Study title: **A new method for identifying sensory changes in painful chemotherapy-induced peripheral neuropathy (CIPN): a feasibility study**
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RESEARCH PROTOCOL

A new method for identifying sensory changes in painful chemotherapy-induced peripheral neuropathy (CIPN): a feasibility study

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Feasibility Study of New Method of Diagnostic and Prediction of Painful CIPN

Protocol Revision History

Initial Approval Version	**8/3/2018**
Amendment #1 Version	**11/12/2018**
Amendment #2 Version	**4/26/2019**
Amendment #3 Version	**6/6/2019**
Amendment #4 Version	**6/25/2019**
Amendment #5 Version	**7/31/2019**
Amendment #6 Version	**8/20/2020**

1. SYNOPSIS

Study Title	A new method for identifying sensory changes in painful chemotherapy-induced peripheral neuropathy (CIPN): a feasibility study
Objective	In this other interventional study, we will test the utility of the Diode Laser fiber type Selective Stimulator (DLss) to identify sensory changes that are unique to patients with painful chemotherapy induced peripheral neuropathy (CIPN) vs. controls.
Hypothesis	Painful symptoms of CIPN develop in patients with differential nerve damage to Aδ vs C-type peripheral nerve fibers. We hypothesize that Aδ:C fiber threshold ratio, as measured by the DLss, will be different between patients with painful CIPN compared to control patients who received a similar regimen of chemotherapy, but did not develop painful CIPN. The confirmation of hypothesis may lead to a novel approach for early detection of CIPN.
Study Period	Enrollment: September 1, 2018 – March 1, 2020 Planned completion: April 1, 2020
Number of Patients	Subjects: 20 evaluable patients with painful CIPN following treatment with oxaliplatin, cisplatin, paclitaxel, docetaxel (or any combination of above) Controls: 20 controls matched by the type of chemotherapy received, who did not develop painful CIPN.
Study Treatment	The study procedure will include a one-time visit for sensory assessments, as proposed in Measurements section.
Study Design	Other Interventional study
Inclusion and Exclusion Criteria	Inclusion criteria: ***Group: A Painful CIPN group*** 1. Age >18 2. Distal symmetric pain distribution (both feet, with or without pain in hands). 3. The pain appeared during or up to 12 weeks after treatment with oxaliplatin, cisplatin, paclitaxel, docetaxel or any combination of these. 4. Score of 4 or more on DN4 neuropathic pain questionnaire 5. Pain duration > 2 months. 6. Patient report of average daily pain intensity in the last week ≥3 on 0-10 Numerical Rating Scale (NRS). 7. Able and willing to sign an IRB-approved written informed consent. ***Group B: Control group:*** 1. Age >18 2. History of cancer diagnosis, previously treated with at least 8 infusions of chemotherapy regimen that included oxaliplatin, or at least 6 infusions of

	chemotherapy regimen that included cisplatin, paclitaxel or docetaxel, or any combination of these. 3. No ongoing pain in distal symmetric distribution • Subjects with symptoms and signs such as mild numbness, or vibration sensation loss are eligible to be included in the control group. • Subjects with pain duration of less than 2 years after treatment, have been pain free for a year or more, and are not currently receiving treatment for painful CIPN are eligible to be included in the control group. 4. Able and willing to sign an IRB-approved written informed consent. * Subjects in the control group will be matched by the type of previous chemotherapy to the subjects in the Painful CIPN group. An additional attempt will be made to match controls by sex, age, cancer diagnosis, and cumulative neurotoxic chemotherapy dose. <u>Exclusion criteria:</u> Group A & B 1. History of pre-existing painful distal symmetric polyneuropathy prior to chemotherapy. 2. Alternative etiology exists for the distal painful symptoms. 3. Current or previous treatment with a vinca alkaloid (e.g. vincristine, vinblastine), bortezomib, or another agent which may cause major peripheral neurotoxicity. 4. Pregnancy 5. Concomitant medication as follows: • Patients receiving chronic daily opioids, topical lidocaine (on feet) or topical capsaicin (on feet) will be excluded. • Patients receiving PRN analgesics, including acetaminophen, NSAIDs or short-acting opioids, will be required not to take them 48h before testing, at for at least five half-lives of the specific analgesic, at the discretion of the investigators.
Measurements	<u>The following tests will be performed at baseline:</u> 1. Spontaneous pain at baseline on 0-10 Numerical Rating Scale (NRS); 2. Assessment of pain symptoms on Neuropathic pain Symptom Inventory (NPSI) and Brief Pain Inventory (BPI). 3. Assessment of mood on hospital anxiety and depression scale (HADS) 4. Quantitative sensory testing (QST): thermal detection and pain thresholds, mechanical detection threshold, temporal summation (TS), and conditioned pain modulation (CPM). <u>The following tests may be performed in a subset of participants:</u> 5. Diode Laser fiber type Selective Stimulator (DLss) to assess Aδ:C fiber threshold ratio and cutaneous flare response.

