AWARD NUMBER: W81XWH-16-1-0098

TITLE: Antilysophosphatidic Acid Antibodies in the Treatment of Post-TBI Neuropathic Pain

PRINCIPAL INVESTIGATOR: David C. Yeomans, PhD

CONTRACTING ORGANIZATION: Stanford University
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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Lysophosphotidic acid (LPA) is a lipid imflammatory mediator that is released following nerve injury, including injury to the brain. LPA has been implicated in the development and maintenance of pain and other deleterious sequelae to brain injury. The broad, long-term goal of this project is to evaluate the therapeutic potential of a novel humanized anti-LPA antibody (Lpathomab) for attenuating post-traumatic brain injury (TBI) associated pain. Thus, we studied the effect of Lpathomab, comparing the utility of nasal, intravenous, and subcutaneous administration, in preventing long-term pain sequelae due to fluid percussion- induced neurotrauma in rats. We successfully implemented the rat model in our laboratory, which produced significant behavioral hypersensitivity. We then demonstrated, and published that nasally applied Lpathomab concentrated in the brain and inhibited post-TBI hypersensitivity. Due to technical issues, we did not complete testing of IV or IM application of Lpathomab or measure LPA in CSF.

15. SUBJECT TERMS
monoclonal antibody, lipid inflammatory mediator, analgesia, lysophosphatidic acid

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRDC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

INTRODUCTION

Lysophosphotidic acid (LPA) is a lipid imflammatory mediator that is released following nerve injury, including injury to the brain. LPA has been implicated in the development and maintenance of pain and other deleterious sequelae to brain injury. The broad, long-term goal of this project is to evaluate the therapeutic potential of a novel humanized anti-LPA antibody (Lpathomab) for attenuating post-traumatic brain injury (TBI) associated pain. Thus, we are studying the effect of Lpathomab, comparing the utility of nasal, intravenous, and subcutaneous administration, in preventing long-term pain sequelae due to fluid percussion-induced neurotrauma in rats.

This grant was originally awarded to LPath, Inc, a small biotech in San Diego, with Roger Sabbadini as principle investigator. Stanford University was the primary contractor, with David Yeomans, a Stanford Professor, as Co-PI. Unfortunately, during the reporting period, LPath went out of business, and Dr. Yeomans and Sabbadini requested transfer of the grant to Stanford, and PI-ship to Dr. Yeomans. This request was made in September of 2016 to transfer the grant as of November, 2016. However, Stanford did not receive a notice of allowance until March, 2018. Meanwhile, LPath went out of business as of January, 2017 – and all LPath email addresses became dead. Thus, if notices were sent to LPath for submitting an annual report, these were not received. In the mean time, Dr. Yeomans and Dr. Sabbadini have continued to work on the project and file quarterly reports with the idea that the funds would be transferred. The transfer notice is included in the appendix.

KEY WORDS

monoclonal antibody, lipid inflammatory mediator, analgesia, lysophosphatidic acid

ACCOMPLISHMENTS

Goals:

Traumatic brain injury (TBI) is the most common major injury suffered by warfighters in Iraq and Afghanistan, and frequency results in multiple symptoms of which chronic pain is the most common. Reviews have placed the prevalence of chronic pain following TBI at 88% in blast exposed soldiers with TBI in OIF/OEF and 65% in non-blast exposed soldiers with TBI from the same conflicts (Gironda, 2009). Another general review of military veterans' medical charts places the prevalence of pain complaints at 50% in the general VA population (Clark, 2002) with 62.6% of TBI of patients reported taking narcotic analgesics (Ponsford, 2011). Lysophosphatidic acid (LPA), a bioactive lipid which is known to be increased in TBI and block of LPA effects using an anti-LPA antibody has been shown to provide neural protection after TBI and to block pain after peripheral nerve injury. The broad, long-term goal of this project is to evaluate the therapeutic potential of a novel anti-LPA antibody (Lpathomab) for attenuating post-TBI pain. Thus, we propose to study the effect of Lpathomab, using 2 different application procedures, in preventing long-term pain sequelae due to fluid percussion-induced neurotrauma.

Statistical significance was set to $p < 0.05$ for all statistical tests, and p values were adjusted for multiple comparisons using Bonferroni correction. Data are reported as the mean and SEM. A 2-tailed Student t test for unpaired samples was used for comparison between ELISA data. For time series analysis and between group distinctions, a 2-way repeated measures analysis of variance with post hoc Bonferroni testing was used. All statistics were done with GraphPad Prism 5 (GraphPad Software, Inc, La Jolla, CA).

1. LPA in cerebral spinal fluid (CSF) after TBI

Research Plan:

Concentration of LPA in CSF of sham and cFP TBI rats was evaluated by ELISA assay. Two groups of animals, including 12 cFP TBI rats and 12 sham cFP rats were anesthetized and surgically prepared for percussion injury. The 12 TBI rats underwent percussion injury wherein the surface of the cortex is exposed to a pressure wave, the remaining 12 rats underwent sham surgery wherein the cortex was exposed, but no pressure wave was introduced. Thirty minutes following TBI, and while still anesthetized, the atlanto-occipital membrane was surgically exposed, and a needle introduced through the membrane for extraction of CSF which were frozen for off-line analysis by ELISA.

Results:

After mild TBI, rats demonstrated significantly ($p< 0.05$, Student's t test) higher levels of LPA when compared to rats that had undergone sham procedures. Average CSF concentration of LPA 30 minutes after TBI was 4.5 (+/- 0.3 SEM) micromolar vs 1.1 (+/- 0.2 SEM) micromolar for sham rats. These values are consistent with values we previously found for mice that had undergone nerve injury where we observed a 2.8X increase and with where we observed a 5.4 X increase at 24 hours after TBI in humans (Crack et al., 2014).

2. I.V. Administration of Lpathomab anti-LPA antibody

The experiment used four levels (dosages) of Lpathomab (LT3114 - 2.5, 25, 50, and 100 mg/kg) and several types of controls. Sham surgery served as an injury control and injection of a matched control antibody served as an Lpathomab treatment control.

Research Plan:

I.V. Lpathomab Administration and Neurobehavioral assessment

Neurologic function by standard assessment and pain sensitivity by mechanical stimulation and thermal (laser) stimulation were assessed at various times after cFP TBI or sham injury and after I.V. application of Lpathomab or a control antibody. Thus, a dose-response relationship was determined in terms of analgesic efficacy.

Thirty minutes after percussion injury (for TBI rats), or suture closing (for sham rats), animals were briefly anesthetized with inhaled isoflurane. Anesthetized rats were then administered an intravenous (tail vein) injection of Lpathomab, an equivalent amount of matched antibody, or a control solution. Lpathomab dosage levels were based on values found to reduce pain symptoms following neuropathic injury in rats.

Results:

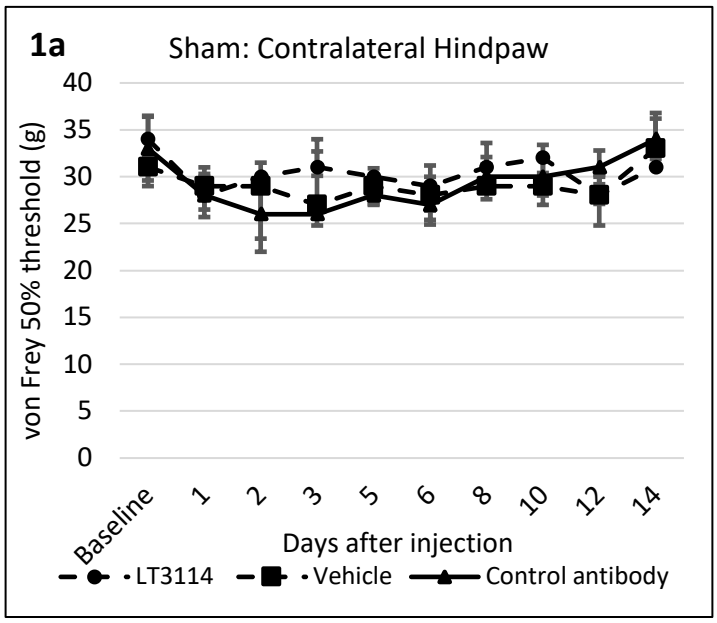
a. Neurological dysfunction severity score

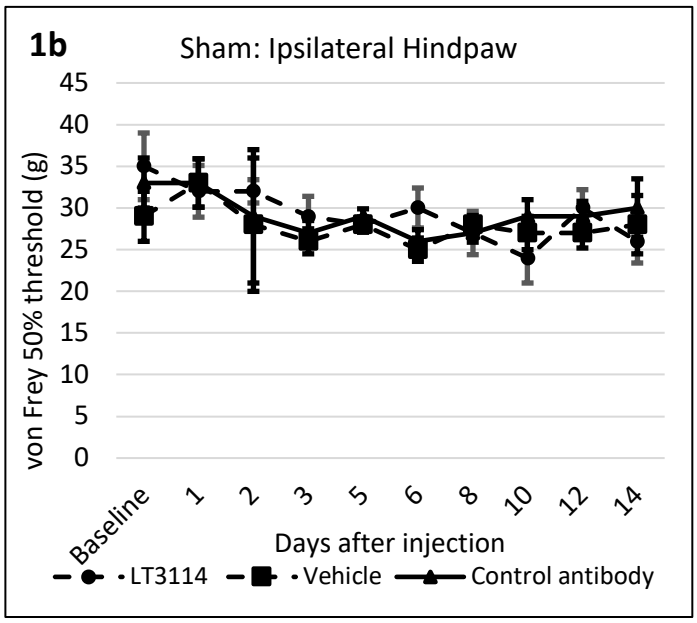
Neurological status after TBI was assessed using a standard neurological severity score described using a 12-level test that assesses, e.g., righting reflex, hemiplegia, and loss of pinna reflex and has been shown to correlate closely with the applied pressure. After TBI, all rats demonstrated no or only mild signs of neurological impairment. Thus, no rats had to be excluded because of preset parameters (elevated neurological severity score (>0) after 1 week or with delayed emergence (>4 minutes after TBI), which would have been indicative of higher grade TBI.

b. Mechanical Allodynia

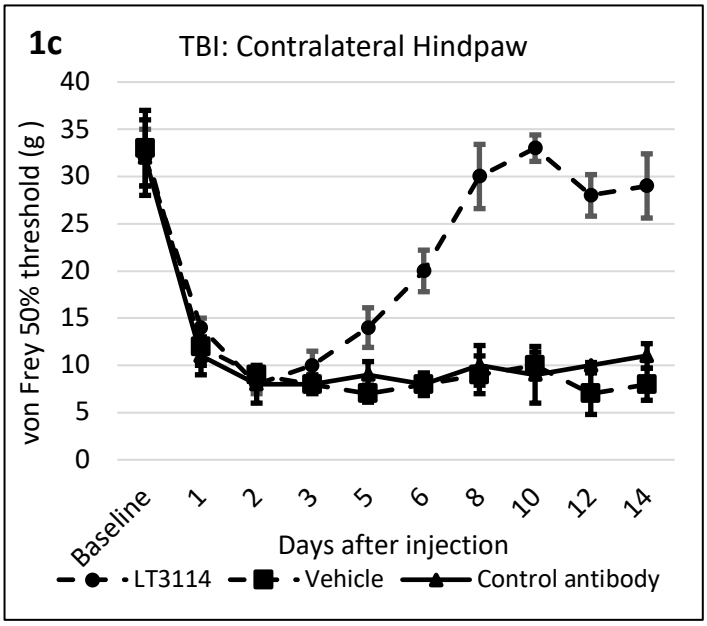
Before the first testing session, animals (N = 6 per group) were habituated for at least 2 hours on a metal mesh (0.5 x 0.5 cm) inside a plastic chamber. One day before TBI, rats were tested for mechanical sensitivity using von Frey filaments (Bioseb, Chaville, France) applied to the plantar surfaces of the hind paws. Fifty percent mechanical withdrawal thresholds to the application of a von Frey probe to the foot was calculated by using the up-down method. To assess mechanical allodynia, an ascending series of von Frey hairs of logarithmically incremental force (0.32-16.31 g) was applied to the midregion of the plantar surface of the hind paw. Each von Frey hair was applied to the test area for 2 to 3 seconds, with a 1- to 2-minute interval between stimuli. Testing was performed on post-TBI day 1, 2, 3, 5, 6, 8, 10, 12, and 14.

