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HRP-592 - Protocol for Human Subject Research with Use of Test Article(s)

Protocol Title:
A Single Dose Pharmaco-Diagnostic for Peripheral Nerve Continuity After Trauma

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1.0 Objectives

1.1 Study Objectives

The purpose of this study is to evaluate the role of single dose 4-aminopyridine (4-AP) on the diagnosis of severing vs non-severing nerve injury after peripheral nerve traction and/or crush injury. The investigational treatment will be used to test the hypothesis that 4-aminopyridine can speed the determination of nerve continuity after peripheral nerve traction and/or crush injuries allowing the identification of incomplete injuries earlier than standard electrodiagnostic (EDX) and clinical assessment.

1.2 Primary Study Endpoints

Primary Aim and Study Endpoint: Return of voluntary function and subjective sensation not present after trauma.

Summary of Specific Aims: This proposal contains two distinct aims to be investigated in two similar but distinct groups of patients. This will be measured by sensory, motor and sudomotor (electrical conduction tests), and electrodiagnositc (EDX) testing.

Aim 1: To examine the mechanistic effect of 4AP on the return of sensorimotor function and EDX sensitivity in the setting of nerve dysfunction from orthopaedic trauma. This aim tests the hypothesis that oral one-time administration of 4AP provides transient return of function and EDX sensitivity to the traumatically denervated limb in alert patients with known limb injuries not involving the central nervous system.

Aim 2: To examine the mechanistic effect of 4AP on the return of sensorimotor function and EDX sensitivity in the setting of iatrogenic nerve injury after surgical intervention. This aim tests the identical hypothesis as in Aim 1 in a distinct group of patients, whose nerve dysfunction is the result of a clinical intervention, and whose function before that intervention was intact.

1.3 Secondary Study Endpoints

Not applicable.

2.0 Background

2.1 Scientific Background and Gaps

Neurological injury in the form of traction or crush to nerves that control muscles and sensory function is common. Because an understanding of these injuries is only now beginning to emerge, research on potential treatments is an important next step. Through experiments performed on animals with the Acorda Therapeutics, Inc. version of the drug (AMPYRA®), 4-aminopyridine (4-AP) has been strikingly effective in ameliorating the effect of a standardized peripheral nerve crush injury. The peripheral nerve injury used in our experiments was a standard model of peripheral nerve injury used to measure recovery in animals and is a model of peripheral nerve traction and crush injury that has been studied for over thirty years. We have found that:

(1) 4-AP administration in a single dose given on day three after the injury led to a drastic reduction in the dysfunction afforded by a crush injury just days after the crush itself.

(2) 4-AP treatment's effect was short-lived after a single dose and was, in effect, diagnostic of the potential to recover in a nerve that was crushed but not shattered.

(3) Severed nerves show no capacity to recover even with 4-AP treatment.

(4) The treatment in a daily regimen led to profound, lasting, permanent improvement in the speed of recovery in these animals.

4-AP is used in some of the most fragile of neurologically-ailing patients and is currently a mainline treatment in the setting of multiple sclerosis [(1)]. Multiple sclerosis patients suffer a demyelinating disorder that causes the stripping of the myelin sheath from around neurons in the peripheral and central nervous system. The myelin covering allows for normal conduction of impulses and, without such covering, impulses are small, impaired, impeded, and ineffective. The recognition that crush injuries to nerves do not simply sever the axonal fibers but also demyelinate some population of nerve cells has led to the idea to study the treatment of peripheral nerve traumatic injuries in humans using 4-AP.

2.2 Previous Data

4-AP has been studied in humans since the early 1980s, and principles of safe usage are extremely well established. For the purposes of this proposal, the immediate release formulation of 4-AP, sometimes called fampridine will be referred to as IR 4-AP. The proposed version of the drug used in this study is an extended release formulation of 4-AP, called dalfampridine, which was marketed under the trade name AMPYRA, by Acorda Therapeutics. Recently, this extended release formulation has become available as a generic, which will be referred to as generic AMPYRA or dalfampridine. Essentially identical principles apply whether 4-AP is provided in an orally available immediate release formulation (IR 4-AP) or an orally available sustained release formulation (dalfampridine). The safety of 4-AP appears to be determined solely by serum levels.

It has long been recognized that the most significant safety concern regarding 4-AP is an increased frequency in seizures, which occurs in a small percentage of patients if serum levels exceed 100 ng/ml. Therefore, dosages are chosen to maintain serum levels that do not exceed 50-60ng/ml. In prior studies, 5mg of study drug were administered once every six hours, for a total dosage per day of 20 mg. This total dose, as indicated by multiple previous studies on immediate release 4-AP, has an excellent safety profile even in the fragile population of patients with multiple sclerosis. A sustained release formulation of 4-AP (AMPYRA®) at this same dose is FDA approved for use in the multiple sclerosis population even with a known risk of seizure activity in these patients.

It is important to note that multiple studies on 4-AP also include patients with chronic stroke, chronic spinal cord injury, transverse myelitis, primary lateral sclerosis, Lambert-Eaton myasthenic syndrome, ocular nystagmus, nonarteritic anterior ischemic optic neuropathy, spinal muscular atrophy, chronic Guillan-Barre syndrome, episodic ataxia Type 2, obstructive sleep apnea and spinocerebellar ataxias. Over 45 clinical trials have been conducted in the US alone (as listed on the Clinicaltrials.gov website). There are also 49 primary publications on clinical trials outcomes on 4-AP, which include patients with multiple sclerosis, chronic spinal cord injury, spinocerebellar ataxias and chronic stroke.

The many trials on 4-AP have been conducted using both immediate release 4-AP and sustained release 4-AP. The difference between the immediate release and sustained release formulations are that the sustained release formulation helps to decrease the peaks and troughs in serum levels that can occur with larger doses of immediate release formulations.

In respect to the use of *immediate release* formulations of 4AP (not proposed for use in this study but relevant for evaluation of safety), the following studies on multiple sclerosis patients are the most pertinent:

1. 4-Aminopyridine in patients with multiple sclerosis: dosage and serum level related to efficacy and safety. Van Diemen HA, Polman CH, Koetsier JC, Van Loenen AC, Nauta JJ, Bertelsmann FW. Clin Neuropharmacol. 1993 Jun;16(3):195-204.

In these studies, nonenteric-coated capsules containing 4-AP or placebo were administered orally for 12 weeks, the starting dosage was 10-20 mg orally per day in 2-3 divided doses. The daily dosage was elevated with 5-15 mg at week two and week six. Blood samples, to determine serum levels at the end of the first and second oral treatment, were taken 1.5-2.5 hours after the latest intake of medication.
Side effects were minimal in these studies and mostly were confined to paresthesias and/or dizziness/lightheadedness and nausea/vomiting and were not considered to be significant by the patients.

The mean dosage given to patients was 31.2 mg per day and ranged from 10-50 mg per day. Thus, the average dose was higher than we have proposed.
Capsules were divided in 2-4 doses and ranged from 0.17- 0.55 mg/ kg. On average, 4AP serum levels increased 1.3 ng/ml per mg 4- AP/day. Side effects were less frequent with oral dosing than with intravenous dosing. When side effects occurred, they mostly occurred within 30-45 minutes after taking the medication and generally resolved within 2-5 hours, with half the patients not experiencing any side effects.

The rapid resolution of side effects with orally administered 4-AP in an immediate release formulation was very favorable.
One patient, who received three oral doses daily at twice the concentration that are proposed (i.e., 10mg per capsule instead of 5 mg/capsule), was closely studied at multiple time intervals in this very early study. Following an initial rapid climb in serum levels to 114 ng/ml, subsequent doses caused increases to lower levels of 60 ng/mL or 80 ng/ml.
In general, restlessness, confusion and generalized tonic-clonic seizures have been reported at doses higher than 0.8 mg/kg bodyweight, and such symptoms were not seen when doses of 0.5 mg/kg bodyweight or less were used.

For this IND application, we are assuming a body weight of 70kg, the dosages proposed are <0.3 mg/kg, distributed in 1 dose of 10mg/capsule. No subject will receive a dose greater than 0.5 mg/kg bodyweight. Patients will receive only one pill for the course of the study.

2. The effect of 4-aminopyridine on clinical signs in multiple sclerosis: a randomized, placebo-controlled, double-blind, cross-over study. van Diemen HA, Polman CH, van Dongen TM, van Loenen AC, Nauta JJ, Taphoorn MJ, van Walbeek HK, Koetsier JC. Ann Neurol. 1992 Aug;32(2):123-30.
In this study, patients reported side effects (which were mild when they occurred at all) that occurred within 30-45 minutes after taking the medication, and generally resolved within 2 -5 hours. Severe side effects were not encountered which was thought to be due to the fact that the maximum daily dose was only 0.5 mg/kg of body weight.

3. 4-Aminopyridine improves clinical signs in multiple sclerosis. Stefoski, D., Davis, F.A., Faut, M., Schauf, C.L., 1987.. Ann Neurol 21, 71-77.
In these patients, single doses up to 15 mg 4-AP were administered in an immediate release oral formulation. In this single-dose administration, 70% of the improvements lasted 7 to 10 hours and usually became apparent one hour post-4-AP and culminated three hours or so later.
Serum samples were taken from a heparin lock placed in a peripheral arm vein before 4-AP initiation and at 0.5, 1.0, 1.5, 2, 3, 4, 6, and 8 hours after the dose. Analysis of serum concentrations of levels of 4-AP showed a terminal half-life of approximately 3 ± 1 hours. There was no detectable serum 4-AP in the majority of patients on the morning following the test day, although 7/17 patients had varying carryover levels. These carryover levels were lower than any from the previous day and clinical improvements had reversed to baseline by this time.
Side effects of this dosage (which is three times higher than we propose) seen were mild to moderate consistently

lasted no more than 60-90 minutes. These were generally well tolerated and patients did not consider them disturbing.

4. Orally administered 4-aminopyridine improves clinical signs in multiple sclerosis. Davis FA, Stefoski D, Rush J. Ann Neurol. 1990 Feb;27(2):186-92.
In these studies, 4 AP was delivered orally in a single dose and no serious or bothersome side effects were observed with single oral doses of up to 25 mg. These dosages were up to 2 times greater than any used in this proposal.

5. The effects of 4-aminopyridine in multiple sclerosis patients: results of a randomized, placebo-controlled, double-blind, concentration-controlled, crossover trial. Bever CT Jr, Young D, Anderson PA, Krumholz A, Conway K, Leslie J, Eddington N, Plaisance KI, Panitch HS, Dhib-Jalbut S, et al. Neurology. 1994 Jun;44(6):1054-9.
In patients given anywhere from 5-10 mg of 4-AP after various time points(i.e., q12h, q8h, q6h) serum levels only once exceeded 64 ng/ml in one patient and 56 ng/ml in one patient and otherwise were all between 35-51 ng/ml at the Cmax analysis point. The only time seizures were seen was in a patient where the serum level reached, with high dosage administration, greater than 100 ng/ml. The conclusion of this study was that peak serum levels above 100 ng/ml should be avoided but that at lower levels side effects are minimal.

The studies on multiple sclerosis define the dosages to be used in this proposal, even though the traumatically injured patients are not considered to be a fragile population. The following studies on patients with spinal cord injuries are also relevant, as these patients, like peripheral nerve injury patients, do not have the central nervous system lesions that are prominent in patients with multiple sclerosis.