Statistical Methodology	The primary outcome is the comparison of Aδ:C fiber threshold ratio between patients who have developed painful CIPN, and the control subjects. We will use t-test for inter-group comparison, if the two groups are matched. If groups do not match on key demographic variables, we will perform multivariable analysis while controlling for key variables. In secondary analyses, we will generate Spearman correlation coefficient between the "Aδ:C fiber threshold ratio" and the severity of painful CIPN on NPSI scale. In addition, we will compare the quantitative sensory testing parameters (thermal and mechanical thresholds and CPM) between the groups.

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2. BACKGROUND AND SIGNIFICANCE

2.1. Peripheral Neuropathy

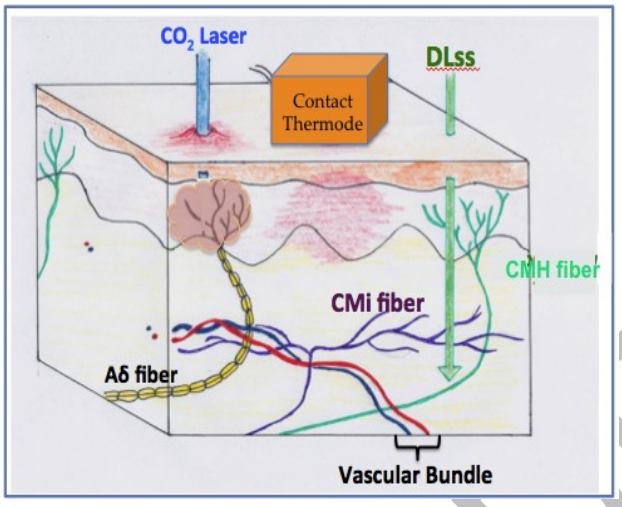
Peripheral neuropathy is a common side effect of chemotherapy, occurring in more than 60% of patients at some point during the course of cancer treatment with commonly used drugs such as taxanes and platinum compounds [41]. The resulting pain, numbness, and weakness can severely diminish quality of life. For many patients, the development of neuropathy leads to dose reduction or/and treatment delay, which may ultimately impact survival. The mechanisms by which chemotherapy-induced nerve damage ultimately leads to pain are poorly understood, because virtually no structural or functional differences in nerve fibers between painless and painful peripheral neuropathy have been identified [44]. As a result, there is no reliable way to predict which patients will develop persistent painful chemotherapy-induced peripheral neuropathy (CIPN) [1,23,24] and consequently, no effective preventative strategy exists so far. The NIH (PA-12-083) has recognized this gap. This SBIR-funded project proposes the use of a new, patented, noninvasive test to interrogate specific subtypes of small diameter nerve fibers in patients with CIPN, addressing the need for an early diagnostic tool and ultimately predicting which patients should be offered early intervention to prevent persistent painful CIPN.