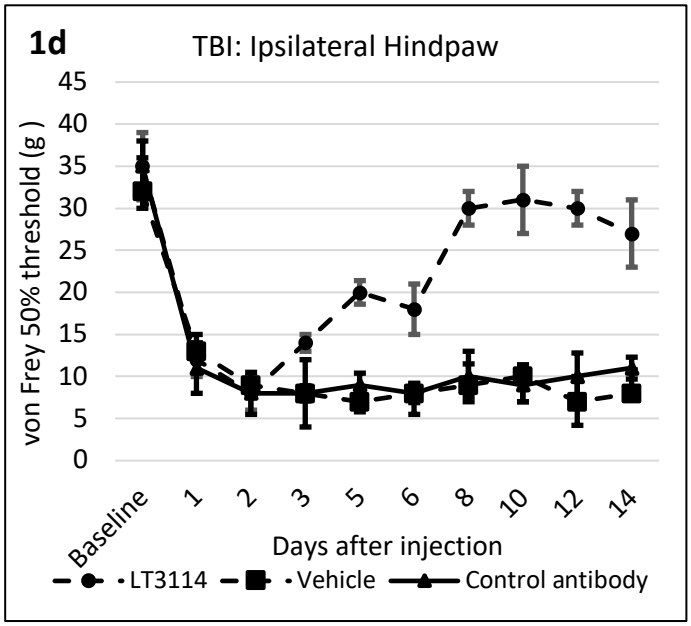
Figures 1a and b shows that there was no change in mechanical withdrawal thresholds for either hindpaw following sham TBI. In addition, no effect was seen on thresholds after IV administration of 100 ug of



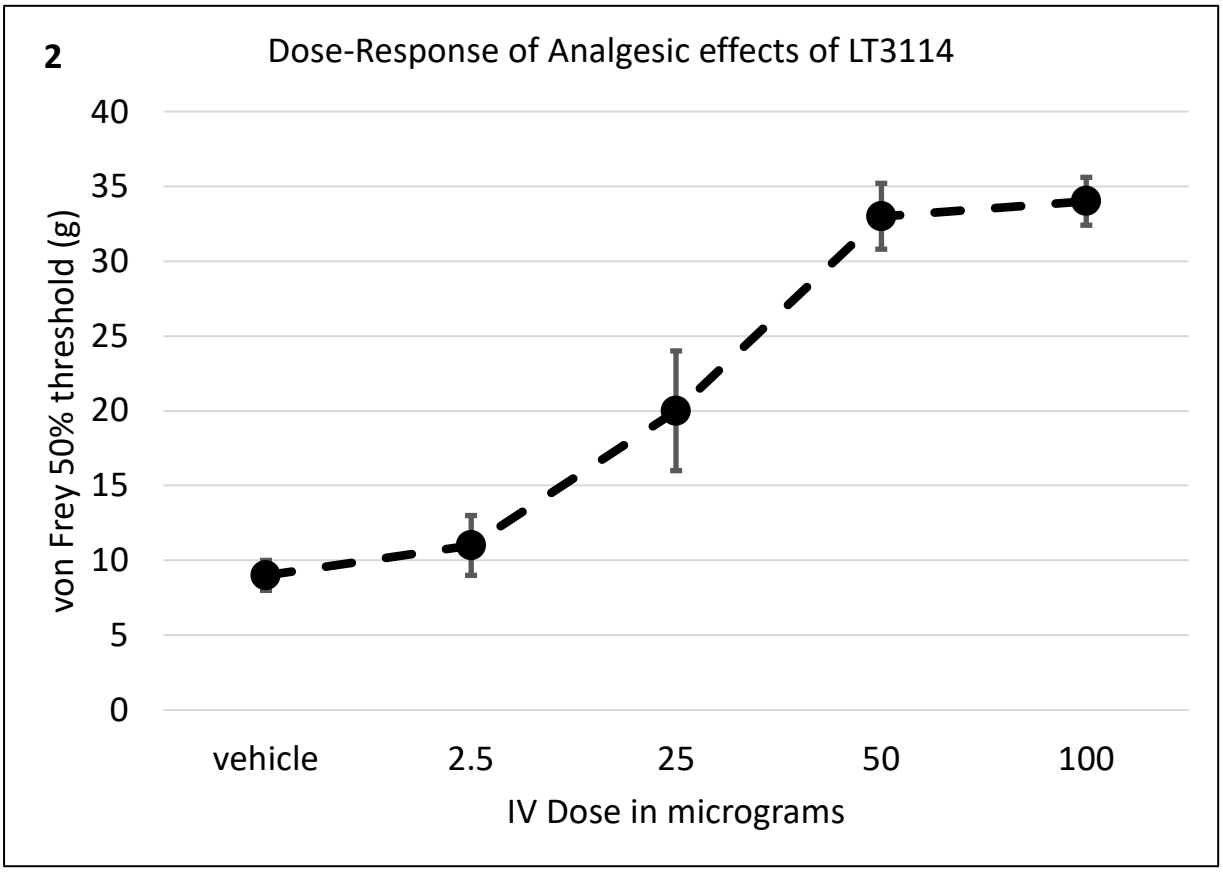


LT3114, control antibody, or vehicle. On the other hand, rats exposed to true TBI showed robust, bilateral mechanical allodynia (Figures 1 c and d) as demonstrated by a significant ($p < 0.05$) decrease in withdrawal thresholds. However, unlike rats that received injections of control antibody or vehicle, rats that received an injection of 100 ug of LT3114 recovered to their pre-TBI levels of mechanical sensitivity over 8 to 10 days following injection. This recovery of normal sensitivity was maintained at least through 14 days.





These anti-allodynic effects were dose-dependent, as is made clear by Figure 2, which represents the average (+/- SEM) von Frey thresholds at 10 days following LT3114 injection.



3. Intranasal (I.N.) Administration of Lpathomab anti-LPA antibody

Research Plan:

a. Intranasal application

Thirty minutes after induction of TBI, anesthesia was induced by isoflurane (3% for induction, 2% for maintaining, 30% O2) in a chamber. Rats were then placed in a supine position on a thermo-regulated surface that maintained body temperature at 37.5°C. Polyethylene tubing (PE-50, 22G) was inserted 0.8 cm into the left nostril. At this point, anesthesia was delivered using facemask. Thereafter, 30 µL of anti-LPA mAb (2.85 mg) or vehicle (200-mM PBS/glycine buffer) was administered over 9.6 minutes (3.125 µL/min) using a syringe pump (GeniePlus; Kent Scientific, Torrington, CT). Preliminary studies showed that this infusion rate minimized respiratory complications while allowing for expeditious delivery of intranasally administered compounds. Rats remained in the same position for additional 5 minutes to allow for uptake of compounds.

Following IN application, rats were tested for neurologic dysfunction as above and, at various time points, for LT-3114 levels in CNS, for neurologic dysfunction, and for changes in mechanical allodynia.

Results:

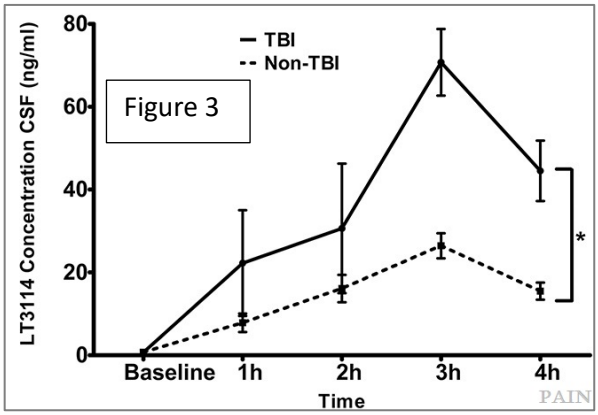
LT-3114 levels in CNS after IN application

Animals with and without TBI (N = 3, per group) were sedated for cisterna magna CSF collection and blood sampling from the tail vein.28 Before intranasal administration of the antibody, baseline samples were taken. Thereafter, CSF and blood samples were collected every hour for 4 hours. Blood was collected in a covered test tube (Becton Dickinson, Franklin Lakes, NJ) and incubated at 30 minutes at room temperature to allow clot formation. Serum was removed by centrifuging at 1000g for 2 minutes and transferred to a 1.5 mL Eppendorf tube.

For neural tissue assessment, animals (N = 2, per group) were euthanized 4 hours after intranasal antibody administration, and brain as well as trigeminal ganglia were harvested and snap frozen. Tissues were dissected, weighted, and homogenized with a Dounce tissue grinder using a neuronal protein extraction reagent (N-PER; Thermo Fisher Scientific, Waltham, MA). To quantify LT3114 tissue, CSF, and serum concentrations, an enzyme-linked immunospecific assay (ELISA) kit (Abnova, Walnut Creek, CA, #KA3817) was used. Samples were diluted according to the ELISA kit manufacturer's protocol or according to the expected concentrations. LT3114 levels were determined using a polynomial standard curve (0.625-40 ng/mL).

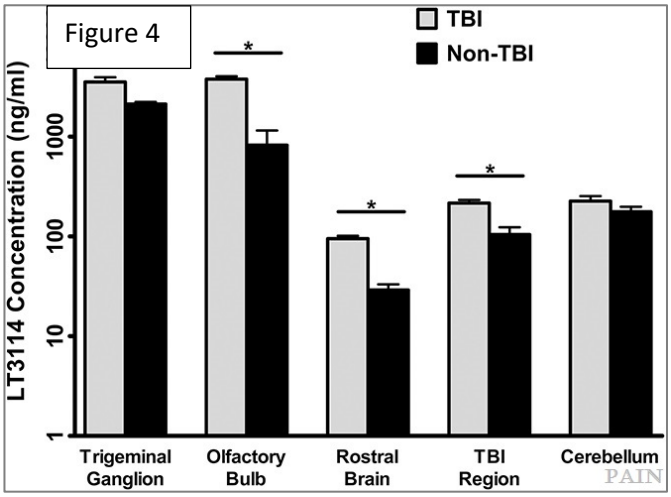
a. Cerebrospinal fluid concentration of LT3114 immunoreactivity

Antibody concentration in CSF was significantly higher in rats with TBI than in animals without injury (Fig. 3, P = 0.0417). The peak concentration levels in both groups were reached 3 hours after LT3114 application. Consistent with the highly vascular nature of the nasal mucosa and the stability of the molecule in blood, the maximum antibody concentration levels in serum were more than 50% higher than those measured in CSF.



b. Brain regions concentrations of LT3114 immunoreactivity

Following intranasal administration of anti-LPA mAb, significant tissue levels were seen in all investigated brain regions (olfactory bulbs, rostral brain region, TBI region, trigeminal ganglia, and cerebellum) (Fig. 4). The highest antibody levels were found in the trigeminal ganglia and olfactory bulbs and were up to 10-fold higher than in the other investigated tissues (Fig. 4). LT3114 levels were significantly higher in TBI rats than in non-TBI rats in the olfactory bulbs (P = 0.0197); there was no significant difference in LT3114 levels in the trigeminal ganglia (P = 0.0804) and the cerebellum (P = 0.2957) between TBI and non-TBI rats. The rostral brain region (bregma 1.3-5.6 mm) and the TBI region (bregma −4.2 to 1.2 mm) also showed significant higher concentrations in rats with TBI (P = 0.0133, P = 0.0478).



c. Effects of IN LT-3114 on severity of neurological injury

After mild TBI, all rats demonstrated no or only mild signs of neurological impairment. Thus, no rats had to be excluded because of preset parameters (elevated neurological severity score [>0]) after 1 week or with delayed emergence (>4 minutes after TBI), which would have been indicative of higher grade TBI.

d. Effect of IN LT-3114 on Mechanical allodynia following TBI

Considering that there is substantial distribution of anti-LPA mAb in the brain after intranasal (IN) administration, we examined whether or not a single IN dose of mAb would have any therapeutic benefit. Figure 5 shows the 14-day time course of the withdrawal response to von Frey filaments after mild TBI in rats that received intranasal LT3114 compared with control rats. For the paw contralateral to the injury, mechanical allodynia was significantly less in the group that received LT3114 compared with the vehicle group (p = 0.0079), suggesting an analgesic effect attributed to a single IN dose of anti-LPA mAb (5A). There was an insignificant trend (P =

0.1762) towards reduced mechanical allodynia on the side ipsilateral to injury for the group that received LT3114 (5B).

Figure 5A

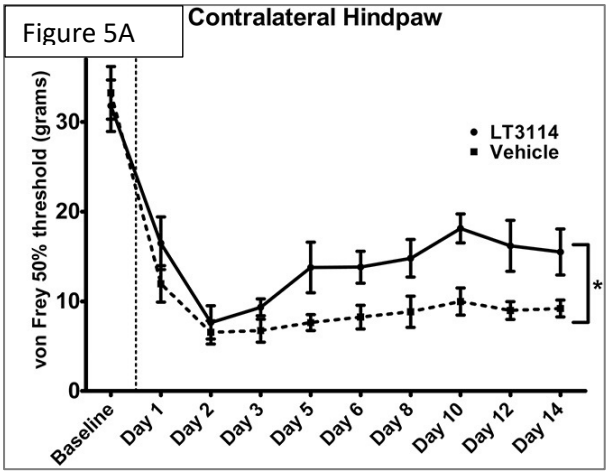
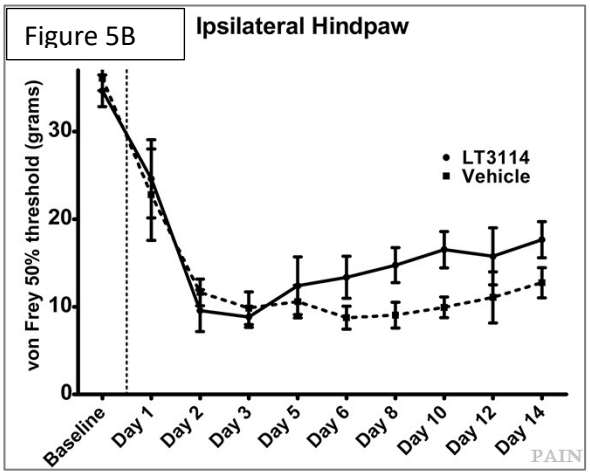


Figure 5B



4. **Training and Professional Development:**

A postdoctoral fellow was trained in the methods used in this study. They also gained knowledge, through mentoring with data analysis, presentation, and manuscript preparation.

5. **Dissemination of Results:**

Some work was presented as a poster at the Society for Neuroscience Annual Conference:

2015 Society for Neuroscience, San Diego, CA "Intranasally delivered Anti-Lysophosphatidic-Acid Antibodies enhances Chronic Pain Recovery after Traumatic Brain Injury in Rats"

In addition we published some results in the journal *Pain*:

Eisenried A, Meidahl AC, Klukinov M, Tzabazis AZ, Sabbadini RA, Yeomans DC. Nervous system delivery of anti-lysophosphatidic acid antibody by nasal application attenuates mechanical allodynia after traumatic brain injury in rats. *Pain*, 2017 Nov;158(11):2181-2188. (attached in appendix)

6. **Next reporting period**

Nothing to Report (final report)

IMPACT

What was the impact on development of the principal discipline(s) of the project?
The results of our work demonstrated that LPA levels are elevated in CSF following TBI – indicating the presence of the target molecule in the target tissue. In addition, these experiments demonstrated that IV application of an antibody can result in decreased pain sensitivity following TBI. Thus, these results give additional confidence that IV or intranasal application of anti-LPA antibody would be useful for the treatment of post-TBI pain.