6. Safety and efficacy of 4-aminopyridine in humans with spinal cord injury: a long-term, controlled trial. Segal JL, Pathak MS, Hernandez JP, Himber PL, Brunnemann SR, Charter RS. Pharmacotherapy. 1999 Jun;19(6):713-23.
In these patients with chronic spinal cord injuries, dosages provided in immediate release oral formulations of 4-AP were 30 mg per day and demonstrated efficacy in multiple patients. Clinically significant adverse effects or measurable toxicity did not occur. Nervousness, giddiness or dizziness, and gastrointestinal upsets manifesting as mild abdominal cramping or nausea were the most frequent side effects. Side effects were transient, self-limiting and seizures or seizure-like activity was not observed.
The authors stated that they have administered an oral formulation in amounts of up to 40 mg/day to more than 60 patients with no significant toxicities or adverse reactions that warranted discontinuing a subject. This is generally the case, and patients with spinal cord injury have been studied at dosages higher than those used in patients with multiple sclerosis.

7. Effect of 4-aminopyridine on gait in ambulatory spinal cord injuries: a double-blind, placebo-controlled, crossover trial. DeForge, D., Nymark, J., Lemaire, E., Gardner, S., Hunt, M., Martel, L., Curran, D., Barbeau, H., 2004.. Spinal cord 42, 674-685.
15 chronic, ambulatory SCI patients were randomized to an initial 2 weeks of 40mg/day, oral medication of either placebo or immediate-release 4-AP and subsequently crossed over to the alternate medication for the following 2 weeks. The study treatment (placebo or 4-AP) started at 5 mg twice daily for 3 days, increased to 10 mg twice daily for 3 days and finally 10 mg four times daily. The subjects were on the full dosage of 40 mg/day within 1 week. Side effects consisted of nausea, dizziness and sleeping difficulties but did not include seizures even though dosages were twice those studied in patients with multiple sclerosis.

8. Sustained improvements in neurological function in spinal cord injured patients treated with oral 4-aminopyridine: three cases. Potter, P.J., Hayes, K.C., Hsieh, J.T., Delaney, G.A., Segal, J.L., 1998.. Spinal cord 36, 147-155.
Potter and colleagues examined three patients with SCI who were administered oral (capsule) IR-4AP (10 mg b.i.d. or t.i.d.) over a 4 month interval. Only trivial side effects (transient light- headedness) were observed.

2.3 Study Rationale

More research is needed to evaluate the role of 4-aminopyridine (4AP) in the course of assessment of nerve continuity after peripheral nerve traction and/crush injuries. The investigational treatment will be used to test the hypothesis that 4AP allows the identification of incomplete injuries earlier than standard electrodiagnostic (EDX) and clinical assessment and may have a role in faster triage.

3.0 Inclusion and Exclusion Criteria

3.1 Inclusion Criteria

- Patients with trauma involving two or less limbs where the continuity of a given peripheral nerve or nerves is unclear on presenting physical examination.
- Closed soft tissue envelope obscuring direct observation of the continuity of the affected nerve.
- Cognitive ability to report sensory and motor deficit during examination.
- Able to complete single day dosing within seven days (168 hours) of nerve injury diagnosis.
- Eligible for standard of care plan of monitoring vs surgical exploration of the nerve.
- Adults subject aged 18-90
- Known limb trauma which resulted in nerve injury (aim 1) or post-operative/post intervention nerve injury (aim 2).
- Ability to give written informed consent.
- Capable of safely undergoing electrodiagnostic testing (EDX).
- Availability for all testing days and main trial day.

3.2 Exclusion Criteria

- Not able to complete dosing within seven days (168 hours) of nerve injury diagnosis
- Distracting injury which prevents adequate examination.
- Plan for surgical exploration of the nerve during the ensuing 48 hours.
- Plan for surgical exploration of the nerve as part of another surgical procedure within 48 hours of evaluation.
- Intoxication during examination or evidence of cognitive deficit that emerges during examination.
- History of multiple sclerosis, stroke or any other diagnosed neurological disorder
- History of hypersensitivity to AMPYRA® or 4-aminopyridine
- Current use of aminopyridine medications, including other compounded 4-AP
- Renal impairment based on calculated GFR (GFR<80 mL/min). This laboratory value is measured in all inpatient trauma patients as part of the standard of care.
- History of difficult compliance with timely follow up or plan to seek care at another institution closer to home.
- Patients outside the age range or unable to consent.
- Patients with a known history of a seizure disorder (4AP overdose can, in selected cases, result in limited seizure activity).
- Patients with a concomitant traumatic brain injury.

- Patients unable to communicate return or loss of sensation.
- Patients unable to exhibit motor control on the affected limb at baseline.
- Patients unwilling to complete the study requirements.
- Patients with injuries too extensive to isolate a single nerve(s) for testing.
- Patients currently taking organic cat-ion transporter 2 (OCT2) inhibitors, e.g. Cimetidine.
- Pregnancy, breastfeeding or incarcerated individuals.

3.3 Early Withdrawal of Subjects

3.3. 1 Criteria for removal from study

- If the subject is unable or unwilling to take the study drug they will be withdrawn from the study.
- Subjects who experience a seizure while taking study medication will be withdrawn from the study.
- The Safety Monitor may withdraw any subject at increased risk using best medical opinion based on reported AEs/SAEs.
- Subjects will be advised in the written informed consent form that they have the right to withdraw from the study at any time without prejudice.

3.3. 2 Follow-up for withdrawn subjects

In order to maintain a sample size of 25-34 subjects per aim (note that there are two aim target groups), those withdrawn from the study will be replaced. Subjects withdrawn for safety reasons will be followed up by regular checking up with them over the phone (and or other tests if necessary).

4.0 Recruitment Methods

4.1 Identification of subjects

The patients will be identified as potential subjects by a co-investigator on the study team while in the emergency department at Hershey Medical Center or during consultation as inpatients. Traumatic peripheral nerve injury (TPNI) patients rarely escape consultation on the orthopaedic or neurosurgical services as patients do not tolerate paralysis without explanation or the initiation of a treatment plan. Clinic (office) presentation is also theoretically possible however, patients presenting to clinics within the 7 day window of inclusion are exceedingly rare in our system. Nonetheless, we will allow inclusion of such patients from orthopaedic surgery, neurosurgery, or neurology clinics of the co-investigators should these rare presentations arise.

4.2 Recruitment process

4.2. 1 How potential subjects will be recruited.

Patients will all be consented in face to face interviews inside the Penn State Milton S. Hershey Medical Center. To minimize the opportunity for coercion a study coordinator listed on the study will conduct the consent process, when available. The subject will be told about the study and given ample time to ask questions and review the consent form before deciding about participation. All subjects will provide written informed consent before beginning study measures or receiving study medications and all will be given a copy of the signed consent form.

4.2. 2 Where potential subjects will be recruited.

As patients in Hershey Medical Center as described in section 4.1

4.2. 3 When potential subjects will be recruited.

At time of treatment from one of the co-investigators listed on the study.

4.2. 4 Describe the eligibility screening process and indicate whether the screening process will occur before or after obtaining informed consent. Screening begins when the investigator obtains information about or from a prospective participant in order to determine their eligibility. In some studies, these procedures may not take place unless HIPAA Authorization is obtained OR a waiver of HIPAA Authorization when applicable for the screening procedures is approved by the IRB. [*For FDA regulated studies, consent for any screening activities would need to be obtained prior to screening unless specifically waived by the IRB.]*
No further eligibility questions will be asked in addition to verification of the inclusion and exclusion criteria. The study team will screen the EHR to confirm inclusion and exclusion criteria prior to consent.

5.0 Consent Process and Documentation

5.1 Consent Process:

Check all applicable boxes below:

☒ **Informed consent will be sought and documented with a written consent form *[Complete Sections 5.2 and 5.6]***

☐ **Implied or verbal consent will be obtained – subjects will not sign a consent form (waiver of written documentation of consent) *[Complete Sections 5.2, 5.3 and 5.6]***

☐ **Informed consent will be sought but some of the elements of informed consent will be omitted or altered (e.g., deception). *[Complete section 5.2, 5.4 and 5.6]***

☐ **Informed consent will not be obtained – request to completely waive the informed consent requirement. *[Complete Section 5.5]***

5.2 Obtaining Informed Consent

5.2. 1 Timing and Location of Consent

Patients will all be consented in face to face interviews inside the Penn State Milton S. Hershey Medical Center. This can take place in the ER, inpatient, and possibly clinics of investigators listed on the study.

5.2. 2 Coercion or Undue Influence during Consent

To minimize the opportunity for coercion a non-conflicted study coordinator or co-investigator will conduct the consent process, when available. Because of potential conflicts, Dr. Elfar will only participate in the explanation of the protocol, risks and benefits, answering potential subject's questions about the study, and reviewing if the potential subject meets criteria for the study. The subject will be told about the study and given ample time to ask questions and review the consent form before deciding about

participation. It will be reviewed that deciding not to participate in the research will not affect treatment.

5.3 Waiver of Written Documentation of Consent

5.3. 1 Indicate which of the following conditions applies to this research:

☐ The research presents no more that minimal risk of harm to subjects and involves no procedures for which written consent is normally required outside of the research context.

OR

☐ The only record linking the subject and the research would be the consent document and the principal risk would be potential harm resulting from a breach of confidentiality. Each subject will be asked whether the subject wants documentation linking the subject with the research, and the subject's wishes will govern. (*Note: This condition is not applicable for FDA-regulated research. If this category is chosen, include copies of a consent form and /or parental permission form for participants who want written documentation linking them to the research.)*

OR

☐ If the subjects or legally authorized representatives are members of a distinct cultural group or community in which signing forms is not the norm, that the research presents no more than minimal risk of harm to subjects and provided there is an appropriate alternative mechanism for documenting that informed consent was obtained. (*Note: This condition is not applicable for FDA-regulated research.)*

Describe the alternative mechanism for documenting that informed consent was obtained:

NA

5.3. 2 Indicate what materials, if any, will be used to inform potential subjects about the research (e.g., a letter accompanying a questionnaire, verbal script, implied consent form, or summary explanation of the research)

NA

5.4 Informed consent will be sought but some of the elements of informed consent will be omitted or altered (e.g., deception).

5.4. 1 Indicate the elements of informed consent to be omitted or altered

NA

5.4. 2 Indicate why the research could not practicably be carried out without the omission or alteration of consent elements

NA

5.4. 3 Describe why the research involves no more than minimal risk to subjects.

NA

5.4. 4 Describe why the alteration/omission will not adversely affect the rights and welfare of subjects.

NA

5.4. 5 If the research involves using identifiable private information or identifiable biospecimens, describe why the research could not be practicably be carried out without using such information or biospecimens in an identifiable format.

NA

5.4. 6 Debriefing

NA

5.5 Informed consent will not be obtained – request to completely waive the informed consent requirement

5.5. 1 Indicate why the research could not practicably be carried out without the waiver of consent

NA

5.5. 2 Describe why the research involves no more than minimal risk to subjects.

NA

5.5. 3 Describe why the alteration/omission will not adversely affect the rights and welfare of subjects.