Two types of nerve fibers found within the epidermal and dermal skin layers, lightly myelinated Aδ and small unmyelinated C fibers, transmit nociceptive (pain) information. In certain types of painful neuropathies, such as painful diabetic neuropathy and CIPN, there is a dramatic dying back or degeneration of these epidermal fibers [21,31,32,34]. These patients have on one hand ongoing pain, and on the other hand often demonstrate significantly increased pain thresholds (i.e. lower sensitivity to evoked pain) when tested with currently available methods, which primarily activate epidermal fibers, such as the CO_2 laser or contact heat thermodes [32,37]. In experimental models, ablation of the nociceptive fibers leads to loss of pain sensitivity, rather than pain, suggesting *nerve fiber loss alone is not sufficient to explain the development of pain [27]*.

In contrast, spontaneous activity of small fibers is associated with painful peripheral neuropathy (PPN) in animals and humans [10,20,51]. Recently, a specific subtype of C fibers, the C mechano-insensitive (CMi) fibers, were found to be spontaneously active in patients with painful CIPN [20] as well as in other types of PPN [42]. CMi fibers are located primarily in the dermis, have widely branching afferent arbors, are relatively insensitive to mechanical stimuli, but respond to noxious heat and chemicals [25]. When activated, these fibers release chemokines that can cause vasodilation and may, in turn, sensitize surrounding fibers. In a rat model of paclitaxel-induced painful neuropathy, intra-epidermal nerve fiber degeneration was prominent, but deeper sub-epidermal axon bundles, where CMi reside, were spared [5]. These characteristics suggest that CMi fibers play a critical role in generating peripheral pain in CIPN.

There are several practical limitations to studying small nerve fibers, particularly CMi fibers, in patients suffering from CIPN. Current diagnostic tests are invasive, extremely time consuming, unable to selectively activate small fiber subtypes, or cannot safely stimulate deep fibers. Conventional electromyography and nerve conduction studies only provide information about large fibers. Microneurography can reliably distinguish small fiber types but it requires significant expertise and hours of invasive testing, which is impractical for broad clinical use. Skin biopsy can be used to quantify small fiber density, but does not always correlate with CIPN symptomatology

[16,31]. Noninvasive techniques, such as the quantitative sensory testing (QST) battery with radiant heat or contact thermodes or with the laser evoked potentials (based on CO_2 laser) do not selectively activate C versus Aδ, and can only safely be used to interrogate superficially located fibers [6,22,48] (see **Figure 1**). CMi fibers require current densities five times higher than epidermal polymodal nociceptors [49]. Similarly, the amount of radiant (e.g., non-coherent) heat required to activate CMi fibers results in surface temperatures that are about 7 °C higher than the activation threshold of C polymodal nociceptors [40,49] Consequently, both electrical and radiant heat stimuli activate C polymodal nociceptors at intensities well below those for activating CMi fibers, rendering them non-selective and of limited use in evaluating CMi status in patients. In other words, currently available psychophysical tests do not allow for the assessment of CMi fiber function.



In contrast, the Diode Laser fiber type Selective Stimulator (DLss) selectively stimulates Aδ and C fibers at much greater depths than existing techniques, which makes it an ideal method to assess small fiber subtypes separately. Thus, this technology opens the possibility for bedside clinical testing of a broad array of small fiber neuropathies.

Figure 1. Cross-section of skin demonstrating deep, uniform penetration of the DLss. The CO_2 laser uses energy levels that damage skin when penetrating beyond the epidermis. Thermal contact diodes emit distributive heat and require damaging levels of heat to active CMi fibers.

2.2. Preliminary Work

In a series of 16 patients with PPN (painful diabetic neuropathy and painful CIPN), the DLss Aδ fiber protocol revealed significantly increased pain thresholds compared to healthy volunteers, but C-fiber protocol pain thresholds were similar to those recorded in healthy volunteers [29]. This is in distinct contrast to radiant or surface heating methods, which produce significantly higher heat pain thresholds in DPN patients [3,32]. The difference in threshold measurements between the DLss and other heating methods cannot be explained by activation of the same fiber type. Instead, it is likely that heat pain thresholds measured using the DLss are the result of deeper CMi fiber activation, whereas radiant or surface heating evokes pain via activation of epidermal C polymodal nociceptors (see **Figure 2**). The six patients with painful CIPN tested in our small pilot had a statistically significant difference in the Aδ:C pain threshold ratio when compared patients with normal volunteers (see **Figure 3**). The range of pain stimulation was set up from pain detection to mild pain and well tolerated by both group of patients. No patients withdrew from this study due to pain caused by the