What was the impact on other disciplines?
These results can impact other disciplines in that they show that Intranasal and intravenous application of an antibody can have therapeutic effects.

What was the impact on technology transfer?
Nothing to report at this time.

What was the impact on society beyond science and technology?
Nothing to report at this time.

CHANGES/PROBLEMS

Changes in approach and reasons for change
We have changed the order of the tasks secondary to problems that arose during the previous reporting period.

Actual or anticipated problems or delays and actions or plans to resolve them
We have run into a serious problem with the reliability of the rat TBI model. We believe that we have fixed this problem, but it is still possible that additional delays will occur because of this.

Changes that had a significant impact on expenditures
We have delayed initiating the next round of experiments while we determine the cause of the problem with reliability. This has caused a delay during which we have used both salary and animals while we try to figure out the issue.

Significant changes in the use of care of human subjects, vertebrate animals, biohazards, and/or select agents
None to report at this time.

PRODUCTS

Publications, conference papers, and presentations
We have presented some of this work at the Annual Society for Neuroscience Conference:

2015 Society for Neuroscience, San Diego, CA "Intranasally delivered Anti-Lysophosphatidic-Acid Antibodies enhances Chronic Pain Recovery after Traumatic Brain Injury in Rats"

We have also published our work in the leading pain journal, *Pain*:

Eisenried A, Meidahl ACN, Klukinov M, Tzabazis AZ, Sabbadini RA, Clark JD, Yeomans DC. Nervous system delivery of antilysophosphatidic acid antibody by nasal application attenuates mechanical allodynia after traumatic brain injury in rats. *Pain*. 2017 Nov;158(11):2181-21.

Website(s) and other Internet site(s)
None to report at this time.

Technologies or techniques
None to report at this time.

Inventions, patent applications, and/or licenses
None to report at this time.

Other Products
None to report at this time.

PARTICIPANTS AND OTHER COLLABORATING ORGANIZATIONS

Name	Roger Sabbadini, PhD
Project Role	Principal Investigator
Researcher Identifier	
Nearest person month worked	3.0
Contribution to Project	Provides overall direction to the project
Funding Support	LPath Inc.

Name	David C. Yeomans, PhD
Project Role	Co-Principal Investigator
Researcher Identifier	0000-0002-9389-8539
Nearest person month worked	3
Contribution to Project	Provides overall direction to the Stanford component of the research
Funding Support	NIH, DOD

Name	Michael Nemenov, PhD
Project Role	Collaborator
Researcher Identifier	
Nearest person month worked	2.4
Contribution to Project	Provided expertise around assessment of effects of TBI on thermal pain sensitivity
Funding Support	NIH

Name	Lena Weber, MD
Project Role	Postdoctoral Fellow
Researcher Identifier	
Nearest person month worked	12
Contribution to Project	Performed animal surgery and testing procedures
Funding Support	Stanford University

Name	Mikhail Klukinov, MD
Project Role	Senior Research Scientist
Researcher Identifier	
Nearest person month worked	7.08
Contribution to Project	Helped with behavioral testing of animals, ran biochemical analyses
Funding Support	Stanford University

No change in active support of key personnel
No other organizations were involved as partners

SPECIAL REPORTING REQUIREMENTS

An updated quad chart is included as Appendix 1

APPENDICES

Appendix 1: updated quad chart

Appendix 2: published journal article

Appendix 3: Approved transfer - amendment of solicitation/modification of contract

Anti-Lysophosphatidic Acid Antibodies in the Treatment of Post-TBI Neuropathic Pain

MR141271 Task Title: Task 6: Test effects of Intranasal application of LPA antibody in TBI rats

W81XWH-16-1-0098

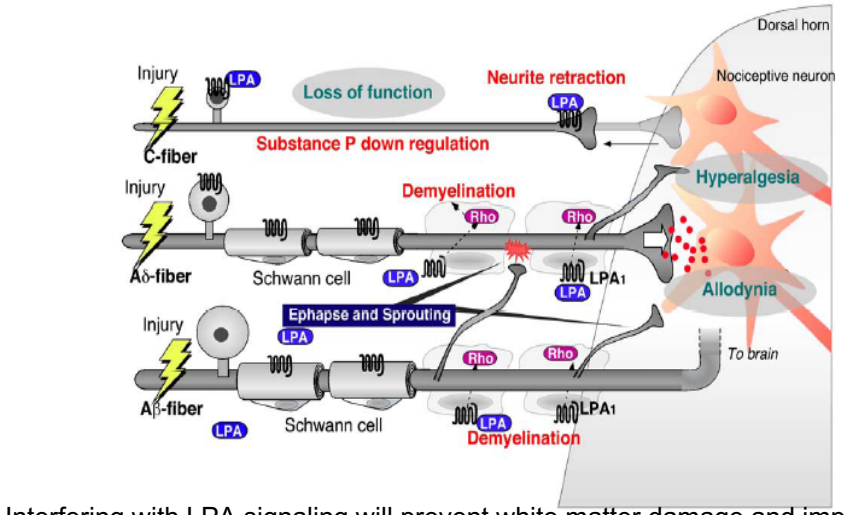
PI: David C. Yeomans **Org:** Stanford University **Award Amount: $1,446,655.00**

Study/Product Aims

- **Specific Aim 1: Investigate the efficacy of intravenous (I.V.) injection of humanized anti-LPA mAb (LT3114, Lpathomab-h) in the central fluid percussion (cFP) injury model of TBI in the rat by measuring several neuropathic pain behaviors following cFP TBI.**
- **Specific Aim 2: To determine the optimal mode of administration by comparing effects of intravenous (I.V.) administration, garnered from Specific Aim 1, to the effects of subcutaneous (S.C.) or intranasal (I.N.) dosing in preventing or ameliorating post-injury pain as well as cognitive deficits.**

Approach

Evaluate the efficacy of anti-LPA antibody for protection against post-TBI neuropathic pain: Rats will subjected to cFP injury followed by anti-LPA antibody treatment and we will correlate with mechanical allodynia and thermal hyperalgesia as well as other neurobehavioral measures



Interfering with LPA signaling will prevent white matter damage and improve pain responses. H. Ueda / Pharmacology & Therapeutics 109 (2006) 57–77

Timeline and Cost

Activities by CY	2016	2017	2018	2019
Investigate the efficacy of Lpathomab in cFP model of post-TBI neuropathic pain		■	■	■
Determine best route of administration (sc, iv, intranasal) and therapeutic time window		■	■	■
Submit FDA and IRB approval for TBI pain as an extension of Lpath's anticipated neuropathic pain IND				■
Estimated Total Budget ($K, direct + indirect)	212	428	513	294

Updated: 02/22/2020

CY16 Milestones accomplished

✔**Put animal protocols in place**

✔**Train personnel and conduct pilot in vivo studies for model development**

CY17 Goals

✔**Analyze of LPA in cerebral spinal fluid (CSF) after injury**

✔**Investigate I.N. Lpathomab Administration and Neurobehavioral assessment**

✔**Investigate I.V. Lpathomab Administration and Neurobehavioral assessment**

CY18 Goals

☐ **Investigate S. C. Lpathomab Administration and Neurobehavioral assessment**

CY19 Goals

✔**Optimize I. N Lpathomab Administration and Neurobehavioral assessment**

☐ **Determination of optimal administration method**

Budget Expenditure to Date

Actual Expenditure: $1,155,760.01

Research Paper

PAIN®

Nervous system delivery of antilysophosphatidic acid antibody by nasal application attenuates mechanical allodynia after traumatic brain injury in rats

Andreas Eisenried[a,b], Anders C.N. Meidahl[a], Michael Klukinov[a], Alexander Z. Tzabazis[a], Roger A. Sabbadini[a], J. David Clark[a], David C. Yeomans[a,*]

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Abstract

Lysophosphatidic acid (LPA) is a bioactive lipid that impacts neurological outcomes after neurotrauma by inhibiting neuroregeneration, promoting inflammation, and contributing to behavioral deficits. Blocking LPA signaling with a novel anti-LPA monoclonal antibody (mAb) is neuroprotective after traumatic brain injury (TBI) if given to injured animals whose blood–brain barrier (BBB) has been compromised. It is hypothesized that the anti-LPA mAb could improve chronic pain initiated by TBI. However, poor brain penetration after systemic application of the antibody makes access to the central nervous system (CNS) problematic in situations where the BBB is intact. Our experiments investigated whether intranasal delivery of the anti-LPA mAb could bypass the BBB, allowing for direct entry of the antibody to certain areas of the CNS. When the humanized anti-LPA mAb, LT3114, was intranasally applied to injured rats within 30 minutes after mild TBI using the central lateral percussion model, enzyme-linked immunospecific assay and immunohistochemistry demonstrated antibody uptake to several areas in the CNS, including the area of cortical injury, the corpus callosum, cerebellum, and the subventricular region. Compared with control rats that received LT3114 but no TBI, TBI rats demonstrated significantly higher concentrations of intranasally administered LT3114 antibody in some tissues. In behavioral studies, a significant attenuation of mechanical allodynia after TBI was observed in the anti-LPA treatment group ($P = 0.0079$), when compared with vehicle controls within 14 days after TBI. These results suggest that intranasal application of the anti-LPA antibody directly accesses CNS sites involved in TBI-related pain and that this access attenuates pain sequelae to the neurotrauma.

Keywords: Naso-cerebral, Trigeminal pain, Monoclonal antibody, Intranasal

1. Introduction

Chronic pain is a common problem after traumatic brain injury (TBI). Migraine-type or tension-type headaches can persist for months or even years after TBI and can lead to a greatly reduced quality of life. Patients with TBI frequently exhibit cephalic and even extracephalic mechanical allodynia.[21,29,34] Similarly, we and others have demonstrated long-lasting periorbital and extracephalic mechanical allodynia in rodent TBI models.[7,25]

Lysophosphatidic acid (LPA) is an extracellular bioactive lipid that is known to act on all central nervous system (CNS) cell types through specific LPA receptors (LPA_{1-6}), resulting in the activation of classic G signaling pathways. Importantly, LPA appears to be a key mediator of acute and chronic pain after injury.[41,42,45] For example, thermal hyperalgesia and mechanical allodynia following partial sciatic nerve injury could be mitigated by the LPA_1 receptor antagonist, Ki-16,425, in $LPA1^{(-/-)}$ mice, or in mice treated with antisense to LPA_1, and in mice deficient in the gene for autotaxin (atx ±), the enzyme that synthesizes LPA.[17,18,23]

In addition to LPA_1, LPA_5 is also hypothesized to play a role in the more chronic aspects of neuropathic pain, as LPA_5 colocalizes with transient receptor potential cation channel subfamily V member 1 (TRPV1)-positive dorsal root ganglia neurons, which have been shown to be critical in the pathogenesis of neuropathic pain.[30] Similarly, LPA_3 may also be involved in the initial pain responses, as both LPA_1 and LPA_3 knockout mice exhibit almost complete elimination of paclitaxel-induced neuropathic pain responses.[41]

Lysophosphatidic acid appears to play a critical role in response to neurotrauma.[10] Thus, expression of LPA receptors is upregulated in the CNS following TBI in both mice and humans.[11,15] Similarly, we have demonstrated significantly elevated LPA levels in cerebrospinal fluid (CSF) samples of patients with TBI and mice compared with healthy controls,[4] and blocking the extracellular LPA signaling with a single dose of an intravenously administered murine anti-LPA antibody (mAb) reduced lesion size and improved behavioral deficits[4] in both mouse models of both TBI and spinal cord injury (SCI).[14]

Usually, the blood–brain barrier (BBB) prevents the access of large molecules to the CNS. This barrier limits the ability of

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

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therapeutic antibodies to gain access to potential targets in the brain unless the BBB integrity is disrupted by injury such as a consequence of TBI. Recently, several studies have shown that the BBB can be circumvented therapeutically as intranasally delivered antibodies or antibody fragments can reach higher brain concentrations than intravenously administered antibodies.[13,35,43] Penetration of large molecules might even be enhanced since the BBB itself has been shown to be temporarily compromised after TBI.[1] Consequently, intranasal administration of an antibody shortly after a TBI could penetrate the brain both by the nasal–cerebral route but also because of injury-induced disruption of the BBB.

This study investigated (1) intranasally administered anti-LPA mAb concentrates in the nervous system, CSF, and serum of rats with and without TBI, (2) the distribution pattern of anti-LPA mAb within the CNS, and (3) the effect of nasal application of anti-LPA mAB on post-TBI pain-associated behavior.

2. Materials and methods

2.1. Animals

Four- to 6-week-old male Sprague-Dawley (320 ± 25 g; Harlan Laboratories, Livermore, CA) rats were housed in groups of 2 in a controlled environment (temperature: 21.5 ± 4.5°C, relative humidity: 35%-55%, 14/10-hour light/dark cycle). Rats were allowed to habituate for at least 1 week before the study and had free access to a standard laboratory diet and tap water. They were randomly and in a blinded manner assigned to anti-LPA mAb or vehicle group. All procedures were approved by the Stanford University Institutional Animal Care and Use Committee.