NA

5.5. 4 If the research involves using identifiable private information or identifiable biospecimens, describe why the research could not be practicably be carried out without using such information or biospecimens in an identifiable format.

NA

5.5. 5 Additional pertinent information after participation

NA

5.6 Consent – Other Considerations

5.6. 1 Non-English-Speaking Subjects

Not applicable.

5.6. 2 Cognitively Impaired Adults

5.6.2.1 Capability of Providing Consent

The PI will determine if there is a possibility of incapability to provide consent.

5.6.2.2 Adults Unable to Consent

Not applicable.

5.6.2.3 Assent of Adults Unable to Consent

Not applicable.

5.6. 3 Subjects who are not yet adults (infants, children, teenagers)

5.6.3.1 Parental Permission

Not applicable.

5.6.3.2 Assent of subjects who are not yet adults

Not applicable.

6.0 HIPAA Research Authorization and/or Waiver or Alteration of Authorization

6.1 Authorization and/or Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

Check all that apply:

☐ **Not applicable, no identifiable protected health information (PHI) is accessed, used or disclosed in this study.** *[Mark all parts of sections 6.2 and 6.3 as not applicable]*

☒ **Authorization will be obtained and documented as part of the consent process.** *[If this is the only box checked, mark sections 6.2 and 6.3 as not applicable]*

☒ **Partial waiver is requested for recruitment purposes only (Check this box if patients' medical records will be accessed to determine eligibility before consent/authorization has been obtained).** *[Complete all parts of sections 6.2 and 6.3]*

☐ **Full waiver is requested for entire research study (e.g., medical record review studies).** *[Complete all parts of sections 6.2 and 6.3]*

☐ **Alteration is requested to waive requirement for written documentation of authorization (verbal authorization will be obtained).** *[Complete all parts of sections 6.2 and 6.3]*

6.2 Waiver or Alteration of Authorization for the Uses and Disclosures of PHI

6.2. 1 Access, use or disclosure of PHI representing no more than a minimal risk to the privacy of the individual

6.2 .1.1 Plan to protect PHI from improper use or disclosure

Information is included in the "Confidentiality, Privacy and Data Management" section of this protocol.

6.2 .1.2 Plan to destroy identifiers or a justification for retaining identifiers
Identifiers will be destroyed at the closure of the study by the PI.

6.2. 2 Explanation for why the research could not practicably be conducted without access to and use of PHI

During treatment an investigator will review the inclusion and exclusion criteria with the potential subject to verify they meet criteria for enrollment. Information must be obtained from the patient's electronic medical record during recruitment to determine eligibility and, in some cases, to confirm information discussed with the subject in regards to their medical history.

6.2. 3 Explanation for why the research could not practicably be conducted without the waiver or alteration of authorization

PHI will need to be accessed to review the medical record to verify the subject meets criteria for enrollment. This is necessary to protect the safety of the subjects
The waiver is requested only for recruitment to determine subject eligibility to ensure that no medical conditions that fall into the exclusion criteria are present and would thus preclude enrollment. This waiver will minimize the enrollment of subjects' who may ultimately fail to meet the study inclusion/exclusion criteria.

6.3 Waiver or alteration of authorization statements of agreement

Protected health information obtained as part of this research will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research study, or for other permitted uses and disclosures according to federal regulations.

The research team will collect only information essential to the study and in accord with the 'Minimum Necessary' standard (information reasonably necessary to accomplish the objectives of the research) per federal regulations.

Access to the information will be limited, to the greatest extent possible, within the research team. All disclosures or releases of identifiable information granted under this waiver will be accounted for and documented.

7.0 Study Design and Procedures

7.1 Study Design

This is a pilot, single-center study that will be conducted at the Penn State College of Medicine at Hershey. It is a double-blind trial design. **Group A** will receive the study drug on the main trial day, and **Group B** will receive placebo. These groups will exist for both aims (both types of included patients).

Rationale for Study Design
Both subject and provider will be blinded to group assignment to avoid selection bias.

The timing of the design comes from the treatment of trauma patients with nerve continuity which is ambiguous. Currently no test for nerve continuity is available for 6 weeks post injury. This allows the proposed intervention to occur in a time when diagnostics are not available (within days of the injury).

Animal models also reveal lasting positive effects beyond presence of 4-AP on myelin. Demyelination and myeloprotection after injury is poorly understood in any model. We have seen changes in mice which evolve over weeks to months with treatment. This has provided support for continuing treatment for our chosen timeline.

Rationale for Dosage
Both the 4-AP and the placebo treatments will be administered orally. In order to conduct the trial according to the strictest safety concerns, the 4-AP dosage will be a one-time dose of 10mg/day which reflects current FDA recommendations for 4-AP (in the form of AMPYRA®) taken by individuals with multiple sclerosis. This dose has also been found to be effective in previous trials on chronic spinal cord injury but is below dosages that have caused increased dropout rates due to side effects[38]. Our initial trial approval is sought for the single dose of 10mg which is well within the range of approved dosages for 4AP.

In order to maintain serum levels in a safe range in this proposal the dosage in any individual capsule has been set to the starting dose used in the more fragile (and therefore susceptible to side effects) group of patients currently treated with MS. The dose is therefore set to a single dose of 10mg and the dosage is trialed based on our initial work at a one-time dose of 10mg (one pill). The tablet will be taken whole, without food, in the Clinical Research Center.

7.2 Study Procedures
This is a pilot, single-center, inpatient Clinical Research Center study that will be conducted at Penn State Hershey. Subjects will be randomized to treatment or placebo for each aim. Both the subject and investigator will be blinded to group assignment for the duration of the trial.

Study Participant Source: Study participants will come from two sources. The first will be traumatized patients presenting with nerve dysfunction. These study participants are referred to as study participants for aim 1 of this trial. These patients often have concomitant orthopaedic injuries, which require stabilization. If the nerve was dysfunctional before intervention, then the patient will be included as a recruit for aim 1. The second source is patients who suffer nerve dysfunction after a surgery or intervention where nerve injury may not have been expected, as is the case with routine fracture fixation or joint arthroplasty procedures. In such cases, post-intervention examination may reveal a loss of nerve function where the continuity of the nerve again cannot be established. These patients, owing to their intact nerve function before the intervention, will be included as recruits to aim 2. In point of fact, both groups of patients have the same phenotypic condition, namely the loss of nerve function where the continuity of the nerve is not demonstrable. However, they are grouped into differing aims because the latter group suffers nerve dysfunction as the result of an intervention or surgical procedure and critically, these patients will have a clear pre-intervention examination without any nerve dysfunction.

Study participant **screening procedures** include the interview and chart review of patients to ensure that they match the inclusion and exclusion criteria listed in section 3.1 and 3.2. This will be performed on first interview with the patient on presentation for both Aim 1 and Aim 2 in the hospital (either inpatient ward or emergency department).

Potential study participants will be pre-screened for eligibility by reviewing the EMR to confirm inclusion and exclusion criteria. Study participants meeting pre-screening criteria will be presented with the study

opportunity. After obtaining informed consent, patients will be provided a research diary and instructed on its use.

Research study participant randomization for this trial is segregated by aim. Aim 1 research study participants are randomized to treatment or placebo separately from aim 2 research study participants. Each study participant for each aim is randomly assigned to either treatment or placebo using permuted block randomization.

Testing

Blood Tests for the circulating level of 4AP will be performed using standard high-performance liquid chromatography. This testing is performed after optimizing conditions with serum samples spiked with either isotope labeled 4AP (Penn State Mass Spec method) or 3,4-diaminopyridine (URMC Mass Spec Method) [24]. These samples will be tested after the optimization process (to begin prior to funding this proposal). Samples will be tested at the Penn State Mass Spectroscopy Laboratories at the Milton S. Hershey Medical Center, which is a laboratory currently involved with several on site clinical trials. The test, which is not CLIA certified, will nonetheless allow us to verify our test results in two different methodologies so that future CLIA applications will benefit from work done in this proposal. Samples will be verified at the Penn State College of Medicine Mass Spectroscopy Resource Center, where we have optimized parameters for this specific test in both human and murine serum samples. The effective range of concentrations which we have measured is 5nM to 50mM which spans an order of magnitude below (ineffective) and above (toxic) doses of 4AP in the literature. Each sample will be 5 ml (1 tsp).

Sensory functional testing: The most complete set of clinical examination tests must be employed to achieve our aims at understanding the mechanism of 4AP mediated return of function (or lack thereof). Each nerve in the body contains an expected sensory nerve dermatomal distribution on the skin [25]. Sensory testing of this distribution is performed to test every type of fiber in the sensory portion of the nerve including: Semmes-Weinstein monofilament responses [26,27,39]; static and moving two-point discrimination [5,28]; light-touch sensation (a commonly used subjective assessment used in trauma patients5); and subjective reports of sensation both inside and outside the dermatome. These reports will be scored to reflect 2:1 weight of objective (e.g., monofilament) over subjective (e.g. reported sensation, light touch sensation), according to the same protocols used previously [26-30]. In the hand, individual sides of each finger (e.g., for ulnar, median, and radial nerve distributions) will be tested as in our previous work [26,28-30]. Patients will also be objectively graded on the withdrawal from a pin-prick using a sterile needle 27-guage needle in the nerve distribution which is absent (8 pricks per patient). Patients will also have any relevant (well established) deep-tendon reflexes measured, although these are not available for every possible affected nerve, in contrast with the rest of the tests we employ.

Motor functional testing: As with sensory testing (above), a complete motor assessment which includes all the voluntary motor function tests used in the routine assessment of these patients must be here applied to our trial patients within the 1 hour testing periods in between serum tests. Each potentially traumatized nerve whose continuity is in question has expected muscles which will not function. Each muscle under the control of the nerve in question will be tested using standard muscle strength testing paradigms [5,7]. Muscle strength is assessed by the rater using standard strength testing scales used elsewhere in the literature (1=no firing, 2=firing but no antigravity strength, 3=antigravity strength only, 4=more strength than gravity but less than the rater, 5=equal to rater strength testing).These tests focus on strength, which may be too high a bar for a nerve whose continuity is in question. Therefore we will also have two independent observers observe six trials at voluntary movement in two muscle groups innervated by the nerve in question and score the observations using standard inter-rater statistics [31-33].

Sudomotor functional testing: We will attempt to obtain electrical conductance measurements [34-36] in skin areas known to be innervated by the injured nerve at all time points during muscle testing. This is done by measuring

the resistance using a standard volt meter (no current involved) across skin scores from this test will be compared (as controls) against the same measurements in non-denervated and from clothing and or hair (non-innervated). These are not primarily used in TPNI patients and, when used are chiefly performed on the hands and feet of patients. Several test methods have been used, including industrial rigs with the capability to measure secreted biomarkers from skin patches [37]. This notwithstanding, the applicability of these tests to a nerve injury where the dermatome affected is not enriched with sweat glands (like the hands and feet), is not established.

