 calendar months
c) Point of contact: Ann M O'Mara, Program official
BG 9609 RM 5E444
9609 Medical Center Drive
Rockville, MD 20850
240-276-7074

d) Overlap: There is no scientific or budgetary overlap with this grant.

A new grant from Children's Hospital Boston began 1/1/17:

a) Title: Rodent "Palm Reader" for Detecting CNS activity, drug efficacy, and drug side effects in lab rodents
b) Principal Investigator: Clifford Woolf, MB, BCh, Ph.D.
c) Goals
d) Specific aims: (1) Develop user interface to facilitate implementation of command line software and cloud based analysis of existing data; (2a). Advance hardware/software features to facilitate reduced cost and complexity of commercial end product, oversight of Specific Aim 2b, and development of user manual; (2b) Develop advanced software features for analysis of gait and body movement.

e) Start and end date: 4/1/17-12/31/17
f) Level of effort: 0.12 calendar months
g) Point of contact: Monique Yoakim, Ph.D,
Boston Children's Hospital
300 Longwood Avenue, Mailstop BCH 3183
617-919-3027

h) Overlap: There is no scientific or budgetary overlap with this grant.

a) Title: "Voltage-dependent ion channels controlling firing patterns of central neurons"

Principal Investigator: Bruce P. Bean, Ph.D.

Type: R01 NS036855

b) Funding agency: National Institute of Neurological Disorders and Stroke

c) Goals: The goal of this research is to understand how the firing properties of particular central neurons are produced by particular combinations of ion channels, with a focus on mechanisms of pacemaking of midbrain dopaminergic neurons and mechanisms enabling very rapid firing in cerebellar Purkinje neurons.

d) Specific aims/tasks:

Aim 1. Determine neuron-specific roles of Kv3 channels in controlling firing frequency and firing patterns. We will follow up preliminary experiments in Purkinje neurons showing that inhibition of Kv3 channels results in slowing of firing, and we will use action potential clamp experiments to determine the mechanistic basis of this effect, testing whether it reflects reduced availability of sodium channels or enhancement of other potassium channels by the changes in action potential waveform. We will also compare changes in firing - and the underlying mechanisms - resulting from inhibition of Kv3 channels in CA1 pyramidal neurons and midbrain dopamine neurons.

Aim 2. Determine neuron-specific roles of Kv2 channels in controlling firing frequency and firing patterns. We will follow up preliminary data in hippocampal CA1 pyramidal neurons and midbrain dopamine neurons showing that inhibition of Kv2 channels can either speed or slow firing, depending on the stimulus and the other channels present. We will use action potential clamp to test whether slowing of firing results from reduced availability of sodium channels or activation of other more persistent potassium channels such as BK or SK channels. We will explore functional consequences of enhancement of Kv2 current by mGluR stimulation.

Aim 3. Determine neuron-specific roles of BK channels in controlling firing frequency and firing patterns. We will follow up preliminary data in hippocampal CA1 pyramidal neurons showing reduced firing frequency after block of BK channels and determine the mechanism of this slowing. We will follow up preliminary data showing a major role of BK current in spike repolarization in dopamine neurons, examining how BK channels help control firing patterns. We will examine the timing and amplitude of BK current relative to activating calcium current and explore specificity for calcium entry through particular calcium channel subtypes and whether this specificity varies with neuronal type.

e) Start and end date: 07/01/1997 – 01/31/2019

f) Level of effort: 3.7 cal. months (30.5%)

g) Point of contact:

Cassandra L Fields, Grants Management Specialist

Edmund M Talley, Program Official

Grants Management Branch

National Institutes of Neurological Disorders and Stroke

6001 Executive Boulevard, Suite 3290, MSC 9537

Rockville, MD 20852 (Express Mail)

Bethesda, MD 20892-9537 (Regular Mail)

h) Overlap: There is no overlap with the present proposal.

i) Funding Level:

a) Title: “Silencing airway nociceptors for treatment of cough and airway inflammation”
Principal Investigator: Bruce P. Bean, Ph.D.
Type: Federally funded cooperative agreement

b) Funding agency: NIH through BBIC (Boston Biomedical Innovation Center)

c) Goals To test efficacy of sodium channel blocking compounds for treating cough in guinea-pig model

d) Specific aims/tasks: 1) Work with contract research organization to develop protocols for testing sodium channel blockers against cough in guinea pigs when administered intratracheally or by nebulization 2) Test 4-5 different compounds for efficacy in treating cough in the guinea pig models 3) Do pharmacokinetic studies to assay systemic exposure of blockers administered by nebulization.

e) Start and end date: 10/1/2016 – 9/30/2017

f) Level of effort: 0.12 cal. months (1%)

g) Point of contact:
Ron K. Blackman, Ph.D. | Senior Project Manager
Boston Biomedical Innovation Center
215 First Street, Suite 500; Cambridge, MA 02142
857-307-2441 | rblackman1@partners.org | b-bic.org

h) Overlap: There is no scientific or budgetary overlap with the present application.

i) Funding Level:

What other organizations were involved as partners?