2.2. Lateral fluid percussion

To induce mild TBI in rats, we used a modification of the lateral fluid percussion (FP) model described by McIntosh et al.[26] Rats were deeply anesthetized in a chamber using isoflurane (3% for induction, 2.6% for maintaining, 30% O_2) and then securely fixated in a stereotactic frame. Anesthesia was maintained with a facemask, and a feedback-regulated warming pad was positioned under the rat during surgery to maintain body temperature at 37.5°C. After intraperitoneal application of 25-mg ceftazidime and hair removal with electric hair clippers, a midline incision was made in the scalp and the underlying periosteum removed. Bleeding was controlled by thermocoagulation (Bovie Medical Corp, FL) and cutaneous injection of 50-μg epinephrine. A 5-mm craniotomy was made in the rat skull in the middle between the bregma and the lambda suture and 4 mm right to the sagittal suture using a mini-drill with a trephination bit (Dremel, Racine, WI). Dura was left intact, and the bone flap was preserved for later replacement. After this, a female luer needle hub was attached to the craniotomy opening and temporarily fixed with cyanoacrylate glue (Loctite, Henkel Corporation, Westlake, OH). Dental cement (Henry Schein, Melville, NY) was applied to the skull to further secure the needle hub. After drying of cement, the luer attachment was connected to a custom-built FP device (**Fig. 1**). The device consists of a piston-driven steel cylinder filled with hydraulic fluid. A pressure pulse is created by a pendulum arm striking the piston from a predetermined angle. The pressure pulse is then transferred through a nitrile membrane to a second circuit filled with sterile saline. The pressure wave pushes the saline into the epidural space, quickly displacing dura mater and causing damage of the underlying brain. The second circuit is fitted with a pressure transducer connected to the computer by a CED-1401 analogue to digital converter (Cambridge Electronic Design, Cambridge, United Kingdom). The pressure pulse was recorded using Spike 2 software (Cambridge Electronic Design). For the present study, the FP device was adjusted to consistently deliver a 1.6 atm pressure pulse over 20 ms. After applying the pressure wave, the needle hub and cement were removed, the bone flap was replaced, the wound was closed with clips (Stoelting, Wood Dale, IL), and the rats were allowed to recover in their prewarmed home cage.

2.3. Neurological severity score

Neurological status after TBI was assessed using the neurological severity score described by Shapira et al.[36] Neurological severity score is a 12-level test that assesses, eg, the righting reflex, hemiplegia, and loss of pinna reflex and has been shown to correlate closely with the applied pressure in the lateral FP model.[20]

2.4. Anti–lysophosphatidic acid monoclonal antibody

For this study, we used a preclinical humanized and affinity-matured anti-LPA mAb with a human IgGκ1 framework (LT3114) generously provided by Lpath Incorporated, San Diego, CA. The stock concentration was 105.2 mg/mL. It has been shown that adding specific amino acids including glycine, histidine, or proline enhance CNS uptake of intranasally delivered antibodies.[9] Accordingly, 200-mM glycine buffer was added to a final concentration of the LT3114 antibodies of 95 mg/mL LT3114

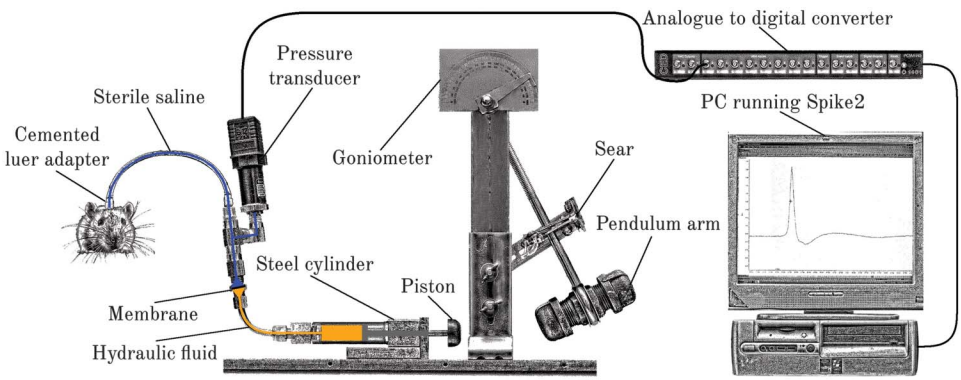

Figure 1. Schema of the lateral fluid percussion device. Experimental setup for the lateral fluid percussion model.

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phosphate-buffered saline (PBS) buffer, resulting in a 190 mM glycine concentration.

2.5. Intranasal application

Thirty minutes after induction of TBI, anesthesia was induced by isoflurane (3% for induction, 2% for maintaining, 30% O_2) in a chamber. Rats were then placed in a supine position on a thermo-regulated surface that maintained body temperature at 37.5°C. Polyethylene tubing (PE-50, 22G) was inserted 0.8 cm into the left nostril (**Fig. 2**). At this point, anesthesia was delivered using facemask. Thereafter, 30 μL of anti-LPA mAb (2.85 mg) or vehicle (200-mM PBS/glycine buffer) was administered over 9.6 minutes (3.125 μL/min) using a syringe pump (GeniePlus; Kent Scientific, Torrington, CT). Preliminary studies showed that this infusion rate minimized respiratory complications while allowing for expeditious delivery of intranasally administered compounds. Rats remained in the same position for additional 5 minutes to allow for uptake of compounds.

2.6. Tissue processing and immunohistochemistry

Animals were euthanized 4 hours after intranasal LT3114 administration and transcardially perfused using 1x PBS followed by 4% paraformaldehyde. Brains, including olfactory bulbs and trigeminal ganglia, were harvested and transferred to 4% paraformaldehyde overnight. For cryoprotection, tissue samples were transferred to 20% sucrose in PBS for 72 hours. Using dry ice cooled isopentane, the samples were flash-frozen and sectioned (15 μm) using a cryostat (Leica, Wetzlar, Germany).

Immunohistochemical tissue staining was performed with a biotinylated goat anti-human kappa polyclonal antibody (Southern Biotech, Birmingham, AL, #2060-08), an avidin–biotin staining kit (Vector Laboratories, Burlingame, CA, #PK-6100) and Vector NovaRED (Vector, #SK-480) as substrate according to the manufacturers' instructions. Sections were counterstained with Mayer hematoxylin (EMS, Hatfield, PA, #26043-06) and dehydrated with ethanol and xylene. Slides were cover slipped with mounting medium (Sigma-Aldrich, St. Louis, MO, #44581), and images were captured on a Leica DMRXA series microscope.

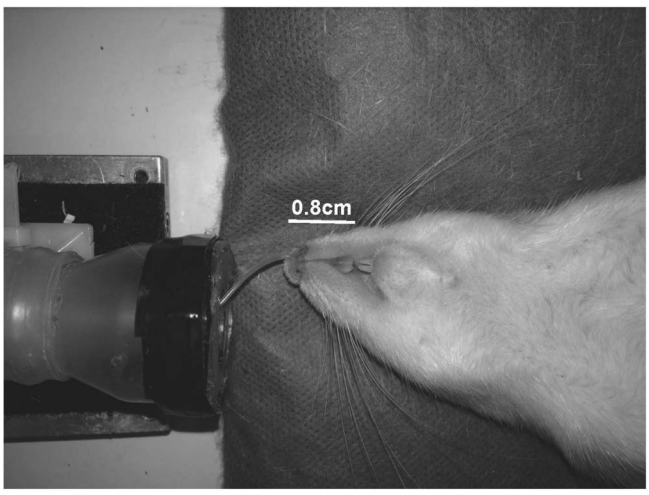

Figure 2. Intranasal delivery of LT3114: anesthetized rat in supine position with a polyethylene tubing inserted 0.8 cm into the left nostril. For demonstration purposes, the facemask delivering anesthetics and oxygen was temporarily removed.

2.7. Quantification of anti–lysophosphatidic acid mAb in brain tissue, cerebrospinal fluid, and serum

Animals with and without TBI (N = 3, per group) were sedated for cisterna magna CSF collection and blood sampling from the tail vein.[28] Before intranasal administration of the antibody, baseline samples were taken. Thereafter, CSF and blood samples were collected every hour for 4 hours. Blood was collected in a covered test tube (Becton Dickinson, Franklin Lakes, NJ) and incubated at 30 minutes at room temperature to allow clot formation. Serum was removed by centrifuging at 1000*g* for 2 minutes and transferred to a 1.5 mL Eppendorf tube.

For neural tissue assessment, animals (N = 2, per group) were euthanized 4 hours after intranasal antibody administration, and brain as well as trigeminal ganglia were harvested and snap frozen. Tissues were dissected, weighted, and homogenized with a Dounce tissue grinder using a neuronal protein extraction reagent (N-PER; Thermo Fisher Scientific, Waltham, MA). To quantify LT3114 tissue, CSF, and serum concentrations, an enzyme-linked immunospecific assay (ELISA) kit (Abnova, Walnut Creek, CA, #KA3817) was used. Samples were diluted according to the ELISA kit manufacturer's protocol or according to the expected concentrations. LT3114 levels were determined using a polynomial standard curve (0.625-40 ng/mL).

2.8. Von Frey mechanical allodynia testing

Before the first testing session, animals (N = 6 per group) were habituated for at least 2 hours on a metal mesh (0.5 × 0.5 cm) inside a plastic chamber. One day before TBI, rats were tested for mechanical sensitivity using von Frey filaments (Bioseb, Chaville, France) applied to the plantar surfaces of the hind paws. Fifty percent mechanical withdrawal thresholds to the application of a von Frey probe to the foot was calculated by using the up–down method of Chaplan et al.[3] To assess mechanical allodynia, an ascending series of von Frey hairs of logarithmically incremental force (0.32-16.31 g) was applied to the midregion of the plantar surface of the hind paw. Each von Frey hair was applied to the test area for 2 to 3 seconds, with a 1- to 2-minute interval between stimuli. If the rats showed no withdrawal to the highest von Frey hair (16.31 g), a von Frey threshold of 25.51 g was assigned, corresponding to the next log increment in potential von Frey probes. Testing was performed on post-TBI day 1, 2, 3, 5, 6, 8, 10, 12, and 14.

2.9. Statistical analysis

Significance was set to $P < 0.05$ for all statistical tests, and *P* values were adjusted for multiple comparisons using Bonferroni correction. Data are reported as the mean and SEM. A 2-tailed Student *t* test for unpaired samples was used for comparison between ELISA data. For time series analysis and between group distinctions, a 2-way repeated measures analysis of variance with post hoc Bonferroni testing was used. All statistics were done with GraphPad Prism 5 (GraphPad Software, Inc, La Jolla, CA).

3. Results

3.1. Traumatic brain injury and severity of neurological injury

After mild TBI, all rats demonstrated no or only mild signs of neurological impairment. Thus, no rats had to be excluded because of preset parameters (elevated neurological severity score [>0]) after 1 week or with delayed emergence (>4 minutes after TBI), which would have been indicative of higher grade TBI.

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3.2. Intranasal delivered antibody uptake

The pharmacokinetics of intranasally administered anti-LPA mAb was investigated to determine a reasonable uptake in the nervous system and to detect differences in this uptake between rats with mild TBI and rats without TBI.

3.2.1. Blood serum concentration of LT3114 immunoreactivity

Figure 3A shows that the highest concentration of the humanized version of the anti-LPA mAb (LT3114) in the serum was reached at the end of the observed time window. The LT3114 concentration was at no time point significantly different between rats that received TBI and rats without TBI.

3.2.2. Cerebrospinal fluid concentration of LT3114 immunoreactivity

Antibody concentration in CSF was significantly higher in rats with TBI than in animals without injury (**Fig. 3B**, $P = 0.0417$). The peak concentration levels in both groups were reached 3 hours after LT3114 application. Consistent with the highly vascular nature of the nasal mucosa and the stability of the molecule in blood, the maximum antibody concentration levels in serum were more than 50% higher than those measured in CSF.

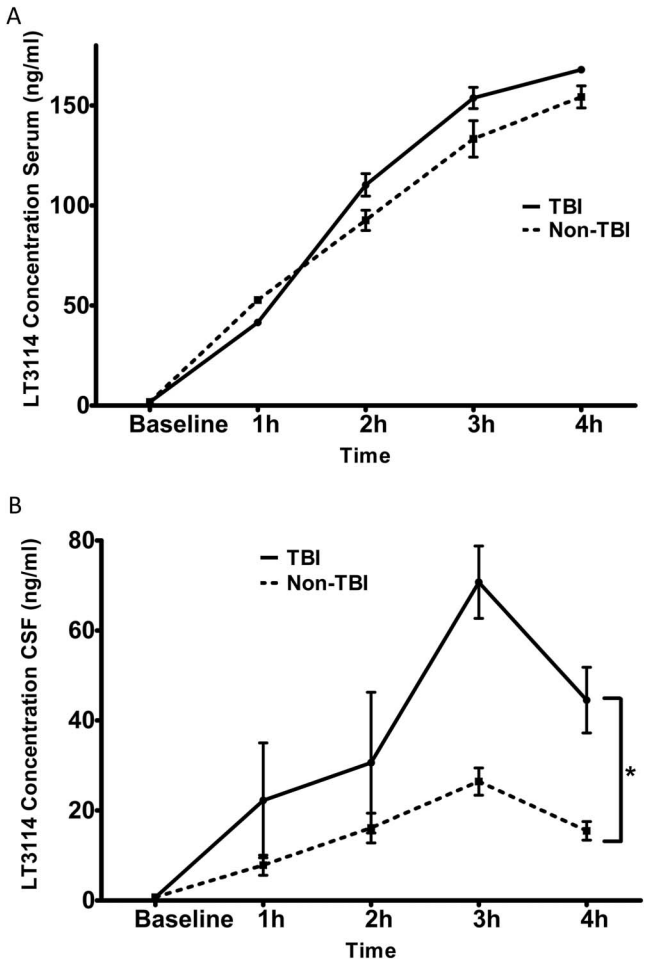

Figure 3. LT3114 concentrations in serum (A) and CSF (B) after intranasal administration in TBI and non-TBI rats. LT3114 levels (average ± SEM) at different time points (0, 1, 2, 3, and 4 hours) after administration and comparison between TBI and non-TBI rats (n = 3 per group). (A) Serum: 2-way repeated measures ANOVA with no significant difference between groups ($P = 0.0597$). (B) CSF: 2-way repeated measures ANOVA with significant difference between groups ($P = 0.0417$). ANOVA, analysis of variance; CSF, cerebrospinal fluid; TBI, traumatic brain injury.