EDX testing: Patients enrolled in the trial will undergo serial EDX testing by a trained member of the study team to specifically assess the continuity of the injured limb nerves. For patients with known single-nerve lesions below the plexus trunk level, the testing will include nerve conduction studies (NCS) and electromyography (EMG). The NCS will include one sensory and one motor nerve (if indicated) with needle exam of two muscles supplied by the motor nerve. EMG will show motor units which will allow for assessment of their size and recruitment, and the presence or absence of active denervation (fibrillation potentials and positive sharp waves) depending on the timeline. It should be noted that our early testing phase is within four days of injury and that these findings of chronic denervation should only be present at 6 and 12 week testing. The NCS will be assessed for latencies, amplitudes, and conduction velocities values. Of particular note is the natural difficulties with the use of EDX in this setting. The presence of non-recordable or absent responses on NCS can be interpreted as abnormal or inconclusive depending on multiple factors including technical limitations. The presence of active denervation and motor unit size and recruitment abnormalities are time-dependent. These do not usually manifest prior to 10-14 days. As such, we expect most of these studies to be inconclusive on the trial day. If, the nerve is believed to be continuous by this test, then the result will be saved and sealed for analysis post-hoc. This test will be performed at baseline before challenge with 4AP or placebo and then hourly for five hours after challenge. There will therefore be a total of six tests performed over the course of the testing day (Figure 1, blue arrows with the 5th post-test being done in conjunction with the Return to Baseline test). Clear indications of continuity will be shared with the main study coordinator at the end of the day and the patient will be informed of the result of the EDX test at the conclusion of the day (but not which group their allocation).

7.2. 1 Visit 1 in Clinical Research Center (CRC) and/or Inpatient setting~8 hours

Trial Logistics and Organizing framework: Patients for this trial are divided into two aims. Patients enrolled for one aim, are excluded from the other aim, because the causes of the injury, which define the aim, are exclusive. Aim 1 is for post traumatic neuropathy. Aim 2 is for post surgical or post intervention neuropathy. Within each aim, there are treated patients and placebo patients. Only one patient will be evaluated at a time. Evaluation takes one day. Patients will be randomized in the Penn State University Clinical Research Center (CRC) or at the bedside, where they will stay for a period of analysis afterwards that lasts a few hours.

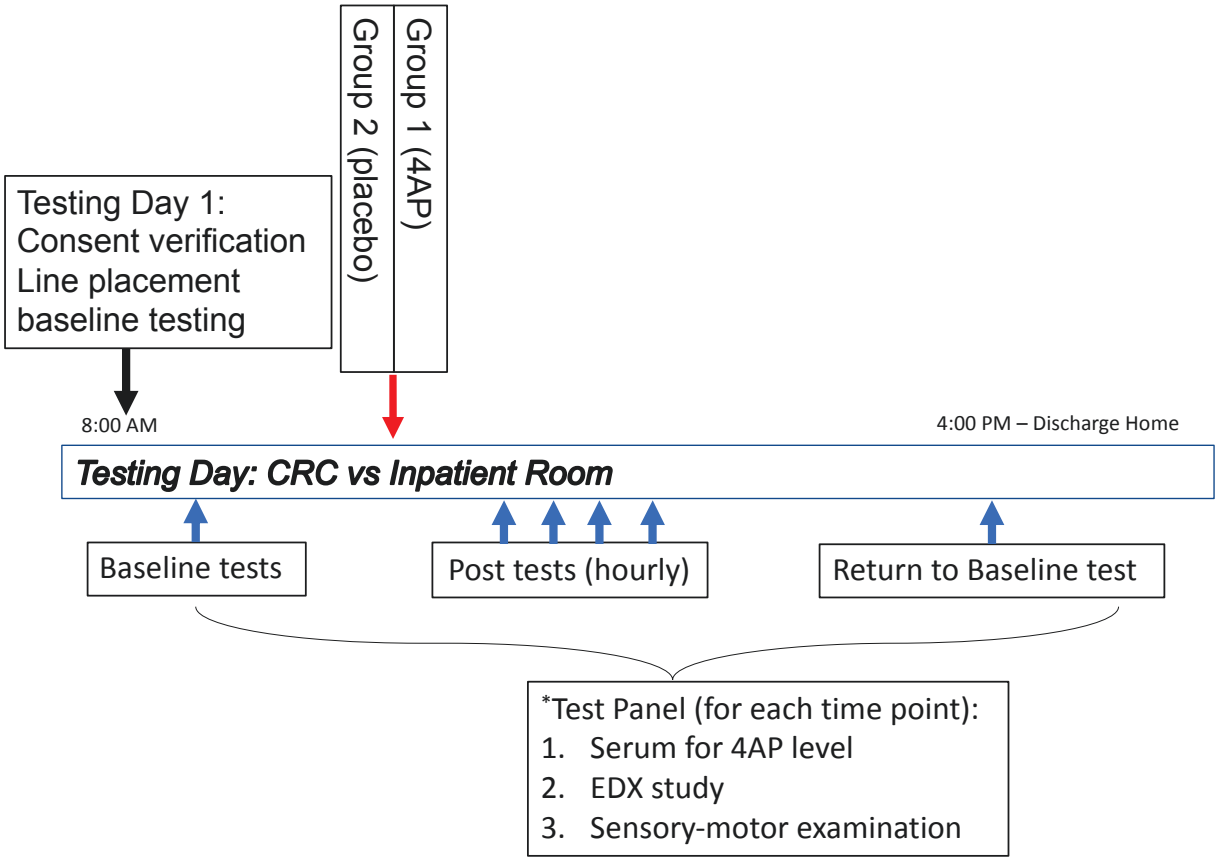
Eligible, consented patients will present for testing from one of three locations (emergency department, inpatient ward, or home). Patients will undergo testing in the CRC and/or bedside under the direction of trial personnel. Testing will be initiated by patients undergoing a thorough sensory and motor evaluation and establishing an intravenous line for blood sampling. A baseline blood sample and EDX study (prior to drug or placebo) and sensorimotor examination will be performed. These tests comprise part of a standardized array of tests performed hourly. This array of tests (serum 4AP level, EDX study, and sensorimotor examination) will be repeated every hour after treatment for five hours (Figure 1) during which the drug will have decreased to a level low enough to have no expected effect. The final hourly test is the return to baseline test. It is likely going to occur at the fifth hourly posttest, but is depicted separately for clarity. Only five tests after dosing will be necessary, based on our expectations from known pharmacokinetic data.

Finally, testing is concluded at the end of this period. Research subjects will be provided a diary and asked to record symptoms as instructed. The subjects will be asked to maintain the diary for 20 weeks and the diaries will be reviewed at each follow up visit and collected at or after the final 20 week visit.

Figure 1 Legend: Planned admission to Penn State Clinical Research Center for testing and monitoring in proposed trial design. Each patient will complete testing in one arm (either 4-AP or placebo) before discharge. Note, standard measures of nerve continuity like EDX are not usually obtained at this period of time because they typically yield no usable information. They are performed during this portion of a trial as a control on our results. Note that the return to baseline test timing is not currently known. As such, this test may not be necessary. We are currently planning on having the final hourly test be the return to baseline test. It is depicted as a separate test to note that we will ensure that the patient has returned to baseline before discharged from the CRC. Based on pharmacokinetic data, we expect that this will occur between the fourth and fifth hourly posttest.

7.2. 2 Follow up visits

Following the initial testing, subjects will be seen for a period of 20 weeks after injury to monitor recovery and progress. Subjects will return for follow-up visits at 2, 6, 12, 18, and 20 weeks post injury. The 2 week visit will coincide with the initial post-operative visit with the treating provider as part of standard of care. The 6, 12, and 18 week visits will be completed in the neurology clinic and EDX testing will be completed as part of standard of care (the current gold standard for continuity and recovery diagnosis). The 20 week visit will be completed in the CRC.



Subjects will receive a physical exam at all follow-up visit. EDX testing will be completed at 6, 12, and 18 week visits only. Subjects will complete a telephone interview at 9 and 15 weeks post injury at which time subjective motor and sensory function will be assessed by asking 1) Can you move your (affected extremity)? Yes or No 2) Can you feel your (affected extremity)? Yes or No. Review of the subject diary will also be completed. The 9, 15, and 20 week visits are not part of standard of care and are being done for research purposes only. Each visit or phone interview will last approximately 30 minutes. Subjects will be instructed to bring the research diary to their follow-up appointments and will be reviewed and evaluated by study personnel. At the final 20 week visit the diary will be collected. Additional follow up might be considered beyond standard of care for diary completion if required.

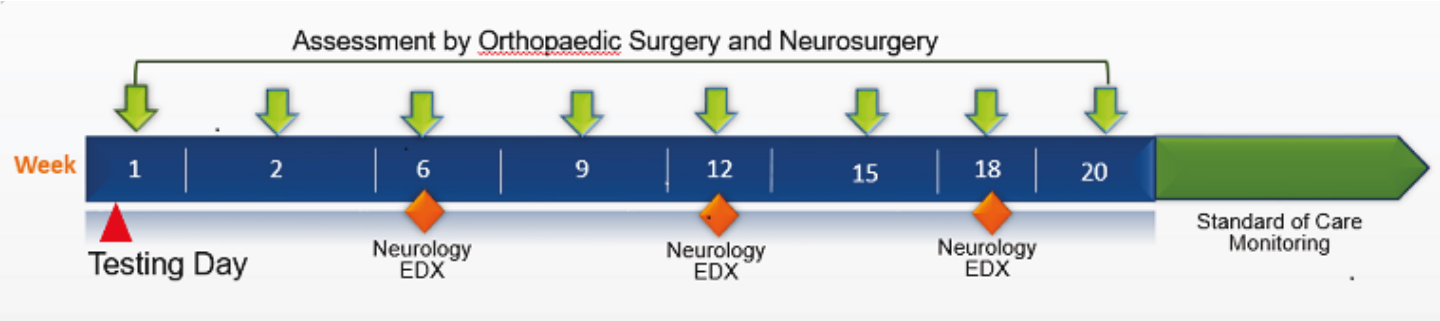


Figure 2 Legend: General timeline of trial for single patient. Note that enrolled patients are allowed to proceed with standard of care exploration of injured nerves at their own discretion (with consultation of their surgeons) all throughout this 20-week period. If the continuity or lack thereof is verified, they continue with assessments as planned. In the figure, week 1 starts after testing day (which is visit 1 in the CRC. The first follow up visit is after testing day.

7.3 Duration of Participation

Enrolled Subjects will be followed for a 20 week period.

7.4 Test Article(s) (Study Drug(s) and/or Study Device(s))

7.4. 1 Description

Generic dalfampridine (4-aminopyridine extended release formulation): 4-AP is an organic compound with the chemical formula $C_5H_4N–NH_2$. The molecule is one of the three isomeric amines of pyridine. It is primarily used to manage some of the symptoms of multiple sclerosis,[3][4] and is indicated for symptomatic improvement of walking in adults with several variations of the disease.[4] 4-AP works as a potassium channel blocker. Electrophysiological studies of demyelinated axons show that augmented potassium currents increase extracellular potassium ion concentration which decreases action potential duration and amplitude which may cause conduction failure. Potassium channel blockade reverses this effect. A study has shown that 4-AP is a potent calcium channel activator and can improve synaptic and neuromuscular function by directly acting on the calcium channel beta subunit.[3]

Subjects will receive one dose of oral dalfampridine 10 mg extended release tablets or an identical appearing placebo pill(see drug insert packet for formulation and testing information). The tablets will be over-encapsulated using Coni-Snap Empty Gelatin Capsules manufactured by Capsugel. USP grade methylcellulose will be used as filler for the active capsules and as the placebo. Placebo capsules will consist of the Con-Snap Empty Gelatin Capsule manufactured by Capsugel and the methylcellulose filler. Both pills will be formulated and tested in conformity with the FDA mandated guidelines for this trial.