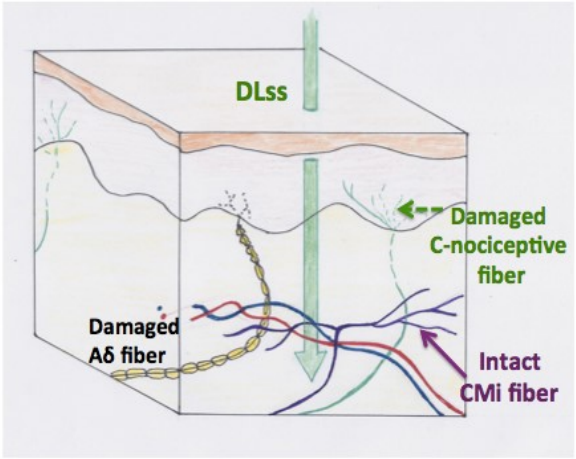


Figure 2. Chemotherapy results in damage or dying back of Aδ and C nociceptive fibers in the epidermis, while dermal fibers are relatively spared. The DLss can penetrate into the dermis. Sparing the CMi in the presence of damage to Aδ results in an increased Aδ:C pain threshold ratio.

testing. No healthy patients in these studies had a ratio >2. This was a small non-uniform sample and was not powered to detect a change in ratios *a priori*. These preliminary data suggest a robust difference between patients with painful CIPN and normal patients. *Therefore, we hypothesize that there is a significant relationship between the Aδ:C fiber threshold ratio and painful CIPN.*

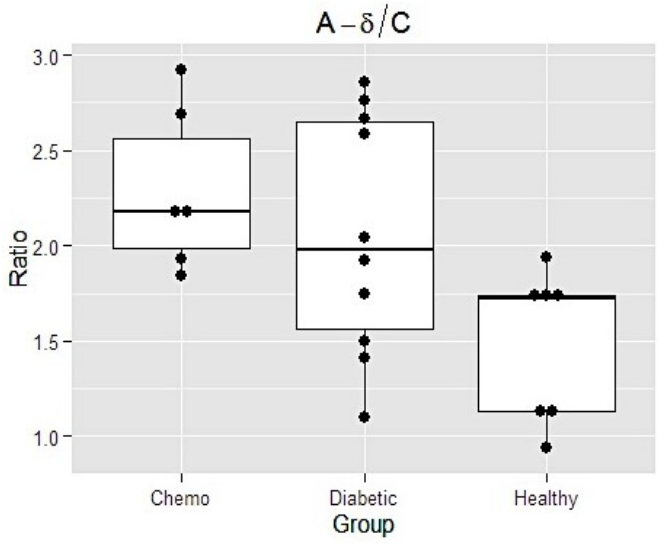


Figure 3. Pain threshold ratio of Aδ to C fibers, activated by DLss for 3 patient groups: healthy, painful diabetic neuropathy, and painful CIPN. P<0.05, one-way ANOVA.

We hypothesize, that this differential *Aδ:C* ratio may be a hallmark of painful (vs painless) CIPN. In this study, we will test the above hypothesis in patients with cancer, who have received chemotherapy which includes oxaliplatin, cisplatin, paclitaxel, docetaxel chemotherapy (or any combination of these). Both the platinum compounds, and the taxanes, individually and in combination, are known to cause painful peripheral neuropathy in a proportion of patients. The mechanism of nerve injury might somewhat differ, but both drug classes ultimately cause painful peripheral distal symmetric neuropathy [8]. Because the proposed underlying pathophysiology of the pain is shared, we feel comfortable grouping these patients. We propose that using the DLss in patients with CIPN will allow for the assessment of changes in small-fiber pain thresholds and correlation of these changes to painful versus painless states. Additionally, we would like to investigate whether the DLss correlates with pain severity in persistent painful neuropathy.