3.2.3. Brain regions concentrations of LT3114 immunoreactivity

Following intranasal administration of anti-LPA mAb, significant tissue levels were seen in all investigated brain regions (olfactory bulbs, rostral brain region, TBI region, trigeminal ganglia, and cerebellum) (**Fig. 4**). The highest antibody levels were found in the trigeminal ganglia and olfactory bulbs and were up to 10-fold higher than in the other investigated tissues (**Fig. 4**). LT3114 levels were significantly higher in TBI rats than in non-TBI rats in the olfactory bulbs ($P = 0.0197$); there was no significant difference in LT3114 levels in the trigeminal ganglia ($P = 0.0804$) and the cerebellum ($P = 0.2957$) between TBI and non-TBI rats. The rostral brain region (bregma 1.3-5.6 mm) and the TBI region (bregma −4.2 to 1.2 mm) also showed significant higher concentrations in rats with TBI ($P = 0.0133$, $P = 0.0478$).

3.3. LT3114 distribution pattern after traumatic brain injury

The distribution of LT3114 immunoreactivity at 4 hours after intranasally administration was examined qualitatively. **Figure 5** demonstrates intense staining in the periventricular and dorsal subventricular zone, particularly on the side ipsilateral to TBI and in the damaged cortex area. In addition, the corpus callosum showed intense LT3114 immunoreactivity; however, no immunoreactivity was observed in the hippocampus area. No LT3114 immunoreactivity was observed in the brain after nasal application in control (non-TBI) rats (data not shown).

3.4. Mechanical allodynia

Considering that there is substantial distribution of anti-LPA mAb in the brain after intranasal (i.n.) administration, we examined whether or not a single i.n. dose of mAb would have any therapeutic benefit. Accordingly, rats were subjected to FP injury (see Methods). Thirty minutes after injury, animals were then given a single dose (30 μL, 2.85 mg) of LT3114 humanized anti-LPA antibody to the left nostril. **Figure 6** shows the 14-day time course of the withdrawal response to von Frey filaments after mild TBI in rats that received intranasal LT3114 compared with control rats. For the paw contralateral to the injury, mechanical allodynia was significantly less in the group that received LT3114 compared with the vehicle group ($P = 0.0079$), suggesting an analgesic effect attributed to a single i.n. dose of anti-LPA mAb. This effect was observed on the ipsilateral side; there was an insignificant trend ($P = 0.1762$) towards reduced mechanical allodynia in the group that received LT3114.

4. Discussion

Along with other post-TBI morbidities, chronic pain conditions remain a major health problem, and no Food and Drug Administration–approved specific drugs are available for the treatment of this pain.[27] Our experiments investigated for the first time that mechanical allodynia after mild TBI in rats can be ameliorated by intranasal administration of an antibody specifically directed against the extracellular bioactive lipid mediator, LPA. Our results indicate that the intranasally delivered antibody reaches measurable levels in the CNS,

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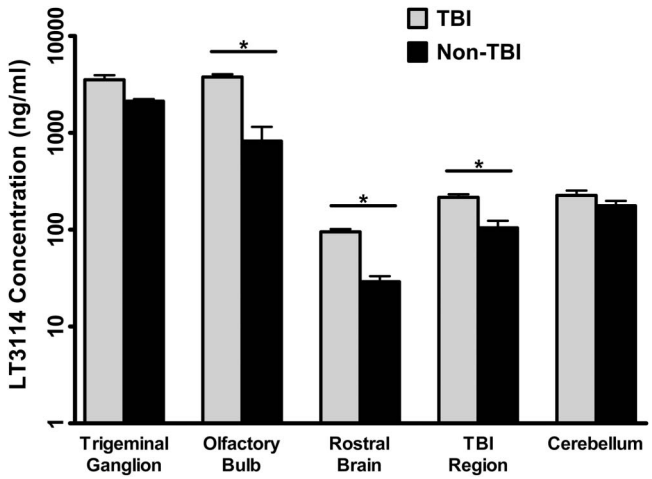

Figure 4. LT3114 concentrations in different CNS tissues and the trigeminal ganglia (average ± SEM) 4 hours after intranasal administration in TBI and non-TBI rats (n = 2 per group). Significant higher antibody concentrations in TBI rats were found in olfactory bulb, rostral brain, and TBI region (2-tailed Student *t* test for unpaired samples, $P = 0.0197$, $P = 0.0133$, $P = 0.0478$, $^*P < 0.05$). CNS, central nervous system; TBI, traumatic brain injury.

and that, following mild TBI, antibody levels seem to be higher in specific neural regions, including the cortical area underlying the TBI injury, as well as the trigeminal system which is critical to craniofacial pain and the periventricular and dorsal subventricular zone, areas that have been demonstrated to be critical to neurogenesis.[2] As mentioned above, mild to moderate TBI usually enhances pain in the contralateral hind paw more severely than the ipsilateral paw; intranasal treatment with the anti-LPA antibody reduced the mechanical allodynia especially in this paw.

4.1. Blocking lysophosphatidic acid signaling

Lysophosphatidic acid plays an important role in the recovery process after TBI and the generation of neuropathic pain conditions. Although LPA receptors show a low expression in normal brain tissue, TBI induces an upregulation of $LPA_{1\text{-}3}$ receptors in the human and rodent brain.[11,15] LPA_1 was found to be expressed by reactive astrocytes and LPA_2 by ependymal cells lining the lateral ventricles in humans.[11] Furthermore, it was observed that both LPA-producing enzymes, autotaxin and phospholipase A_2, are upregulated following brain injury in rats.[32,37] Consistent with this finding was the observation that a substantial increase in LPA itself was observed in the CSF of patients after experiencing a TBI.[4] This "LPA pulse" was also seen in the CSF of mice subjected to the controlled cortical impact model of TBI. Moreover, using the controlled cortical impact model, we also demonstrated neuroprotective effects by blocking the LPA signaling with intravenously administration of a murine anti-LPA mAb when given as a single dose 30 minutes after the TBI.[4] In this study, antibody-treated animals exhibited a reduction in lesion volumes as determined histologically and by magnetic resonance imaging. Importantly, we observed improvements in functional outcome as assessed by the DigiGait system when compared with animals treated with control mAb. Similar neuroprotective effects were seen with the anti-LPA mAb in a mouse SCI model, suggesting that LPA may be an early mediator of neurotrauma.[14,32] Lysophosphatidic acid may negatively impact the longer-term sequelae of events post-TBI is suggested by the finding that LPA is also a proinflammatory mediator in the CNS

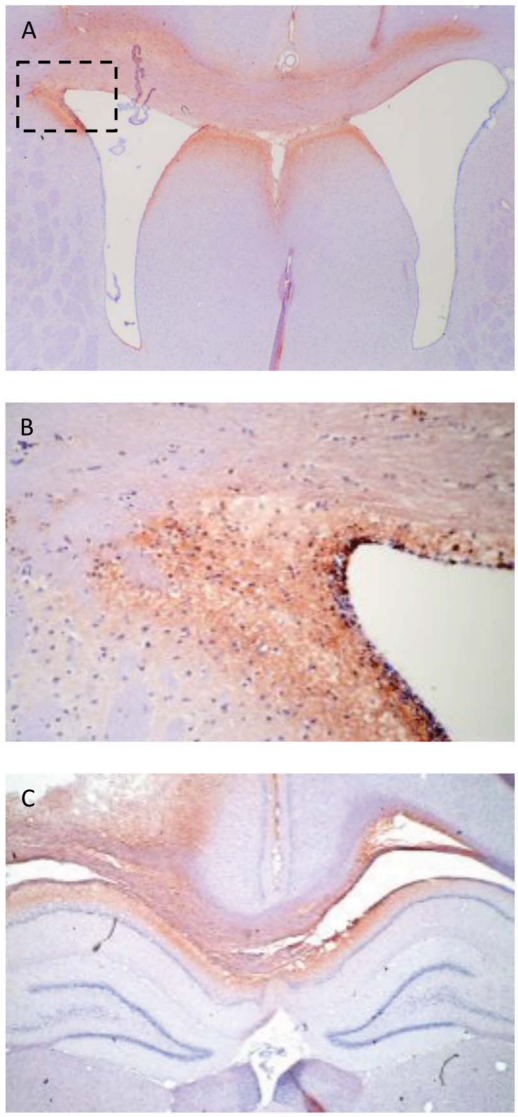

Figure 5. Immunohistochemical staining of the LT3114 (red) distribution in the periventricular CNS region after TBI and intranasal administration. (A and B) Ventricular region with intense staining in the ipsilateral ventral, dorsal subventricular zone and corpus callosum. (C) Immunoreactivity in the ipsilateral TBI region and periventricular zone, but no staining of the hippocampus formation. CNS, central nervous system; TBI, traumatic brain injury.

and a potent inhibitor of human and neuronal stem cell development and differentiation.[10] We have previously demonstrated that the anti-LPA mAb can reverse these.[4,14] In addition to the reduction of inflammatory processes, anti-LPA may have neural regenerative properties. Lesion volume after TBI appears to correlate with the severity of chronic pain states following TBI; anti-LPA administration may reduce post-TBI lesion volume by protecting or enhancing neuronal stem cell development and differentiation (eg, in the parietal cortex)[4] and may thereby reduce chronic pain, possibly by enhancing recovery of sensory fibers in the internal capsule.

Neuropathic pain is an important behavioral consequence of central neurotrauma such as TBI and SCI. The literature suggests that LPA is causally involved in the induction of neuropathic pain induced by peripheral nerve injury and chemotherapeutic agents such as paclitaxel. LPA_1 receptor signaling may lead to demyelination after neurotrauma induced by partial nerve ligation,

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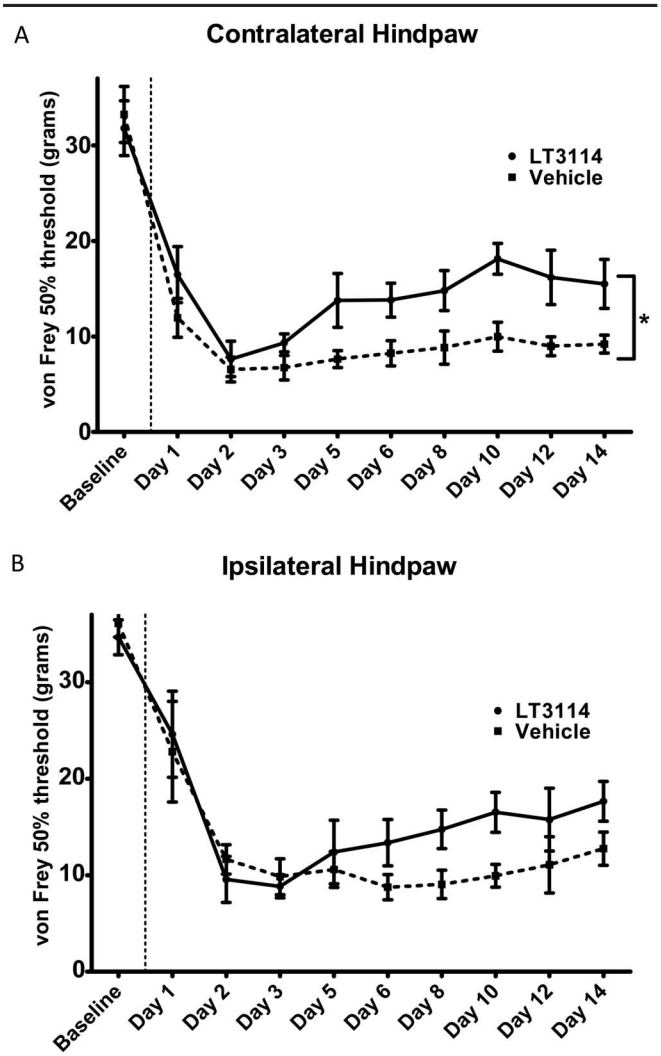

Figure 6. LT3114 effect on the attenuation of mechanical allodynia in hind paws after TBI. (A) Contralateral and (B) ipsilateral von Frey testing at different time points after TBI and LT3114 administration (baseline, day 1, 2, 3, 5, 6, 8, 10, 12, and 14, n = 6 per group). Two-way repeated measures ANOVA between groups: (A) $P = 0.0079$, (B) $P = 0.1762$ (*$P < 0.05$). ANOVA, analysis of variance; TBI, traumatic brain injury.

which likely contributes to observed thermal analgesia and mechanical allodynia.[23] Intrathecal injection of LPA can recapitulate these pain responses—effects that are attenuated or absent in $LPA_1^{(-/-)}$ mice or in mice treated with LPA_1 receptor antagonist, Ki-16,425.[18,23] Central neuropathic pain after cerebral ischemia was also shown to be significantly mitigated in $LPA_1^{(-/-)}$ mice.[16]

Mechanical allodynia may also be induced by central neuroinflammation (reviewed by Ellis and Bennett), which is commonly observed after TBI.[6] Even mild TBI can produce central inflammation and an increase of inflammatory cytokines such as IL-6.[38,44] Blocking LPA signaling with intravenously administered anti-LPA mAb 30 minutes after TBI significantly reduced IL-6 CNS concentrations.[4]