7.4. 2 Treatment Regimen

Investigational Treatment – Group A and group B will both receive the same treatment with active ingredient listed below. Group A will receive the active drug and Group B will receive the placebo. This is *a single-dose study*.

Active Ingredient: 4-aminopyridine (4-AP) 10 mg pill per dose
Inactive Ingredient: See package insert
Dosing schedule = 1 capsule.

Other Agent(s): Placebo

7.4. 3 Method for Assigning Subject to Treatment Groups

Subjects in both aims will be assigned to Group A or Group B using a randomization scheme. Each study participant for each aim is randomly assigned to either treatment or placebo using permuted block randomization.
This randomization will be done using redcap. **This is a single-dose, pilot study.**

7.4. 4 Subject Compliance Monitoring

Not applicable. Study medication will be administered under observation.

7.4. 5 Blinding of the Test Article

Blinding will be performed by the IDS service at Penn State.

7.4. 6 Receiving, Storage, Dispensing and Return

7.4 .6.1 Receipt of Test Article

The study drug and placebo will be maintained by Penn State Investigational Drug Service (IDS). They will store, dispense and monitor all investigational supplies.

7.4 .6.2 Storage

The study drug and placebo will be maintained by Penn State Investigational Drug Service (IDS). They will store, dispense and monitor all investigational supplies. IDS will prepare and dispense study drug and placebo after randomization upon receipt of a valid prescription from the study director, which will be transported to IDS after consent.

7.4 .6.3 Preparation and Dispensing

Dalfampridine (extended release 4-aminopyridine) 10mg tablets will be obtained from Accord Pharmaceuticals by the Investigational Drug Service (IDS) Pharmacy at Penn State Health Milton S. Hershey Medical Center. IDS will then prepare blinded capsules of over-encapsulated Dalfampridine tablets and matching placebo.

7.4 .6.4 Return or Destruction of the Test Article

One 10 mg study capsule will be taken by mouth, whole in the setting of the Clinical Research Center or at the bedside.
In case there is unused product if a subject chooses to withdraw after the med is dispensed, the CRC or research coordinator will return the unused product to the IDS.
Investigational product that remains at end of study will be destroyed by IDS according to Institutional policy.

7.4 .6.5 Prior and Concomitant Therapy

The study drug may not be taken together with other aminopyridine medications, including other compounded 4-AP (sometimes called 4-aminopyridine or dalfampridine).

8.0 Subject Numbers and Statistical Plan

8.1 Number of Subjects

This is a pilot study. The purpose is to gain preliminary data and refine infrastructure and establish finalized routines associated with our work. We hope to recruit 50 patients to this pilot study based on our previous statistical analysis.

8.2 Sample size determination

We cannot provide sample size based on the pilot nature of this study. Our purpose is to generate preliminary data that can further inform our biostatisticians. We also have found that defining our protocol procedures in the CRC will help us define statistical groups for regulatory approval of the crossover trial currently planned.

8.3 Statistical methods

Consultation with the biostatistical team included separate discussions with Dr. Vernon Chinchilli and Dr. Shaohow Zhou. This trial is a pilot study in preparation for an NIH funded randomized controlled trial which is currently in preparation.

Our methods will be to stratify nerve injuries based on injury severity and compare placebo to drug patients using the methods listed below:

This proposal contains two distinct aims to be investigated in two similar groups of patients.

Aim 1: To examine the effect of 4AP on the return of sensorimotor function and EDX sensitivity in the setting of nerve dysfunction from orthopaedic trauma. This aim tests the hypothesis that oral one-time administration of 4AP provides transient return of function and EDX sensitivity to the traumatically denervated limb in alert patients with known limb injuries not involving the central nervous system.

Aim 2: To examine the effect of 4AP on the return of sensorimotor function and EDX sensitivity in the setting of iatrogenic nerve injury after surgical intervention. This aim tests the identical hypothesis as in Aim 1 in a distinct group of patients, whose nerve dysfunction is the result of a clinical intervention, and whose function before that intervention was intact.

Statistical planning was performed in the design of this trial with a qualified biostatistician, who also sits on the Data Safety Monitoring Board for this study. Statistical methods will be used to evaluate both the effect of treatment as well the effects of injury type (either aim 1 or aim 2). Groups will be analyzed separately within each aim.

Categorical variables (as in the presence or absence of sensation) will be compared (between treated and untreated groups for each aim) using Chi-squared testing. Continuous data (as in nerve conduction velocity from EDX) will be compared with standard student's T testing. Grouped data variables will be first compared in linear models (accounting for mixed effects of multiple variables as indicated). Formal recommendations for the statistical methods to be used for posthoc comparisons will be made by the biostatistician designee of the DSMB (Dr. Vernon Chinchilli), who has aided in the planning of this trial and is a non-conflicted member of the trial team.

Information provided by the biostatistician collaborator:

Data analysis for both of these aims is identical, and involves a generalized linear mixed-effects model with a logit link function (118). The separation of these aims is based on the source of the injury, the likelihood of a severed nerve (less likely in aim 2), and the patient's need for an answer to the question. In aim 1, patients may suffer additional harm to a nerve for necessary care but fundamentally, aim 1 contains patients for whom the question of nerve continuity is not the result of a surgical intervention.

Patients will undergo clinical testing for both subjective (return of feeling, motor control) and objective (withdrawal from painful stimuli, deep-tendon reflexes, sudomotor function, muscle firing) measures of function before and after administration of 4AP and placebo (all patients will receive both treatments in random order). Standard monitoring will include clinical observation for voluntary movement, electrodiagnostic testing, and serum tests to correlate circulating 4AP concentrations to diagnostic thresholds and compare those thresholds to those used for therapeutic efficacy in the current approved patient populations. The diagnostic information, in conjunction with formalized inclusion and exclusion criteria, will not affect the current standard treatment paradigm, but will inform our decisions on suspected nerve continuity and surgical exploration.

Patients with intact function prior to operative procedures often suffer trauma to a nerve iatrogenically as a result of a procedure. In these patients, the trauma is often our surgery to correct a fracture or other deformity. We will investigate the possibility of administration of oral 4AP in patients with nerve dysfunction after surgical intervention in the limb. Despite the belief that the nerve is not severed, dysfunction in these patients is indistinguishable from cases where the nerve is later discovered to be iatrogenically severed as part of the procedure. Their testing will be performed identically as in the case of aim 1, with the exception that these patients will have documented presurgical examination of the limb showing function to be intact prior to the index procedure.

9.0 Data and Safety Monitoring Plan

9.1 Periodic evaluation of data

The study Medical Monitor will be named by the DSMB. Dr. Kenneth Taylor and Dr. John Elfar, MD, Professor in the Department of Orthopaedics and Rehabilitation, will be responsible for reporting to the study monitor. A separate study monitor from neurology will be assigned to monitor functional outcomes. This neurologist will be familiar with the study parameters and intervention proposed and will assess EDX outcomes.

For the entire study trial day in the CRC, subjects will be continuously monitored (see trial logistics section above). Thereafter, serial follow-up with phone call backup will be instituted per the protocol depicted in Figure 2 (above). Alternative contacts will be instituted and maintained to allow direct interaction between the study coordinator, principal investigator and patients so that all interactions (in person, by phone or by email) are possible should the need arise. Patients will also keep a diary (distributed at the time of consent) to monitor any symptoms, signs, illnesses or experiences which develop or worsen, whether or not the event is considered related to the study drug. After the first two weeks and for the following 18 weeks, each subject will be queried about any potential AEs at the time of standard visits with the neurologist and study principal investigator.

All subject reports of events that develop or worsen will be reported as Adverse Events and sent to the Safety Monitor (same as the medical monitor), the PI and the neurologist to ensure on-going subject safety. Any adverse medical experience that meets the definition of an SAE will be monitored and reported in the same way. The Adverse Event Tracking Form will be used to document safety monitoring and reporting.

Estimated glomerular filtration rate (GFR) is assessed in trauma patients and will be known before initiating treatment with 4-AP. The relationship between 4AP dosage and estimated GFR levels is based on the importance of renal function in removing 4AP from the system. In patients with mild renal impairment (estimated GFR 51–80 mL/min), 4-AP plasma levels may approach those seen at a dose of 15 mg twice daily, a dose that may be associated with an increased risk of seizures. As mild renal impairment is common after age 50, estimating GFR is particularly important in such patients. Patients with renal impairment (estimated GFR≤80 mL/min) will be excluded from the study based on the fact that this level of impairment is contraindicated

9.2 Data that are reviewed

All clinical outcomes data will be reviewed.

9.3 Method of collection of safety information

Patients will also keep a diary to monitor any symptoms, signs, illnesses or experiences which develop or worsen, whether or not the event is considered related to the study drug. After the first two weeks and for the following 18 weeks, each subject will be queried about any potential AEs at the time of standard visits with the neurologist and study principal investigator.

9.4 Frequency of data collection

Data will be collected at each study visit as outlined in Sections 7.2.1 and 7.2.2 of the protocol.

9.5 Individuals reviewing the data

The Data Safety Monitoring Board will be reviewing the data a minimum of twice per year unless there is an adverse event reported and then a meeting will be convened to review the adverse event unless a meeting has already been scheduled within a week.

9.6 Frequency of review of cumulative data

Twice per year.

9.7 Statistical tests

Harm will be determined based on standard comparison of response to placebo with response to the drug. Each test, provided data from that test is normally distributed, which the data from each of our clinical and diagnostics are, is tabulated and compared with the same response when no active drug is in the system. Since all variables in our outcomes are continuous, the appropriate tests are standard t-tests. Theoretically, ordinal tests will be assayed with the chi-squared method.

9.8 Suspension of research

An adverse event where the patient has an unexpected reaction to the drug will suspend this study. An example of such an event would be a seizure or a drastic increase in pain in the limb after administration of a single dose of the active drug. One other way to stop the trial is by an objection by a member of the data safety monitoring board, which exists to monitor all adverse events in this trial.

10.0 Risks

Loss of confidentiality:
One potential risk is the possible consequences of breach of confidentiality.

Venipuncture:
There is a venipuncture risk drawing blood of a slight pinch or pin prick when the sterile needle enters the skin. The risks include mild discomfort and/or bruising at the site of the puncture. Less common risks include a small blood clot, infection or bleeding at the puncture site, and on rare occasions fainting during the procedure.

4-AP study drug:
In addition, serious risks associated with allergic reaction to the study medicine including shortness of breath or trouble breathing, swelling of the throat or tongue or hives are possible. There is also a serious risk of kidney or bladder infection and seizure.

The following information will be included in the consent form to make sure that the subjects understand the risks:

- The research participant could have a seizure even if they never had a seizure before.

- The chance of having a seizure is higher if too much study medication is taken or if the participant's kidneys have a mild decrease of function, which is common after age 50.
- The participant's kidneys will be checked before start of study medication to determine how well they are working.
- Before taking study medication, the participant should tell the doctor if they have kidney problems.

The study medication can also cause dizziness, confusion and balance problems. Therefore, prior to leaving the CRC, patients planning on driving or operating machinery, should be familiar with how the study medication affects them.