The ultimate goal of this study is to develop a non-invasive, bedside quantitative test that is specific for painful CIPN. If our initial hypothesis is confirmed, the next step would be to design a prospective longitudinal study and assess changes in DLss early after initiation of chemotherapy, to determine whether this approach can help identify early predictive parameters of painful CIPN.

The potential implications of this research are the following:

1. It can help identify patients who are likely to develop painful neuropathy and introduce dose/drug modifications to avoid dose-liming toxicity.
2. For practitioners and patients, this approach could help avoid the use of more invasive, time-consuming tests, and non-small-fiber oriented testing, such as electromyography, for the diagnosis of CIPN.
3. This approach would be also extremely useful for CIPN prevention studies of investigational drugs, so that only high-risk patients are exposed to preventive interventions. Since some patients will not develop CIPN, a stratified approach like this can help avoid unnecessary exposure to investigational agents in patients who are unlikely to benefit from the intervention.
4. While this study focuses on taxane- and platinum- based regimens, the results could be potentially useful for cancer patients receiving other neurotoxic drugs such as vinca alkaloids, or patients with hematologic malignancies treated with proteasome inhibitors such as bortezomib [15].

3. OBJECTIVES

Painful peripheral neuropathy (PPN) is a common sequela of chemotherapy that severely impacts the quality of life in cancer patients [41]. There are currently no biomarkers or methods to predict the development or progression of painful chemotherapy-induced peripheral neuropathy (CIPN). The pathophysiology of CIPN remains insufficiently understood; current treatment and prevention strategies provide only modest benefit to patients [18].

The proposed work will provide valuable information about the development of pain in CIPN caused by common chemotherapies used to treat ovarian, colon, and other cancers. It will provide the basis for a larger study to validate the utility of DLss as a noninvasive bedside test for small-fiber neuropathies and provide a tool for investigators and clinicians to track and predict the development of pain in CIPN.

In this study, we will test the utility of the Diode Laser fiber type Selective Stimulator (DLss) [33] in identifying changes that are specific to painful CIPN, compared to controls who received chemotherapy but did not develop painful neuropathy.

Small-diameter lightly myelinated Aδ and unmyelinated C cutaneous nociceptive fibers transmit pain from the peripheral to central nerve system. Aδ and C fibers are divided by sensitivity: heat, mechanical (tactile), and chemical stimulation, and epidermal versus dermal location. In animals and humans, dying back intra-epidermal fibers lead to reduced pain sensitivity, rather than pain, which suggests fiber loss alone is not sufficient to explain the development of neuropathic pain [2,7,11,32,37,38]. In contrast, abnormally high spontaneous activity of nociceptive fibers, specifically dermal C mechano-insensitive (CMi) fibers, is associated with peripheral ongoing neuropathic pain [25,39,49]([10,20,51]. Therefore, a tool that can measure, track, and predict the development of abnormal function of Aδ and C fibers is a critical unmet medical need.

Most diagnostic tests to study nociceptive fibers are able to measure the loss of pain sensitivity in epidermal fibers [2,7,11,32,37,38]. However, these tests are not fiber-type selective [49]. Only microneurographic recording is able to separate fiber type and to access single fibers [42]. Though due to complexity, microneurography is unpractical in the clinic. In contrast, DLss has been developed and patented to be used at the bedside to safely and selectively stimulate Aδ and C fibers in superficial and deep skin [9,19,20,28,29,42,45-47,51]. Our preliminary DLss data demonstrate that patients with painful CIPN, who have decreased epidermal Aδ and C-fiber densities, have increased Aδ pain thresholds, while C-fiber thresholds are intact [29]. The Aδ:C pain threshold ratio was consistently higher in CIPN than healthy volunteers. To add an objective measure or C-fiber stimulation, we will also add a measurement of cutaneous flare response to DLss stimulation. As we have previously shown, C-fiber stimulation, by releasing vasoactive peptides, can cause cutaneous flare response that can be captured by laser Doppler or thermal imaging [17].