Consistent with these findings, Fujita et al.[12] demonstrated that rat microglia can be activated in vitro by LPA_1 and LPA_3 receptors. Similarly, blocking the LPA signaling with anti-LPA mAb in mice with traumatic spinal injury reduces microglial cell activation close to the lesion.[12] These finding also support the idea that LPA may be an important mediator in the induction of central neuropathic pain, where microglial activation appears to play a prominent role.[46]

4.2. Intranasal application of monoclonal antibodies

Several studies have used intranasal drug application as a promising route for direct delivery of large molecules to the CNS. Substances including nerve growth factor, insulin, insulin-like growth factor 1 and interferon beta were successfully delivered to nervous system by this approach, reaching effective tissue concentrations that can have physiological effects.[8,24,31,40] Xiao et al.[43] showed that therapeutic immunoglobulins (anti-amyloid-β oligomer antibody, 150 kDa) could be detected in the CNS, reducing amyloid plaques and improving cognitive function in mice after intranasal application. A pharmacokinetic study with an intranasally applied tumor necrosis factor-alpha-inhibitory scFv antibody fragment (26.3 kDa) showed a much higher uptake into the brain compared with intravenous application.[13] Our investigations confirm the utility of this approach, as we could also show antibody concentrations in the investigated brain regions after intranasal application and a concomitant attenuation of mechanical allodynia. The main reason for this higher concentration might be the bypass of the BBB and the direct access to the brain through the olfactory or trigeminal route because tight junctions in the BBB can effectively block most molecules greater than 600 Da.[5,35] Although we did not measure concentrations of LT3114 in the CSF or serum after intravenous application, we believe that we would have obtained similar results as Furrer et al.[13] Two distinct neural pathways have been described which provide potential substrates for nasal delivery to neural structures: (1) the olfactory route provides a possible connection between the nasal cavity, the olfactory epithelium, and the subventricular region.[22] The Rostral Migratory Stream is well described in rodents and can explain the high concentrations of intranasally delivered compounds in the olfactory bulbs.[35] (2) The trigeminal route provides a link between the trigeminal nerve endings which innervate the nasal mucosa, the trigeminal ganglia, the brain stem, and the hindbrain including cerebellum and can explain the high concentrations of intranasally delivered compounds in the trigeminal ganglia and subcortical regions.[19]

A third explanation for the relatively high concentrations of the intranasally delivered antibody could be a temporary compromise of the BBB and the cribriform plate: although the BBB seems to effectively block large molecules in healthy rats, the BBB was shown to be compromised after allowing for penetration of larger molecules into the CNS over a period that ranges from 3 kDa to at least 44 kDa.[1,33,39] In experimental animal models of TBI, the duration and the onset of the BBB breakdown depends on the TBI method and varies between 1 and 6 hours for an early peak and Başkaya et al.[1] mention a delayed peak 2 or 3 days after TBI. We also observed levels in the plasma that were significantly higher than this seen in the CSF. In our study, maximum serum levels after intranasal application were measured about 4.5 hours after TBI, ie, 4 hours after intranasal administration of the antibody. Thus, it is possible that serum LT3114 crossed into the CNS when BBB compromise reached a maximum within the 1 to 6 hours window as suggested by other groups. This can be one of the reasons why we could find significantly higher antibody concentrations in several regions of the brain after TBI. However, in addition to the potential for direct access to neural sites, intranasal application also allows minimal systemic distribution, limiting systemic side effects. Other advantages are simplicity of application, noninvasiveness, and potentially higher tissue concentrations in the brain. Thus, the benefit of intranasal drug

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administration compared with intravenous administration needs to be further examined with a direct comparison study.

The humanized anti-LPA mAb used in this study may be an excellent drug candidate for the treatment of central neuropathic pain associated with neurotrauma to the CNS, particularly if intranasal application of the antibody can enhance exposure of the agent to the LPA target in the brain. A single-dose escalation safety study for intravenously applied Lpathomab-3114 (clinicaltrials.gov identifier NCT02341508) has recently been completed showing that the antibody was well tolerated at all doses tested, with no serious adverse events or dose-limiting toxicities observed. The work presented in this manuscript suggests that brain delivery of anti-LPA mAb through nasal application of may represent a novel, noninvasive therapeutic approach to long-term therapy for pain associated with TBI. Should the aforementioned clinical trial prove successful, the results of the current study suggest that additional trials examining nasal application of Lpathomab may be justified.

Conflict of interest statement

Dr Sabbadini is the founder of LPath, Inc. The remaining authors have no conflicts of interest to declare.

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AMENDMENT OF SOLICITATION/MODIFICATION OF CONTRACT

1. CONTRACT ID CODE	PAGE OF PAGES
S	1 | 16

2. AMENDMENT/MODIFICATION NO.	3. EFFECTIVE DATE	4. REQUISITION/PURCHASE REQ. NO.	5. PROJECT NO.(If applicable)
P00001	08-Mar-2018	0010768265-0001	

6. ISSUED BY	CODE	7. ADMINISTERED BY (If other than item 6)	CODE
USA MED RESEARCH ACQ ACTIVITY 820 CHANDLER ST FORT DETRICK MD 21702-5014	W81XWH	See Item 6	

8. NAME AND ADDRESS OF CONTRACTOR (No., Street, County, State and Zip Code)		
LELAND STANFORD JUNIOR UNIVERSITY, THE STANFORD UNIVERSITY 450 SERRA MALL STANFORD CA 94305-2004		9A. AMENDMENT OF SOLICITATION NO.
		9B. DATED (SEE ITEM 11)
	X	10A. MOD. OF CONTRACT/ORDER NO. W81XWH-16-1-0098
	X	10B. DATED (SEE ITEM 13) 15-Apr-2016
CODE 1KN27	FACILITY CODE	

11. THIS ITEM ONLY APPLIES TO AMENDMENTS OF SOLICITATIONS

☐ The above numbered solicitation is amended as set forth in Item 14. The hour and date specified for receipt of Offer ☐ is extended, ☐ is not extended.

Offer must acknowledge receipt of this amendment prior to the hour and date specified in the solicitation or as amended by one of the following methods: (a) By completing Items 8 and 15, and returning _____ copies of the amendment; (b) By acknowledging receipt of this amendment on each copy of the offer submitted; or (c) By separate letter or telegram which includes a reference to the solicitation and amendment numbers. FAILURE OF YOUR ACKNOWLEDGMENT TO BE RECEIVED AT THE PLACE DESIGNATED FOR THE RECEIPT OF OFFERS PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN REJECTION OF YOUR OFFER. If by virtue of this amendment you desire to change an offer already submitted, such change may be made by telegram or letter, provided each telegram or letter makes reference to the solicitation and this amendment, and is received prior to the opening hour and date specified.

12. ACCOUNTING AND APPROPRIATION DATA (If required)

13. THIS ITEM APPLIES ONLY TO MODIFICATIONS OF CONTRACTS/ORDERS. IT MODIFIES THE CONTRACT/ORDER NO. AS DESCRIBED IN ITEM 14.

☐ A. THIS CHANGE ORDER IS ISSUED PURSUANT TO: (Specify authority) THE CHANGES SET FORTH IN ITEM 14 ARE MADE IN THE CONTRACT ORDER NO. IN ITEM 10A.

☐ B. THE ABOVE NUMBERED CONTRACT/ORDER IS MODIFIED TO REFLECT THE ADMINISTRATIVE CHANGES (such as changes in paying office, appropriation date, etc.) SET FORTH IN ITEM 14, PURSUANT TO THE AUTHORITY OF FAR 43.103(B).

☐ C. THIS SUPPLEMENTAL AGREEMENT IS ENTERED INTO PURSUANT TO AUTHORITY OF:

X D. OTHER (Specify type of modification and authority)
Award Transfer, USAMRAA Terms and Conditions

E. IMPORTANT: Contractor ☒ is not, ☐ is required to sign this document and return _____ copies to the issuing office.

14. DESCRIPTION OF AMENDMENT/MODIFICATION (Organized by UCF section headings, including solicitation/contract subject matter where feasible.)
Modification Control Number: cmeinber181789
Effective this date; this award is hereby transferred:

FROM Lpath, Inc.
4025 Sorrento Valley BLVD
San Diego, CA 92121-1404

TO: The Leland Stanford Junior University
3172 Porter Dr.
Palo Alto, CA 94304-1212

Except as provided herein, all terms and conditions of the document referenced in Item 9A or 10A, as heretofore changed, remains unchanged and in full force and effect.

15A. NAME AND TITLE OF SIGNER (Type or print)		16A. NAME AND TITLE OF CONTRACTING OFFICER (Type or print) JASON KUHNS / CONTRACTING OFFICER TEL: 301-619-1861 EMAIL: jason.d.kuhns.civ@mail.mil	
15B. CONTRACTOR/OFFEROR _____ (Signature of person authorized to sign)	15C. DATE SIGNED	16B. UNITED STATES OF AMERICA BY _____ (Signature of Contracting Officer)	16C. DATE SIGNED 07-Mar-2018

SECTION SF 30 BLOCK 14 CONTINUATION PAGE

SUMMARY OF CHANGES

SECTION 00010 - SOLICITATION CONTRACT FORM

DELIVERIES AND PERFORMANCE

The following Delivery Schedule item for CLIN 0001 has been changed from:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	DODAAC / CAGE
POP 15-APR-2016 TO 14-APR-2018	N/A	W03J USA MED RESEARCH MAT CMD W03J USA MED RESEARCH MAT CMD 1077 PATCHEL STREET FORT DETRICK MD 21702-5024 301-619-7416 FOB: Destination	W91ZSQ

To:

DELIVERY DATE	QUANTITY	SHIP TO ADDRESS	DODAAC / CAGE
POP 15-APR-2016 TO 31-OCT-2019	N/A	W03J USA MED RESEARCH MAT CMD W03J USA MED RESEARCH MAT CMD 1077 PATCHEL STREET FORT DETRICK MD 21702-5024 301-619-7416 FOB: Destination	W91ZSQ

The following have been added by full text:

AWARD TRANSFER DETAILS

Award Transfer Details

PI: David Yeomans

Project Title: Antilysophosphatidic Acid Antibodies in the Treatment of Post-TBI Neuropathic Pain

1. Lpath, Inc. submitted a relinquishment statement dated 10 October 2016, incorporated herein by reference, relinquishing this award as of 10 November 2016. Lpath, Inc. hereby relinquishes all future claims under this award.

2. There were no refund checks submitted by Lpath, Inc.

3. The total amount of this award for the full period of performance is $1,446,655
Total amount awarded to Lpath, Inc.: $269,779.26
Total amount awarded to Stanford University: $1,176,875.74

4. The entire period of performance for this award is: 15 April 2016 – 31 October 2019

15 April 2016 – 07 March 2018: Lpath, Inc.
08 March 2018 – 31 October 2019: Stanford University
This award incorporates an approximate 19-month extension through 31 October 2019 with no additional funds.

5. The Final Technical Report to be submitted by Stanford University shall encompass the entire period of performance 15 April 2016 – 31 October 2019.

6. Payment Terms: Cost Reimbursement, Net 30 days.

7. The revised budget dated 16 November 2017 and the revised SOW, dated 16 February 2017, Submitted by Stanford University is incorporated, by reference, into this award.

SECTION 00800 - SPECIAL CONTRACT REQUIREMENTS

The following have been added by full text:

U.S. ARMY MEDICAL RESEARCH ACQUISITION ACTIVITY
AWARD SPECIFIC RESEARCH TERMS AND CONDITIONS
WITH INSTITUTIONS OF HIGHER EDUCATION, HOSPITALS, AND NON-PROFIT ORGANIZATIONS

DIVISION I – AWARD COVER PAGES

A. Award Information

1. **Department of Defense Awarding Office:** USAMRAA
2. **Award number/Project title: W81XWH-16-1-0098/Antilysophosphatidic Acid Antibodies in the Treatment of Post-TBI Neuropathic Pain**
3. **Type of Award: Grant**
4. **Type of Award Action: Modification – Grant Transfer**
5. **i. Brief description of project or program:**
   The DOD has solicited neurosensory research proposals to “support both applied (preclinical) research and clinical studies assessing traumatic brain injury (TBI) within specific Focus Areas of pain management, hearing loss/dysfunction, balance disorders, tinnitus, vision, or physical rehabilitation." The proposed project responds to this solicitation and concerns the development of a new approach to the treatment of acute and chronic pain that is a frequent sequel to head injury. The proposed project responds to this solicitation and concerns the development of a new approach to the treatment of acute and chronic pain that is a frequent sequel to head injury. The prevalence of concussions in warfighters returning from Iraq or Afghanistan has been estimated at approximately 19.6% and the prevalence of posttraumatic craniofacial pain were present in 37% soldiers with 27% diagnosed with chronic daily headache and central TBI neuropathic pain. Chronic pain can contribute to psychological dysfunctions accompanying post-traumatic stress disorder. Current therapies, such as non-steroidal anti-inflammatory drugs (NSAID), opiates, anti-epileptics, and triptans can help some patients, but are far from universally effective and have, in some cases, substantial side-effect and abuse potential. Thus there is an acute need for improved strategies to prevent the development or treat existing chronic pain in civilians, war-fighters and veterans suffering from head trauma. The literature and our preliminary evidence clearly show that lysophosphatidic acid (LPA) plays a prominent role in nerve injury-induced pain in experimental animal models. In the CNS, LPA is synthesized by astrocytes, choroid plexus epithelial cells and inflammatory cells and is released upon cell activation. Its concentration within the brain increases during inflammation, clotting and neurotrauma, at which time it likely potentiates its known roles in astrocyte proliferation, neuronal death, axonal injury and

microglial activation. Our preliminary and published data indicate a specific upregulation of LPA receptors following injury to the adult mouse central nervous system (CNS), where LPA has been shown to induce neuronal apoptosis and to inhibit neural stem/progenitor cell differentiation along a neuronal lineage. In addition, LPA receptors are similarly increased after human brain injury. Moreover, LPA levels are upregulated in the CSF of TBI patients, suggesting.