Risks of EDX tests: The EDX tests that will be performed are low-risk procedures, and complications are rare. There's a small risk of pain (although rarely encountered), bleeding, infection and nerve injury where a needle electrode is inserted.

The member of the study team trained to conduct the EMG will need to know if the participant has certain medical conditions. The participant must tell the study team member and other EMG lab personnel if the subject:

- Has a pacemaker or any other electrical medical device
- Takes blood-thinning medications
- Has hemophilia, a blood-clotting disorder that causes prolonged bleeding

Risks of Randomization: The primary risk of randomization is that the treatment the subjects receive may prove to be less effective or to have more side effects than the other study treatment(s) or other available treatments. A secondary risk of randomization is that the randomization process is somehow in error, and the patient is assigned to the wrong group. This would result in a patient exposed to treatment and labelled as having been exposed to placebo. The only negative effect of this error would be to mask the truly beneficial effects of the treatment. The same masking would occur if a patient assigned to treatment, being given instead placebo. This would have the same masking effect. Neither effect exposes patients in this trial to harm.

Protection Against Risks

Breaches of confidentiality will be reduced by storing the data on secure share drives within Penn States Trial Data Center (part of the Department of Public Health Sciences). Access will be restricted to the investigators, the study coordinator, and any agency legally empowered to demand access to the data and only encrypted, password-protected computers will be used.

Potential risks and discomforts from taking the study medication will be minimized to the greatest extent possible by closely monitoring subjects for the most common side effects including allergic reactions and the less common side effect of seizure while in the CRC and after discharge (see above). Study subjects will be asked by study personnel after the CRC day about any potential symptoms and any evidence of difficulty or adverse event will be evaluated.

Estimated glomerular filtration rate (GFR) is assessed prior to inclusion in this study and in trauma patients as standard of care and will be known before initiating the trial with 4-AP. In patients with mild renal impairment (estimated GFR 51-80 mL/min), 4-AP plasma levels may approach those seen at a dose of 15 mg twice daily, a dose that may be associated with an increased risk of seizures. As mild renal impairment is common after age 50, estimating GFR is particularly important in these subjects. Patients with renal impairment (estimated GFR≤80 mL/min) will be excluded from the study based on the fact that this level of impairment is contraindicated in the use of 4-AP.

4-AP has never been shown to definitively exacerbate renal function in humans and studies in animals only reveal alterations at doses several-fold higher than are used in our proposed study. The relationship between 4AP dosage and

estimated GFR levels is based on the importance of renal function in removing 4AP from the system. Therefore, the risk of 4-AP use in this patient population is directly linked to the risk of changes in estimated GFR. To be on the safe side of renal clearance issues, however, we have taken two specific steps with regards to issues with 4-AP in renal toxicity. First, we have excluded patients with estimated GFR <= 80 mL/min based on the fact that this level of impairment is contraindicated in the use of the study drug. This criteria means that we even have excluded patients whose estimated GFR levels actually met criteria for inclusion in earlier 4-AP trials. Second, we are using lower doses of 4-AP in this study than were used in initial studies for 4-AP for a variety of neurological maladies to further ensure we do not run into toxicity issues. The specific dose we have selected is the starting dose used in multiple sclerosis patients prior to dose escalation.

Randomization errors are protected against in this trial by double checking the randomization protocol in IDS and use of redcap. Further checking and oversight is planned at the DSMB level prior to initiating the trial and after the 80% mark is achieved.

11.0 Potential Benefits to Subjects and Others

11.1 Potential Benefits to Subjects

A known risk in nerve injury is a delay in recovery of function attributable to inability to correctly diagnose a nerve laceration without EDX, which only becomes sensitive to the continuity of the nerve weeks after the injury. It is well known that early surgical exploration and repair of lacerations of the nerve is best in patients with known nerve lacerations and detrimental for patients found at surgery to have an unsevered nerve. As this trial offers no change in the overall standard of care for nerve patients other than a trial of a drug to see if it returns function or sensation (clear indications that the nerve is not severed), this trial does not alter the standard of care for these patients. The benefits are a clear indication of the status of the nerve weeks before the gold standard test can reveal the same information – but this benefit is only realized in the drug group and only if return of function is demonstrated. 4-AP is a known neuro-enhancing agent we hypothesize will potentially return function transiently but long enough to reveal those patients whose nerves are intact but not working. Furthermore, we predict this diagnostic intervention will also limit the difficulty and stigma associated with not knowing if their nerve has been cut, again a direct benefit only realized when continuity is demonstrated. This trial only affects patient decisions to pursue further treatment if return of function is clearly demonstrated. Otherwise, patients might have no return of function either because their nerve was indeed severed in the trauma or if they received placebo. As such, no direct conclusions or alterations in patient care will be based on these results for trial patients. Also, since there are additional research visits included at two week intervals in this trial, recovery will be followed with greater accuracy over time.

11.2 Potential Benefits to Others

Currently in the literature there is no consensus as to the right way to diagnose a nerve with ambiguous continuity. There is agreement that patients must wait until EDX studies become sensitive, but even within the literature, the time for the first use of this test varies from three to eight weeks with most practitioners agreeing with a standard six week first EDX study. The current evidence is not sufficient to support the routine use of surgical exploration or watch-and-wait strategies for these injuries. Practitioners simply choose which option for a nerve injury based on their clinical guess as to the status of the nerve. This strategy represents the alternative to participation.

We, therefore, propose to allow the current standard of care to be the alternative to participation. In this case, a patient would be treated as all patients with this injury and differs from trial patients only in the fact that trial patients may have more information about the state of their nerve with which to make a more informed treatment decision with their treating surgeon.

12.0 Sharing Results with Subjects

The results of this study include both subjective and objective measures. It is impossible to hide the subjective measures from the patients given the nature of our measurements. We will prevent active discussion of objective measures until final follow up at 20 weeks. Patients will be free to use their subjective assessments of the return of function in their standard-of-care treatment decisions. We will not inform other physicians except at the express consent of patients to do so. Subjective responses to treatment can be discussed, again at the patient's request, with treating physicians before final follow up. Objective test results will not be discussed with treating physicians until 20 weeks, again, only with patient consent to do so. In cases where the patient's decision to seek further surgical exploration of an injured peripheral nerve based on subjective return of function (or lack of return) seems ill-advised to members of the study team, a patient conference will be arranged in conjunction with the ethicist and DSMB. Such cases where the patient intends to monitor a nerve where our subjective findings suggest clear evidence that the nerve requires surgical exploration, are expected to be rare. Other such cases, where the patient intends to elect for surgical exploration of a nerve which is believed, based on the results of the study, to be in continuity, may be slightly more common. In both of these cases, the patient's decision in conjunction with the treating surgeon (who is purposefully excluded from the study team to avoid any conflicts) is held to be paramount. Conferences of the type described above will be held in selected cases where the evidence from the trial is deemed to be clearly in conflict with the decision taken by the patient – only to ensure adequate education, again with prior consultation with the DSMB. Unblinding will be discussed at this time as well.

13.0 Subject Payment and/or Travel Reimbursements

Subjects will be compensated $80 after completion of Visit 1. This is based on the standard hourly rate ($10 per hour). Subjects completing visit 1 in the CRC will be provided with a $10 meal card. This will be done through Greenphire ClinCard.

14.0 Economic Burden to Subjects

14.1 Costs

The subject will not incur any additional costs as a result of participation in this study. The study drug and placebo will be provided to the subject at no charge. The subject or the subject's insurance will be responsible for all standard of care costs associated with treatment for the nerve injury. Testing completed in the CRC for visit 20 weeks will be covered by study funding and will not impact insurance coverage. The EDX testing done on visit 1 and 6, 12, and 18 weeks post injury are considered standard of care and will be changed to the subject's insurance accordingly.

14.2 Compensation for research-related injury

It is the policy of the institution to provide neither financial compensation nor free medical treatment for research-related injury. In the event of injury resulting from this research, medical treatment is available but will be provided at the usual charge. Costs for the treatment of research-related injuries will be charged to subjects or their insurance carriers.

15.0 Resources Available

15.1 Facilities and locations

This research will be conducted at the Penn State Milton S. Hershey Medical Center. Specifically, the trial day (when patients are given 4AP or placebo and monitored) is conducted in the Penn State Hershey Clinical Research Center (https://research.med.psu.edu/research-support/crc). Patients will be followed in the Penn State Hershey Outpatient Clinics in both the Orthopaedic Surgery and Neurology Departments. No patients will be treated, seen or recruited at any site outside of the Penn State Hershey Facility.

15.2 Feasibility of recruiting the required number of subjects

We have undertaken several different forms of analysis aimed assessing the feasibility of this study. These efforts combine in-depth studies of patient selection, sources, availability and estimates of the likelihood of recruitment if a patient is found.

Patient Selection: Patients eligible for this trial must exhibit the loss of nerve function from recent trauma or post-operative state (within 7 days of injury) where the state of nerve continuity is unknown. These patients do not currently qualify for objective EDX because of known limitations of that electrophysiologic testing. We will, as part of the trial perform these tests nonetheless to ensure that they are still not usable with and without 4AP treatment. Patients may have multiple nerves effected with at least one, defined and EDX testable nerve among them. Patients must have a functional loss that they themselves can appreciate at the time of enrollment because some of the employed tests are subjective. Patients must report the capacity to use and sense with that nerve before trauma, and must be willing to undergo a test which may expose only some functions and not others (see below). Moreover, patients must be willing to have a test of a return of function which, in all likelihood will be fleeting – lasting only so long as the circulating level of 4AP, allows the impulse conduction in the traumatized nerve before being cleared and excreted.

Patient Source: Patients will come from two sources. The first will be traumatized patients presenting with nerve dysfunction. These patients often have concomitant orthopaedic injuries, which require stabilization. If the nerve was dysfunctional before intervention, then the patient will be included as a recruit for aim 1. The second source is patients who suffer nerve dysfunction after a surgery or intervention where nerve injury may not have been expected, as is the case with routine fracture fixation or joint arthroplasty procedures. In such cases, post-intervention examination may reveal a loss of nerve function where the continuity of the nerve again cannot be established. These patients, owing to their intact nerve function before the intervention, will be included as recruits to aim 2. In point of fact, both groups of patients have the same phenotypic condition, namely the loss of nerve function where the continuity of the nerve is not demonstrable. However, they are grouped into differing aims because the latter group suffers nerve dysfunction as the result of an intervention or surgical procedure and critically, these patients will have a clear pre-intervention examination without any nerve dysfunction.

Splitting aims is necessary because of the differing means by which the patient is injured and the different circumstances of injury. Surgeons will often tell patients who would qualify for aim 2 that they are absolutely sure that the nerve is not severed, altering the pre-test/pre-trial likelihood that the patient may feel a return of function even if exposed to the placebo. We therefore intend to keep the recruitment and counselling of aim 2 patients separate from those who present after traumatic, non-iatrogenic nerve injury.