If there is nothing significant to report during this reporting period, state “Nothing to Report.”

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

Provide the following information for each partnership:

Organization Name:

Location of Organization: (if foreign location list country)

Partner’s contribution to the project (identify one or more)

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

We received grants supporting *in vivo* drug development assays for charged sodium blockers from the Boston Biomedical Innovation Center (NIH funded) in the amount of \$250,000, and a similar drug development grant from an anonymous foundation, also for \$250,000. These grants are both in support of the parallel development of this technology for the treatment of cough by silencing nociceptors in the lung.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

Nothing to Report.

QUAD CHARTS:

Will be submitted with this report as a separate attachment

9. APPENDICES:

Nothing to Report.



Development of Novel Local Analgesics for Management of Acute Injury Pain



Log Number: MR141339 Task Title: Year 2 Quarter 4 Quad Chart

Award Number: W81XWH-15-1-0480

PI: CJ Woolf & B Bean

Org: Boston Children's Hospital / Harvard Medical School

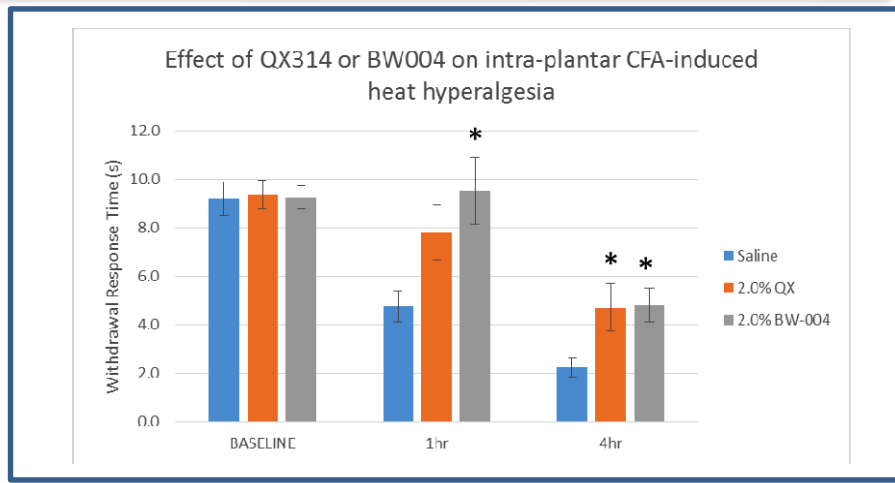
Award Amount: \$1,500,000

Study/Product Aim(s)

- Synthesize and test compounds for intracellular but not extracellular Na-channel blocking activity
- Assess permeation through TRPV1 channels and test for CNS and CVS toxicity
- Demonstrate long lasting selective analgesic activity in inflammation and tissue injury models
- Study systemic redistribution and tolerability

Approach

Our objective is to develop a local/regional analgesic by targeting small charged sodium channel blockers into nociceptor neurons through large pore TRP channels.



Accomplishment: Our new compound BW-004 blocks inflammatory pain in rats (intraplantar CFA model) comparable to or better than our tool compound, QX-314.

Timeline and Cost

Activities	CY	15	16	17	18
Produce candidate charged compounds			█	█	█
Evaluate pore permeation and blocking activity			█	█	█
Test analgesic activity of leads in vivo			█	█	█
Biodistribution, safety and toxicity testing			█	█	█
Estimated Budget (\$K)			\$500	\$500	\$500

Goals/Milestones:

- CY15-17 Goal** – Produce set of charged sodium channel blocker new chemical entities (NCEs).
- CY15-18 Goal** – Identify most potent intracellular sodium channel blockers capable of permeation through TRPV1.
- CY15-18 Goal** – Determine permeation through TRPV1 in DRGs.
- CY15-18 Goal** – Obtain NCEs in sufficient quantities for *in vivo* testing
- CY16-18 Goal** – Test for NCEs without extracellular CNS/ CVS activity.
- CY16-18 Goal** – Evaluate/rank NCEs for analgesic activity, selectivity.
- CY16-18 Goal** – Determine analgesic IC₅₀ for the selected NCEs.
- CY16-18 Goal** – Evaluate local and systemic safety and tolerability

Comments/Challenges/Issues/Concerns

- No substantial challenges, issues or concerns
- Difference in actual vs. projected expenditure due to deferred costs

Budget Expenditure to Date

Projected Expenditure: \$999,795
Actual Expenditure: \$999,795

Updated: September 2017