ii. Funding Overview

	Federal funds	Cost Sharing	Total amount
a. Obligated or deobligated this action	$0		$0
b. Cumulative obligations to date, including this and previous actions	$1,446,655		$1,446,655
c. Planned project costs in the currently approved budget through the end of the period of performance, to include any future incremental funding obligations	$1,176,875.74		$1,176,875.74
d. Total value, which includes any unexercised options for which amounts were established in the award	$1,446,655		$1,446,655

6. **Obligation/Effective Date:** 15 April 2016
7. **Period of performance:** 15 April 2016 – 31 October 2019
8. **Authorities:** This award is made under the authority of 10 U.S.C. 2358.
9. **Catalog of Federal Domestic Assistance Number:** 12.420-Military Medical Research and Development
10. **Project Performance Information:**
    i. This award is for research and development**.** Construction activities under this award are not authorized. (Reference Department of the Army Pamphlet 420-11, dated 18 March 2010, for the definition of construction activities.)
    ii. Statement of Work and Budget: The revised Statement of Work (SOW) dated 16 February 2017 and the revised budget dated 16 November 2017 for your application submitted in response to the Fiscal Year 2014 DoD Clinical and Rehabilitative Medicine Research Program/Joint Program Committee 8, Neurosensory and Rehabilitation Research Award Program Announcement (Funding Opportunity Announcement Number W81XWH-14-CRMRP-NSRRA, which closed 11 February 2015) are incorporated herein by reference. You may rebudget allowable costs in accordance with applicable cost principles and in accordance with the prior approval requirements as stated in this award. Additional terms and conditions applicable to this award are in Division II and Division III.
    iii. The following terms and conditions are incorporated herein by reference:
        a. Division III - USAMRAA Addendum to the DoD R&D General Terms and Conditions available at http://www.usamraa.army.mil/Pages/Resources.aspx.
        b. The DoD R&D General Terms and Conditions (September 2017), available at http://www.onr.navy.mil/Contracts-Grants/submit-proposal/grants-proposal/grants-terms-conditions.aspx.
    iv. These USAMRAA Award Specific Research Terms and Conditions are in addition to the terms and conditions incorporated above. Any inconsistencies in the requirements of this award will be resolved in the following order:
        a. Federal statutes
        b. Federal regulations
        c. 2 CFR part 200 with amendments, as modified and supplemented by DoD's interim implementation found in 2 CFR part 1103
        d. Division II - USAMRAA Award Specific Research Terms and Conditions
        e. Division III – USAMRAA Addendum to the DoD R&D General Terms and Conditions

f. DoD R&D General Terms and Conditions (September 2017)

v. **Grants Administration Office**

Grants Specialist: Christopher Meinberg
Phone: 301-619-2657
Email: christopher.l.meinberg.civ@mail.mil
Assistance Agreement Branch Email: usarmy.detrick.medcom-usamraa.mbx.aa1@mail.mil

vi. **Grants Officer's Representative**

Congressionally Directed Medical Research Program Office
Phone: 301-619-7071
Email: usarmy.detrick.medcom-cdmrp.mbx.cdmrp-reporting@mail.mil

B. Recipient Information

1. **Unique Entity Identifier:** 009214214
2. **Recipient Business Name and Address:** The Leland Stanford Junior University
3. **Name and Title of Authorized Representative:** Natalie Muzzio
   a. Phone: 650-724-0907
   b. Email: muzzio@stanford.edu
4. **Principal Investigator and Organization:** David Yeomans
   a. Phone: 650-725-5864
   b. Email: dcyeomans@stanford.edu
5. **Recipient's Indirect Cost Rate at the Start of the Performance Period:**
   57%, Predetermined, MTDC, 02 August 2016
   Negotiation Agency: Department of the Navy

   83%, Predetermined, Animal Care, 02 August 2016
   Negotiation Agency: Department of the Navy

C. **Additional Information:**

1. **Award Modification:** The only method by which the award may be modified is by a formal, written modification signed by the USAMRAA Grants Officer. No other communications, whether oral or in writing, are valid to change the terms and conditions of this award. Awards will not be modified to provide additional funds for such purposes as reimbursement for unrecovered indirect costs resulting from the establishment of final negotiated rates or for increases in salaries, fringe benefits, changes in exchange rates, or other costs.
2. **Expiration of Funds**: Funds obligated on this award are available for use for a limited period based on the fiscal year (FY) of the funds. That time is considered when establishing your period of performance. **This award is funded with FY15 funds in the amount of $1,446,655 which will expire for use on September 30, 2021**. You must monitor the established milestones, timelines, expenditures and invoicing to make sure the project is on schedule and that you voucher promptly. If you have not submitted a final SF270 and been paid before the expiration date of these funds, any excess funds will be deobligated from the award at that time.

DIVISION II – AWARD SPECIFIC RESEARCH TERMS AND CONDITIONS

Federal Interagency Traumatic Brain Injury Research Informatics System
This award involves research in the area of traumatic brain injury (TBI). The Department of Defense, in collaboration with the National Institutes of Health, has developed the Federal Interagency Traumatic Brain Injury Research (FITBIR) Informatics System, a central repository and resource for sharing data to promote collaboration, accelerate research, and advance knowledge on the characterization, prevention, diagnosis, and treatment of TBI. FITBIR provides a common platform and standardized format for data collection, retrieval and archiving, while allowing for flexibility in data entry and analysis.

The Principal Investigator is expected to share his/her data via FITBIR in accordance with FITBIR policy and procedures found at https://fitbir.nih.gov/jsp/about/policy.jsp. If the PI feels (s) he cannot submit data to the system, (s) he must coordinate with the GOR.

Interim (In-Progress) Progress Review

In addition to quarterly, annual, and final technical progress reports, the PI shall prepare for and participate in at least one Interim Progress Review (IPR) for each year of the project's term of award. Generally, the IPR will last no longer than two days and require no more than two overnight stays. It most likely will be held in the Fort Detrick, Maryland area, but may occur elsewhere in the U.S. The invitation and format for the IPR will be provided by the GOR at least 90 days prior to the scheduled date.

Quarterly Technical Reports

a. For each year of the award, the PI must submit Quarterly Technical Progress Reports covering research results (positive and negative data) over a three month period (quarter). A reporting quarter begins with the start date of the award and restarts annually from that date for the entire period of performance. A Quarterly Technical Progress Report for the fourth quarter each year is not required, as the Annual Technical Report must incorporate all four quarters of progress.

b. Quarterly reports are the most immediate and direct contact between the PI and the Grants Officer's Representative (GOR). The reports provide the means for keeping the US Army Medical Research and Materiel Command (USAMRMC) advised of developments and problems as the research effort proceeds. The reports also provide a measure against which funding decisions are made.

c. Prepare all Quarterly reports in accordance with the Quarterly Technical Progress Report format, available at http://www.usamraa.army.mil/Pages/Resources.aspx. Each item of the report format must be completed.

d. Each report must be submitted electronically, within 30 days after the end of each quarter, to the Grants Specialist and the GOR at the e-mail addresses specified in the front pages of this award. Name your file with your award number, followed by Year X Quarter Y Report (example: W81XWH-18-1-0000 Year 1 Quarter 1 Report.) If you have questions, contact the GOR.

e. Special Requirements for Quarterly Technical Reports (must be submitted as an appendix to the quarterly report)

    Quad Charts: The Quad Chart (available on https://www.usamraa.army.mil) must be updated and submitted as an appendix.

Special Requirements for Annual/Final Technical Reports

Special Requirements for Annual/Final Reports (must be submitted as an appendix to the annual/final report)

Quad Charts: The Quad Chart (available on https://www.usamraa.army.mil) must be updated and submitted as an appendix.

Title to Equipment - Conditional

Title to the infrared diode laser (Lasmed model LS110) acquired with award funds vests upon acquisition in you, subject to the conditions of Section A of PROP Article I of the DoD R&D General T&Cs. This equipment is non-exempt property and title is conditional. Upon completion of the award or when the equipment is no longer needed in performance of the award, you must request disposition instructions from the USAMRAA Grants Officer.

DIVISION III- USAMRAA ADDENDUM TO THE DoD GENERAL TERMS AND CONDITIONS AND USAMRAA PROGRAMMATIC REQUIREMENTS

Preamble

This award incorporates by reference the Department of Defense (DoD) Research and Development Terms and Conditions available at https://www.onr.navy.mil/Contracts-Grants/submit-proposal/grants-proposal/grants-terms-conditions . The USAMRAA Addendum to the DoD R&D General Terms and Conditions provides additional content relevant to USAMRAA awards for sections of specified articles from those general research terms and conditions. **The five asterisks indicate that there is content from the DoD R&D General Research Terms and Conditions within the identified parts and articles that remains unchanged and is not restated in this document. To understand the requirement for a given article, the DoD R&D General Research Terms and Conditions must be read in tandem with this USAMRAA Addendum.** The second portion of this addendum is comprised of the programmatic requirements portion of the general terms and conditions that apply to USAMRAA awards subject to the DoD R&D General Terms and Conditions.

USAMRAA Addendum to the DoD R&D General Terms and Conditions

* * * * *

Part I: Definitions

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Section D. Definitions

* * * * *

43. Intangible Property

* * * * *

c. For purposes of USAMRAA awards, software is also considered intangible property.

* * * * *

Part 2: Financial and Program Management (FMS Articles)

* * * * *

FMS Article II. Payments

* * * * *

Section C. Electronic Funds Transfer and other payment procedural instructions of information

* * * * *

2. Other payment procedural instructions or information

a. Request for Payments

i. Payments. Payments will be made to you upon receipt of a "grant voucher" (used for both grants and cooperative agreements) submitted through the Wide Area Work Flow (WAWF) e-Business Suite in accordance with the Contract Line Item Number (CLIN) structure set forth in this award. The Defense Finance and Accounting Service (DFAS) will generally make payments within 30 calendar days after we receive the request for reimbursement unless we reasonably believe the request is improper.

ii. You must select "advance" or "reimbursement" on the grant voucher in WAWF.

iii. In order to conserve administrative resources for both parties, you are encouraged to voucher no more frequently than monthly. Failure to voucher at least quarterly may raise concerns about research progress and the need for continued funding.

iv. All payments will be made by Electronic Funds Transfer (EFT) to the bank account registered in the System for Award Management (SAM) (available at https://www.sam.gov). You must maintain the currency about yourself in SAM, including information necessary to facilitate payment via EFT. We cannot be held responsible for any misdirection or loss of payment which occurs as a result of your failure to maintain correct/current EFT information within your SAM registration. Failure to update SAM ensuring active account status will result in nonpayment.

b. Electronic Payment Instructions

i. The Wide Area Work Flow (WAWF) e-Business Suite is the required method to electronically process your requests for payments. Once on the WAWF e-Business Suite web site, select the Invoicing, Receipt, Acceptance, and Property Transfer (iRAPT) button to electronically submit "grant vouchers" (used for both grants and cooperative agreements). You must (i) register to use WAWF at https://wawf.eb.mil and (ii) ensure an electronic business point of contact (POC) is designated in the System for Award Management (SAM) site at https://www.sam.gov within ten (10) calendar days prior to requesting a payment for this award. The Award specific Research Terms and Conditions will include additional instructions on how to submit grant vouchers and who to contact for assistance if needed.

ii. Questions concerning specific payments should be directed to the Defense Finance and Accounting Service (DFAS), Indianapolis, at 1-888-332-7366, unless a different office is specified in Division II in your award specific terms and conditions. **You can also access payment and receipt information using the "myInvoice" button in WAWF at** https://wawf.eb.mil. The award number or grant voucher number will be required to inquire about the status of the payment.

iii. The following codes and information are required to initiate the grant voucher and assure successful flow of WAWF documents.

TYPE OF DOCUMENT: **Grant Voucher** ***(Used for both grants and cooperative agreements)***

CAGE CODE: **Enter Your Cage Code**

ISSUE BY DODAAC: **W81XWH**

ADMIN BY DODAAC: **W81XWH**

INSPECT BY DODAAC: **W81XWH**

ACCEPT BY DODAAC: **W81XWH**

SHIP TO DODAAC: **W81XWH**

LOCAL PROCESSING OFFICE DODAAC: **Not Applicable**

PAYMENT OFFICE FISCAL STATION CODE: **Unless otherwise specified in Division II in your award specific terms and conditions enter Fiscal Station DODAAC as HQ0490 = DFAS Indianapolis**

EMAIL POINTS OF CONTACT LISTING:

INSPECTOR: **Submit to Assistance Agreement Branch Email identified in the Division I, 10.v.**
ACCEPTOR**: Submit to Assistance Agreement Branch Email identified in the Division I, 10.v.**
RECEIVING OFFICE POC: **Submit to Assistance Agreement Branch Email identified in the Division I, 10.v.**
GRANT ADMINISTRATOR: **Leave Blank**
GRANTS OFFICER: **Leave Blank**
ADDITIONAL CONTACT: **Submit to Assistance Agreement Branch Email identified in the Division I, 10.v.**

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FMS Article IV. Revision of budget and program plans.

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Section B. Revisions requiring prior approval.

1**. Non-Construction Activities**

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e**. USAMRAA Specific Prior Approval Requirements**

i. The transfer (relocation) of the PI and or research project to another entity.
ii. Reimbursing a DoD Military Treatment Facility (MTF) for costs incurred if the MTF is involved in the award. Reimbursing these costs is generally prohibited and only approved under unusual and extraordinary circumstances.