Patient Selection and Availability Patient Availability and Cohort Query Analytics: Ambiguous nerve continuity is common in our medical center. Initial estimates, based on stakeholder provider interviews,

indicate that there are sufficient cohorts to conduct this trial in our single institution with matched cohorts divided into sub-categories based on the extent of nerve involvement. Annually, presenting patients (aim 1) with nerve deficits and those whose nerve continuity becomes obscure after intervention (aim 2) number at least 24 and 50 respectively. Our proposed recruitment estimates, corrected for exclusion criteria and inability to consent, indicate that diagnostic trials on 4AP can be completed in 24 months with full follow up for our gold standard electrodiagnostic endpoint (10 weeks). Thus, within two years, we can answer this highly relevant clinical question unambiguously in our single center.

15.3 PI Time devoted to conducting the research

Dr. Taylor is allotted time to work on his research in agreement with his contract in the Department of Orthopaedics.

15.4 Availability of medical or psychological resources

Medical or psychological care will be referred to resources here at Penn State Hershey Medical Center.

15.5 Process for informing Study Team

As detailed in the team composition/budget justification, our team is composed of several different types of clinicians and scientists. This entire team will be housed at a single medical center (not at satellite facilities). In addition to quarterly meetings, the entire team will attend a yearly retreat to discuss issues surrounding this trial.

This communication time notwithstanding, the investigators will also communicate regularly, either by phone or in person, to discuss study progress, updates, and responsibilities. Communication will occur weekly during the first six months of the project to facilitate successful implementation of the project protocol. After six months, communication will be bi-weekly for the duration of the project period. Both investigators and the principal investigator will share their respective research results with key personnel. The study team will work together to discuss any changes in the direction of the research projects.

16.0 Other Approvals

16.1 Other Approvals from External Entities

Not applicable

16.2 Internal PSU Committee Approvals

Check all that apply:

☐ Anatomic Pathology – **Penn State** Health **only** – Research involves the collection of tissues or use of pathologic specimens. Upload a copy of "HRP-902 - Human Tissue For Research Form" in CATS IRB.

☐ Animal Care and Use – **All campuses** – Human research involves animals and humans or the use of human tissues in animals

☒ Biosafety – **All campuses** – Research involves biohazardous materials (human biological specimens in a PSU research lab, biological toxins, carcinogens, infectious agents, recombinant viruses or DNA or gene therapy).

☐ Clinical Laboratories – **Penn State Health only** – Collection, processing and/or storage of extra tubes of body fluid specimens for research purposes by the Clinical Laboratories; and/or use of body fluids that had been collected for clinical purposes but are no longer needed for clinical use. Upload a copy of "HRP-901 - Human Body Fluids for Research Form" in CATS IRB.

☒ Clinical Research Center (CRC) Advisory Committee – **All campuses** – Research involves the use of CRC services in any way.

☒ Conflict of Interest Review – **All campuses** – Research has one or more of study team members indicated as having a financial interest.

☐ Radiation Safety – **Penn State Health only** – Research involves research-related radiation procedures. All research involving radiation procedures (standard of care and/or research-related) must upload a copy of "HRP-903 - Radiation Review Form" in CATS IRB.

☒ IND/IDE Audit – **All campuses** – Research in which the PSU researcher holds the IND or IDE or intends to hold the IND or IDE.

☒ Scientific Review – **Penn State Health only** – All investigator-written research studies requiring review by the convened IRB must provide documentation of scientific review with the IRB submission. The scientific review requirement may be fulfilled by one of the following: (1) external peer-review process; (2) department/institute scientific review committee; or (3) scientific review by the Clinical Research Center Advisory committee. NOTE: Review by the Penn State Health Cancer Institute (PSCI) Protocol Review Committee or the PSCI Disease Team is required if the study involves cancer prevention studies or cancer patients, records and/or tissues. For more information about this requirement see the IRB website.

17.0 Multi-Site Study

17.1 Other sites
not applicable

17.2 Communication Plans
not applicable

17.3 Data Submission and Security Plan
not applicable

17.4 Subject Enrollment
not applicable

17.5 Reporting of Adverse Events and New Information
not applicable

17.6 Audit and Monitoring Plans
not applicable

18.0 Adverse Event Reporting

18.1 Adverse Event Definitions

For drug studies, incorporate the following definitions into the below responses, as written:	
Adverse event	Any untoward medical occurrence associated with the use of the drug in humans, whether or not considered drug related
Adverse reaction	Any adverse event caused by a drug
Suspected adverse reaction	Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than "adverse reaction". • *Reasonable possibility*. For the purpose of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event.
Serious adverse event or Serious suspected adverse reaction	Serious adverse event or Serious suspected adverse reaction: An adverse event or suspected adverse reaction that in the view of either the investigator or sponsor, it results in any of the following outcomes: Death, a life-threatening adverse event, inpatient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or a congenital anomaly/birth defect. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
Life-threatening adverse event or life-threatening suspected adverse reaction	An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator (i.e., the study site principal investigator) or Sponsor, its occurrence places the patient or research subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that had it occurred in a more severe form, might have caused death.
Unexpected adverse event or Unexpected suspected adverse reaction.	An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure, general investigational plan, clinical protocol, or elsewhere in the current IND application; or is not listed at the specificity or severity that has been previously observed and/or specified.

For device studies, incorporate the following definitions into the below responses, as written:	
Unanticipated adverse device effect	Any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or IDE application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

18.2 Recording of Adverse Events

Adverse events will be assessed at every visit after consent is obtained.
All adverse events (serious or non-serious) and abnormal test findings observed or reported to study team believed to be associated with the study drug(s) or device(s) will be followed until the event (or its sequelae) or the abnormal test finding resolves or stabilizes at a level acceptable to the investigator.

An abnormal test finding will be classified as an adverse event if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms

- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy

NOTE: Simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an adverse event.

- The test finding leads to a change in study drug dosing or discontinuation of subject participation in the clinical research study

The test finding is considered an adverse event by the investigator.

18.3 Causality and Severity Assessments

The investigator will promptly review documented adverse events and abnormal test findings to determine 1) if the abnormal test finding should be classified as an adverse event; 2) if there is a reasonable possibility that the adverse event was caused by the study drug(s) or device(s); and 3) if the adverse event meets the criteria for a serious adverse event.

If the investigator's final determination of causality is "unknown and of questionable relationship to the study drug(s) or device(s)", the adverse event will be classified as associated with the use of the study drug(s) or device(s) for reporting purposes. If the investigator's final determination of causality is "unknown but not related to the study drug(s) or device(s)", this determination and the rationale for the determination will be documented in the respective subject's case history.

Data collected and events reviewed by Dr. Elfar will also be reviewed by a non-conflicted investigator on the study.

18.4 Reporting of Adverse Reactions and Unanticipated Problems to the FDA

18.4. 1 Written IND/IDE Safety Reports

The Sponsor-Investigator will submit a written IND Safety Report (i.e., completed FDA Form 3500A) to the responsible new drug review division of the FDA for any observed or volunteered adverse event that is determined to be a serious and unexpected, suspected adverse reaction. Each IND Safety Report will be prominently labeled, "IND Safety Report", and a copy will be provided to all participating investigators (if applicable) and sub-investigators.

Written IND Safety Reports will be submitted to the FDA as soon as possible and, in no event, later than 15 calendar days following the Sponsor-Investigator's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

For each written IND Safety Report, the Sponsor-Investigator will identify all previously submitted IND Safety Reports that addressed a similar suspected adverse reaction experience and will provide an analysis of the significance of newly reported, suspected adverse reaction in light of the previous, similar report(s) or any other relevant information.

Relevant follow-up information to an IND Safety Report will be submitted to the applicable review division of the FDA as soon as the information is available and will be identified as such (i.e., "Follow-up IND Safety Report").

If the results of the Sponsor-Investigator's follow-up investigation show that an adverse event that was initially determined to not require a written IND Safety Report does, in fact, meet the requirements for reporting; the Sponsor-Investigator will submit a written IND Safety Report as soon as possible, but in no event later than 15 calendar days, after the determination was made.

18.4. 2 Telephoned IND Safety Reports – Fatal or Life-threatening Suspected Adverse Reactions

In addition to the subsequent submission of a written IND Safety Report (i.e., completed FDA Form 3500A), the Sponsor-Investigator will notify the responsible review division of the FDA by telephone or facsimile transmission of any unexpected, fatal or life-threatening suspected adverse reaction.

The telephone or facsimile transmission of applicable IND Safety Reports will be made as soon as possible but in no event later than 7 calendar days after the Sponsor-Investigator's receipt of the respective adverse event information and determination that it meets the respective criteria for reporting.

18.5 Reporting Adverse Reactions and Unanticipated Problems to the Responsible IRB

In accordance with applicable policies of The Pennsylvania State University Institutional Review Board (IRB), the investigator will report, to the IRB, any observed or reported harm (adverse event) experienced by a subject or other individual, which in the opinion of the investigator is determined to be (1) unexpected; and (2) probably related to the research procedures. Harms (adverse events) will be submitted to the IRB in accordance with the IRB policies and procedures.

18.6 Unblinding Procedures

In the case of a medical emergency the un-blinded IDS can disclose the randomization group. Contact information will be listed in the subject's electronic medical record (EMR). If such action is necessary, the Investigator and Safety Monitor will review the incident to determine if the subject should be withdrawn from the study. All emergency drug disclosures will also be reviewed at DSMC meetings to assure the continuing safety of research subjects.

18.7 Stopping Rules

Our safety analysis plan includes routine review (urgently within 48 hours) of every adverse event to the data safety monitoring board. The board will render a decision on suspension of the trial based on the available data in an unbiased manner. Examples of reasons for such a stoppage include adverse events that are not explainable, and patient dissatisfaction with the trial progression. The rules for stopping are not confined to adverse events, as defined by the drug information insert. The data safety monitoring board has the power to stop the trial for any reason surrounding safety, even if that includes investigating a particular patient's response to the trial drug or evaluation of the circulating levels of the drug at the time of an adverse event. Stopping rules include: Seizure, increased pain not treatable in the clinical research center, clear demonstration of a positive result which alters the statistical numbers for the trial, clear indications of deviations from the protocol, inadequate reporting by the trial team, and incomplete documentation of results.

19.0 Study Monitoring, Auditing and Inspecting

19.1 Study Monitoring Plan

19.1. 1 Quality Assurance and Quality Control

This is a single site study with low risk. 4AP is an FDA approved drug used in a patient population known to be more susceptible to its side-effect profile than the currently proposed study population. Nonetheless, we are implementing a full data and study monitoring apparatus to ensure study compliance within our single institution.

Elements of this monitoring body are a fully functional and active Data Safety Monitoring Board including a bio-ethicist to review each consented patient as well as a standardized system for

the reporting of adverse events (see explanation of each of these features below). Furthermore, we expect, invite and encourage institutional as well as outside monitoring of our trial execution through standardized means to ensure quality study execution.

The PI will monitor the study and ensure that this study is conducted, and that the data are generated, documented (recorded), and reported, in compliance with this protocol, with institutional and IRB policies, with Good Clinical Practice guidelines and any other applicable regulatory requirements.

The study will be monitored by the Clinical Trial Monitoring Team from the Department of Public Health Sciences at Penn State Hershey College of Medicine. The monitors will provide an independent review of the regulatory and subject records and the data collected to assure compliance with the protocol, GCP, and applicable federal regulations. The monitoring will occur at regular bimonthly intervals after the enrollment of the first subject and the times will be predetermined by the monitoring plan developed by the Clinical Trial Monitoring Team.

19.1. 2 Safety Monitoring

A Data Safety Monitoring Board (DSMB) will be constructed of individuals with varying backgrounds to allow for a holistic approach to data monitoring. One of the goals will be to completely and comprehensively capture any adverse events and process information to the FDA and regulatory bodies at Penn State in a straightforward manner.

We intend to include an ethicist as a lay member of the DSMB as well as key members of the Clinical and Translational Science Institute (CTSI). The CTSI places the highest priority on ensuring the safety of patients participating in clinical trials. The initial review of trial protocol lies with the CTSI Scientific Review Committee (SRC) to ensure that the trial, regardless of sponsorship or support, contains adequate plans for data and safety monitoring. The CTSI is therefore a natural component of our Data and Safety Monitoring Board (DSMB), which will be responsible for monitoring this investigator-initiated trial (IIT).

The DSMB has the responsibility for continuing review and monitoring of the study. The DSMB's review and oversight are written in to the IRB-approved protocol for such trials. The DSMB provides oversight of study progress and safety by review of the following information:

- Rates of accrual and retention.
- Frequency and severity of adverse events (AEs) and serious adverse events (SAEs).
- Response rates, where appropriate.
- New information related to the trial, i.e., published scientific reports or other developments that may affect subject safety or ethical concerns.
- Any changes to the anticipated risk/benefit ratio of the study that would affect its continuation.
- Protocol deviations and violations (although this is the subject of independent audits by the IRB as well).
- Matters that pertain to serious errors or potential misconduct by any of the investigators or research staff, i.e., breaches in confidentiality, research fraud.
- Subject complaints.
- Conflicts of interest.

The DSMB will be empowered to recommend a halt of the study should there be deviations from established gender or race metrics of presenting patients in this "consecutive consent" design. The timeline for review of trials by the DSMB is determined at the outset of the study with approval by the SRC. The frequency of DSMB review required for this protocol at six

months but will include an additional review after every ten patients recruited (whichever is most frequent). These details are recorded by the clinical coordinator, who prepares the skeleton of the report of the DSMB, which then adds the interpretation and evaluation sections of the report and then submits that to the IRB and study PI at least once yearly (but likely twice, depending on recruitment).

Roles in the reporting of adverse event(s) (AE(s)): The **Principal Investigator** will confirm that all adverse events (AE) are correctly entered into the AE case report forms by the coordinator; be available to answer any questions that the coordinators may have concerning AEs; and will notify the IRB, FDA, sponsor and/or DSMB of all applicable AEs as appropriate. All assessments of AEs will be made by a licensed medical professional who is an investigator on the research.

The **Research Coordinator** will complete the appropriate report form and logs; assist the PI to prepare reports and notify the IRB, FDA and/or DSMB of all Unanticipated Problems/SAE's.

The **Data Monitor** will confirm that the AEs are correctly entered into the case report forms. The Monitor will also confirm that the adverse events are consistent with the source documents and are reported to the appropriate regulatory bodies as required.

The ethicist will review every proposed consenting trial patient and report results monthly to the balance of the DSMB. Accrual will be monitored monthly by the DSMB based on this report.

Data Management and Monitoring: Monthly monitoring by the co-investigator (from the Department of Public Health Sciences) will also be performed as per the mechanism offered by the Penn State Clinical Trial Data Management Center (housed within the same department). Trial outcome data will be held in a secure server within that department and an interim statistical analyses will be undertaken at the approximate midpoint for each specific aim (13 patients) via the Obrien-Fleming group sequential method to monitor the trial data. Early achievement of the trial result will be identified by this mechanism and written reports of progress towards this achievement will be relayed to the DSMB by this co-investigator. Quality control of serum data will be ensured through dual laboratory certification of serum results. The same control of clinical data will be ensured through dual independent assessment of each test on the patient during testing periods, and interpretation of EDX results by a qualified specialized blinded participating clinician. Analysis for any given patient's data will be contained to the timeline in Figure 2.

20.0 Future Undetermined Research: Data and Specimen Banking

20.1 Data and/or specimens being stored

not applicable

20.2 Location of storage

not applicable

20.3 Duration of storage

not applicable

20.4 Access to data and/or specimens
not applicable

20.5 Procedures to release data or specimens
not applicable

20.6 Process for returning results
not applicable

21.0 References

1. Robinson LR. Role of neurophysiologic evaluation in diagnosis. J Am Acad Orthop Surg. May-Jun 2000;8(3):190-199.
2. Robinson LR. Traumatic injury to peripheral nerves. Muscle Nerve. Jun 2000;23(6):863-873.
3. Lerner A, Soudry M. *Armed Conflict Injuries to the Extremities: A Treatment Manual.* Springer Science & Business Media; 2011.
4. Lundborg G. *Nerve Injury and Repair: Regeneration, Reconstruction, and Cortical Remodeling.* Elsevier/Churchill Livingstone; 2005.
5. Brushart TM. *Nerve Repair.* Oxford University Press; 2011.
6. Mackinnon SE, Dellon AL. *Surgery of the Peripheral Nerve.* Thieme Medical Publishers; 1988.
7. Birch R. *Surgical Disorders of the Peripheral Nerves.* Springer London; 2011.
8. Wilbourn AJ. The electrodiagnostic examination with peripheral nerve injuries. Clinics in plastic surgery. 2003;30(2):139-154.
9. Seddon HJ, Medawar PB, Smith H. Rate of regeneration of peripheral nerves in man. The Journal of physiology. 1943;102(2):191-215.
10. Seddon HJ. Nerve grafting. Annals of the Royal College of Surgeons of England. 1963;32:269-280.
11. Sunderland S, Bradley KC. Denervation atrophy of the distal stump of a severed nerve. The Journal of comparative neurology. 1950;93(3):401-409.
12. Sunderland S. A classification of peripheral nerve injuries producing loss of function. Brain : a journal of neurology. 1951;74(4):491-516.
13. Sir SS. *Nerves And Nerve Injuries.* Churchill Livingstone; 1978.
14. Grass G, Kabir K, Ohse J, Rangger C, Besch L, Mathiak G. Primary Exploration of Radial Nerve is Not Required for Radial Nerve Palsy while Treating Humerus Shaft Fractures with Unreamed Humerus Nails (UHN). Open Orthop J. 2011;5:319-323.
15. Korompilias AV, Lykissas MG, Kostas-Agnantis IP, Vekris MD, Soucacos PN, Beris AE. Approach to radial nerve palsy caused by humerus shaft fracture: is primary exploration necessary? Injury. Mar 2013;44(3):323-326.
16. Niver GE, Ilyas AM. Management of radial nerve palsy following fractures of the humerus. Orthop Clin North Am. Jul 2013;44(3):419-424, x.
17. Li Y, Ning G, Wu Q, Wu Q, Li Y, Feng S. Review of literature of radial nerve injuries associated with humeral fractures-an integrated management strategy. PLoS One. 2013;8(11):e78576.
18. Rocchi M, Tarallo L, Mugnai R, Adani R. Humerus shaft fracture complicated by radial nerve palsy: Is surgical exploration necessary? Musculoskelet Surg. Dec 2016;100(Suppl 1):53-60.
19. Han SH, Hong IT, Lee HJ, Lee SJ, Kim U, Kim DW. Primary exploration for radial nerve palsy associated with unstable closed humeral shaft fracture. Ulus Travma Acil Cerrahi Derg. Sep 2017;23(5):405-409.
20. Chang G, Ilyas AM. Radial Nerve Palsy After Humeral Shaft Fractures: The Case for Early Exploration and a New Classification to Guide Treatment and Prognosis. Hand Clin. Feb 2018;34(1):105-112.

21. METRC Nerve Study: https://metrc.org/researchstudies/54-infopages/crsd/610-nervestudydescription – (personal communication, with interim results review performed in 3/2018).
22. Perry JD. Electrodiagnosis in musculo-skeletal disease. Best Pract Res Clin Rheumatol. Jun 2005;19(3):453-466.
23. Korte N, Schenk HC, Grothe C, Tipold A, Haastert-Talini K. Evaluation of periodic electrodiagnostic measurements to monitor motor recovery after different peripheral nerve lesions in the rat. Muscle Nerve. Jul 2011;44(1):63-73.
24. Capacio BR, Byers CE, Matthews RL, Chang FC. A method for determining 4-aminopyridine in plasma: pharmacokinetics in anaesthetized guinea pigs after intravenous administration. Biomedical chromatography : BMC.10(3):111-116.
25. Netter FH. *Atlas of Human Anatomy, Professional Edition E-Book: including NetterReference.com Access with Full Downloadable Image Bank.* Elsevier Health Sciences; 2014.
26. Elfar JC, Yaseen Z, Stern PJ, Kiefhaber TR. Individual finger sensibility in carpal tunnel syndrome. J Hand Surg Am. Nov 2010;35(11):1807-1812.
27. Sahin F, Atalay NS, Akkaya N, Ercidogan O, Basakci B, Kuran B. The correlation of neurophysiological findings with clinical and functional status in patients following traumatic nerve injury. J Hand Surg Eur Vol. Feb 2014;39(2):199-206.
28. Sims SE, Engel L, Hammert WC, Elfar JC. Hand Sensibility, Strength, and Laxity of High-Level Musicians Compared to Nonmusicians. J Hand Surg Am. Oct 2015;40(10):1996-2002 e1995.
29. Chimenti PC, McIntyre AW, Childs SM, Hammert WC, Elfar JC. Prospective cohort study of symptom resolution outside of the ulnar nerve distribution following cubital tunnel release. Hand (N Y). Jun 2015;10(2):177-183.
30. Chimenti PC, McIntyre AW, Childs SM, Hammert WC, Elfar JC. Combined Cubital and Carpal Tunnel Release Results in Symptom Resolution Outside of the Median or Ulnar Nerve Distributions. Open Orthop J. 2016;10:111-119.
31. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. Feb 8 1986;1(8476):307-310.
32. Cohen J. A coefficient for agreement for nominal scales. Educational and Psychological Measurement. 1960;20:37-46.
33. Fleiss JL. Measuring nominal scale agreement among many raters. Psychological Bulletin. 1971;76:378-382.
34. Kim J, Farahmand M, Dunn C, et al. Evaporimeter and Bubble-Imaging Measures of Sweat Gland Secretion Rates. PLoS One. 2016;11(10):e0165254.
35. Kiistala R, Kiistala U, Parkkinen MU, Mustakallio KK. Local sweat stimulation with the skin prick technique. Acta Derm Venereol. 1984;64(5):384-388.
36. Park SJ, Tamura T. Distribution of evaporation rate on human body surface. Ann Physiol Anthropol. Nov 1992;11(6):593-609.
37. Buchmann SJ, Penzlin AI, Kubasch ML, Illigens BM, Siepmann T. Assessment of sudomotor function. Clin Auton Res. May 8 2018.
38. Cardenas DD, Ditunno J, Graziani V, et al. Phase 2 trial of sustained-release fampridine in chronic spinal cord injury. Spinal Cord. Feb 2007;45(2):158-168.
39. Sims SEG, Engel L, Hammert WC, Elfar JC. Hand sensibility, strength, and laxity of high-level musicians compared to nonmusicians. Journal of Hand Surgery. 2015 2015;40(10):1996-2002.

22.0 Confidentiality, Privacy and Data Management

See the Research Data Plan Review Form