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Section C. Pre-award costs, carry forward of unobligated balances, and one-time no-cost extensions.

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3**. No-cost Extension of the Period of Performance**

a. You may initiate one time, without prior approval, a no-cost extension to the expiration date of the award for a period of up to 12 months, as long as the no-cost extension does not involve a change in the approved objectives or scope of the project. You must notify the USAMRAA Grants Officer in writing at least 30 calendar days prior to the expiration date of the award. The notification must state the additional time needed, the reasons for the extension, and the work to be completed during the extension period. You must be current with all financial and technical reporting requirements and be in compliance with all other terms and conditions of the award. This one-time no-cost extension may not be exercised merely for the purpose of using unobligated balances. An official modification to the award document must be issued by the USAMRAA Grants Officer to extend the period of performance.

b. Reference “Expiration of Funds” in Division I Award Cover Pages to understand the impact of the availability of funds on award extensions.

c. Collaborating awards (two or more USAMRAA-issued awards completing the same Statement of Work) may have to have identical periods of performance. Each collaborating recipient’s business office must contact the Grants Officer assigned to the awards regarding extensions.

d. Any subsequent no-cost extensions require prior approval from the USAMRAA Grants Officer.

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Part 5. Financial Programmatic, and Property Reporting (REP Article)

REP Article I. Performance management, monitoring, and reporting.

Section A. Required reporting form, format, or data elements for interim and final performance reports.

1. Annual Technical Report

    a. Annual reports are required and must be prepared in accordance with the Research Performance Progress Report (RPPR). The RPPR is the uniform format for reporting performance progress on Federally-funded research projects and research-related activities. Annual reports must provide a complete summary of the research results (positive or negative) to date in direct alignment to the approved Statement of Work (SOW). The importance of the report to decisions relating to continued support of the research cannot be over-emphasized.
    b. Special Requirements for Annual Reports-Refer to Division II.

2. Final Technical Report

    a. A final report must be prepared in accordance with the RPPR. The report must summarize the entire research effort, citing data in the annual reports and appended publications.
    b. Special Requirements for Final Reports-Refer to Division II.

3. Format

    Prepare the annual and final reports in accordance with the RPPR format, available at http://www.usamraa.army.mil/Pages/Resources.aspx. Although there is no page limitation for the reports, each report must be of sufficient length to provide a thorough description of the accomplishments with respect to the approved SOW

Section B. Frequency, reporting periods, and due dates for interim performance reports.

An annual technical report must be submitted within 30 calendar days of the anniversary date of the award for the preceding 12 month period. If the award period of performance is extended by the USAMRAA Grants Officer, then an annual report must still be submitted within 30 days of the anniversary date of the award.

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Section F. Performance Reporting Procedures

Annual and Final Technical Reports, in electronic format (PDF or Word file only), must be submitted to https://ers.amedd.army.mil.

Additional information is available on the Researcher Resources website, available at https://mrmc.amedd.army.mil/index.cfm?pageid=researcher_resources.technical_reporting

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REP Article II. Financial Reporting.

Section A. Required reporting form, format, or date elements for interim and final financial reports.

You must submit financial reports on the Standard Form 425 (SF425) "Federal Financial Report."

Section B. Interim financial reports; frequency, reporting periods, and due dates.

The Federal Financial Reporting period end dates fall on the end of the calendar year for annual reports (12/31). You must submit annual reports no later than 90 days after the end of the calendar year.

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Section E. Where and how to submit financial reports.

1. You must submit all interim SF425 reports electronically through the web site https://www.usamraa.army.mil/Pages/SF425.aspx. The form and instructions can be obtained on this site.

2. Do not report multiple awards on one report. Each award must be reported separately on its own SF425.

3. Do not combine multiple SF425s into one submission. Each form must be saved as a separate PDF and submitted individually.

4. You must submit Final SF425 reports electronically to usarmy.detrick.medcom-usamraa.mbx.closeout@mail.mil

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REP Article III. Reporting on Property.

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Section D. Intangible Property.

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1**. Inventions developed under this award.**

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a. Patents and Inventions Reporting Requirements

i. iEdison and annual reporting. You must electronically file Invention Disclosures and Patent Applications using the Interagency Edison (iEdison) system through the National Institutes of Health (https://s-edison.info.nih.gov/iEdison) within the times specified for reporting.
ii. Report of Inventions and Subcontracts. A final DD Form 882 is required and must be submitted electronically within 120 days of end of the term of award. List all inventions made during the term of the award or state "none," as applicable. The award will NOT be closed until you have met all reporting requirements. Submit the final DD882 reports electronically to usarmy.detrick.medcom-usamraa.mbx.closeout@mail.mil

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Part 6: Other Administrative Requirements (OAR Articles)

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OAR Article III Remedies and termination.

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Section B. Remedies for non-compliance.

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f. If you are delinquent on technical reporting requirements for other USAMRAA-sponsored awards, no new awards will be issued to you until all delinquent reports have been submitted.

g. Failure to submit required Technical Reports or Federal Financial Reports (SF425s) may delay payments or result in nonpayment.

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OAR Article IV. Claims, disputes, and appeals.

Section A. Definitions

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2. Grant Appeal Authority- Lamont G. Kapec Deputy Chief of Staff, Procurement and Head of the Contracting Activity HQDA Office of the Surgeon General and U.S. Army Medical Command, 7700 Arlington Boulevard Falls Church, VA 22042-5140

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OAR Article VI. Closeout

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Section B. Refunds of Unobligated balances.

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a) Make check payable to the U.S. Treasury and mail to:
USAMRAA
Attn: MCMR-AAP-C
(Insert Federal Award No. W81XWH-16-1-0098
820 Chandler Street
Fort Detrick, Maryland 21702-5014

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Section D. Accounting for Property.

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a) **Property Acquired with Award Funds, if applicable** [Reference PROP Article IV of the DoD R&D General Terms and Conditions (September 2017).]

i. If equipment under this award is exempt property, you must provide a cumulative listing of exempt equipment acquired with award funds. Submit this on your organization's letterhead. Submit to: Assistance Agreement Branch Email identified in the Division I, 10.v.

ii. If supplies under this award are exempt, you must submit a statement that: (i) there is, or is not, a residual inventory of unused supplies exceeding $5,000 in total aggregate value; and (ii) if there is, state whether or not the unused items will be needed on other Federally sponsored

projects or programs. Submit this on your organization's letterhead. Submit to Assistance Agreement Branch Email identified in the Division I, 10.v.

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Part 8: National Policy Requirements

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NP Article III. National policy requirements concerning live organisms.

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Section B. Other requirements concerning live organisms

1. **Research Involving Recombinant DNA Molecules** By signing the award or accepting funds under the award, you assure that all work involving the use of recombinant DNA will be in compliance with guidance provided at https://osp.od.nih.gov/biotechnology/biosafety-and-recombinant-dna-activities/.

2. **Prohibition of Use of Laboratory Animals**

Notwithstanding any other terms and conditions contained in this award or incorporated by reference herein, the recipient is expressly forbidden to use or subcontract for the use of laboratory animals in any manner whatsoever without the express written approval of the USAMRMC, Animal Care and Use Review Office (ACURO). Written authorization to begin research under applicable protocol(s) proposed for this award will be issued in the form of an approval letter from the USAMRMC ACURO to the recipient with a copy to the USAMRAA Grants Officer. Furthermore, modifications to already approved protocols require approval by ACURO prior to implementation. For each fiscal year, the recipient must maintain, and upon request from ACURO, submit animal usage information.

Noncompliance with any of these terms and conditions may result in withholding of funds and/or the termination of the award.

The Animal Care and Use Office requirements can be accessed at https://mrmc.amedd.army.mil/index.cfm?pageid=research_protections.acuro.

3. **Prohibition of Use of Human Subjects**

Research under this award involving the use of human subjects, to include research involving the secondary use of human biospecimens and/or human data, cannot begin until the USAMRMC's Office of Research Protections (ORP) provides authorization that the research may proceed. The USAMRMC ORP will issue written approval to begin research under separate notification to you. Written approval to proceed from the USAMRMC ORP is also required for any subrecipient that will use funds from this award to conduct research involving human subjects.

The USAMRMC ORP conducts site visits as part of its responsibility for compliance oversight. Accurate and complete study records must be maintained and made available to representatives of the USAMRMC as a part of their responsibility to protect human subjects in research. Research records must be stored in a confidential manner so as to protect the confidentiality of subject information.

The recipient is required to adhere to the following reporting requirements:

Submission of substantive modifications to the protocol, continuing review documentation, and the final report as outlined in the USAMRMC ORP approval memorandum.

Unanticipated problems involving risks to subjects or others, subject deaths related to participation in the research, clinical holds (voluntary or involuntary), and suspension or termination of this research by the IRB, the institution, the Sponsor, or regulatory agencies, must be promptly reported to the USAMRMC ORP.

Change in subject status when a previously enrolled human subject becomes a prisoner must be promptly reported to the USAMRMC ORP HRPO.

The knowledge of any pending compliance inspection/visits by the FDA, ORP, or other government agency concerning this clinical investigation or research, the issuance of Inspection Reports, FDA Form 483, warning letters or actions taken by any Regulatory Agencies, and any instances of serious or continuing noncompliance with regulatory requirements that relate to this clinical investigation or research, must be reported immediately to the USAMRMC ORP.

Non-compliance with these terms and conditions may result in withholding of funds and/or the termination of the award.

DoD requirements for human subjects research, including 32 CFR Part 219, DoD Instruction 3216.02, and USAMRMC ORP Human Research Protection Office submission instructions can be accessed at https://mrmc.amedd.army.mil/index.cfm?pageid=research_protections.hrpo.

4. **Prohibition of Use of Human Cadavers**

Research, development, testing and evaluation (RDT&E), education or training activities involving human cadaveric specimens under this award shall not begin until approval is granted in accordance with the Army Policy for Use of Human Cadavers for RDT&E, Education, or Training, 20 April 2012 (https://mrmc.amedd.army.mil/index.cfm?pageid=research_protections.overview).

The USAMRMC Office of Research Protections (ORP) is the Action Office (usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil) for this policy. Approval must be obtained from the USAMRMC ORP. Award recipients must coordinate with the supporting/funding Army organization to ensure that proper approvals are obtained. ORP will issue written approvals to begin under separate notification to the recipient. Written approval to proceed from the USAMRMC ORP is also required for any subrecipient that will use funds from this award to conduct RDT&E, education or training involving human cadaveric specimens.

Recipients must promptly report problems related to the conduct of the activity involving cadavers or the procurement, inventory, use, storage, transfer, transportation, and disposition of cadavers to the USAMRMC ORP.

Recipients must maintain complete records of the activity.

The USAMRMC or designees must be permitted to observe the activity upon request and/or audit activity records to ensure compliance with the approved protocol or applicable regulatory requirements.

Non-compliance with these terms and conditions may result in withholding of funds and/or the termination of the award.

Programmatic Requirements Portion of the General Terms and Conditions

Publication, Acknowledgement, and Public Release

a. Publication. You are encouraged to publish results of the research, unless classified, in appropriate media. Submit one copy of each paper to the GOR **simultaneously** with its submission for publication. Forward copies of all publications resulting from the research to the USAMRAA Grants Officer or Grants Specialist as they become available, even though publication may in fact occur subsequent to the termination date of the award. (See Section C of the DoD R&D General Terms and Conditions for the charging of publication costs incurred after the period of performance.)

b. Acknowledgment. You agree that in the release of information relating to this award such release will include the statements below, as applicable. "Information" includes, but is not limited to, news releases, articles, manuscripts, brochures, advertisements, still and motion pictures, speeches, trade association meetings, and symposia.

   i. "The U.S. Army Medical Research Acquisition Activity, 820 Chandler Street, Fort Detrick MD 21702-5014 is the awarding and administering acquisition office" and;

   ii. "This work was supported by the Office of the Assistant Secretary of Defense for Health Affairs through the Clinical and Rehabilitative Medicine Research Program/Joint Program Committee 8 under Award No. W81XWH-16-1-0098. Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the Department of Defense."

   iii. "In conducting research using animals, the investigator(s) adheres to the laws of the United States and regulations of the Department of Agriculture."

   iv. "In the conduct of research utilizing recombinant DNA, the investigator adhered to NIH Guidelines for research involving recombinant DNA molecules."

   v. "In the conduct of research involving hazardous organisms or toxins, the investigator adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories."

c. Public release. Prior to release to the public, you must notify the USAMRAA Grants Officer and the GOR of the following: planned news releases, planned publicity, advertising material concerning project work, and planned presentations to scientific meetings. This provision is not intended to restrict dissemination of research information; the purpose is to inform the USAMRMC of planned public release of information on USAMRMC-funded research in order to adequately respond to inquiries and to be alerted to the possibility of inadvertent release of information.

   Failure to include the above statements and adhere to the above regulations, when required, may result in loss of funding and/or termination of this award.

2. **National Security**

The award is intended for unclassified, publicly releasable research. You will not be granted access to classified information. We do not expect that the results of the research project will involve classified information. If, however, in conducting the activities supported under the award, you or the PI is concerned that any of the research results involve potentially classifiable information that may warrant Government restrictions on the dissemination of the results, you must promptly notify the USAMRAA Grants Officer.

3. **Use of Non-Federal Personnel**

Some USAMRMC program offices use contractor personnel to assist the GORs with review of technical reports. All review processes are conducted confidentially. Contractor personnel are required to sign agreements to protect the confidentiality of the information. Violations by reviewers that compromise the confidentiality of the reviews may result in suspension or debarment of the individual or contractor from Federal awards.

The following have been deleted:

USAMRAA-XXXX-0002	AA T&C with For-Profits (Nov 2015)	NOV 2015

(End of Summary of Changes)