Based on these preliminary data, *we propose that the Aδ:C fiber threshold ratio, as measured by the DLss, can differentiate between patients with painful CIPN vs control patients who received similar chemotherapy but did not develop painful neuropathy.*

The key objective of the study is to demonstrate that the Aδ:C fiber threshold ratio is significantly higher in patients with painful CIPN than in patients who did not develop painful CIPN following similar cancer chemotherapy.

Ultimately, we hypothesize that this will assist in identification of patients early in their chemotherapy treatment who are likely to develop painful neuropathy: 1) so that these may benefit from a change in their treatment to prevent CIPN , and 2) to target CIPN preventative intervention in more personalized manner to avoid unnecessary drug exposure in low risk patients.

4. PATIENT SELECTION

4.1. Inclusion Criteria

Group A: Painful CIPN group

1. Age >18
2. Distal symmetric pain distribution (both feet, with or without pain in hands).
3. The pain appeared during or up to 12 weeks after treatment with oxaliplatin, cisplatin, paclitaxel, docetaxel or any combination of these.
4. Score of 4 or more on DN4 neuropathic pain questionnaire
5. Pain duration > 2 months.
6. Patient report of average daily pain intensity in the last week ≥3 on 0-10 Numerical Rating Scale (NRS).
7. Able and willing to sign an IRB-approved written informed consent.

Group B: Control group:

1. Age >18
2. History of cancer diagnosis, previously treated with at least 8 infusions of chemotherapy regimen that included oxaliplatin, or at least 6 infusions of chemotherapy regimen that included cisplatin, paclitaxel or docetaxel or any combination of these.
3. No ongoing pain in distal symmetric distribution.
    - Subjects with symptoms and signs such as mild numbness, or vibration sensation loss are eligible to be included in the control group.
    - Subjects with pain duration of less than 2 years after treatment, have been pain free for a year or more, and are not currently receiving treatment for painful CIPN are eligible to be included in the control group.
4. Able and willing to sign an IRB-approved written informed consent.

* Subjects in the control group will be matched by the type of previous chemotherapy to the subjects in the Painful CIPN group. An additional attempt will be made to match controls by sex, age, cancer diagnosis, and cumulative neurotoxic chemotherapy dose.

4.2. Exclusion Criteria

1. History of pre-existing painful distal symmetric polyneuropathy prior to chemotherapy.
2. Alternative etiology exists for the distal painful symptoms.
3. Current or previous treatment with a vinca alkaloid (e.g. vincristine, vinblastine), bortezomib, or another agent which may cause major peripheral neurotoxicity.
4. Pregnant
5. Concomitant medication as follows:
    - Patients receiving chronic daily opioids, topical lidocaine (on feet) or topical capsaicin (on feet) will be excluded.
    - Patients receiving PRN analgesics, including acetaminophen, NSAIDs or short-acting opioids, will be required not to take them 48h before testing, or for at least five half-lives of the specific analgesic, at the discretion of the investigators.

4.3. Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

5. REGISTRATION PROCEDURES

5.1.Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below:

1. Registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

5.2.Patient Registration in the Siteman Cancer Center OnCore Database

All patients must be registered through the Siteman Cancer Center OnCore database.

5.3.Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. All data will be recorded with this identification number on the appropriate CRFs.

6. STUDY DESIGN

The study is an interventional study. It will include 2 groups, 20 evaluable patients with painful CIPN following treatment with oxaliplatin, cisplatin, paclitaxel, docetaxel and 20 controls matched by the type of chemotherapy received, who did not develop painful CIPN.

7. STUDY PROCEDURES

7.1. Study Period

This is an open-label, interventional protocol testing a diagnostic device for the characterization of chemotherapy induced peripheral neuropathy. Patients will be stratified into Group A or Group B after completing the pain questionnaires. *There is no difference in testing protocols between these groups.*

Patients will be studied at a Washington University School of Medicine facility. After obtaining signed informed consent, we will collect patient demographic data from the medical record including details on cancer type and status, chemotherapy regimen(s) and cumulative doses, history of chronic pain, concomitant diseases and medication. Spontaneous pain at baseline on 0-10 Numerical Rating Scale (NRS) and for the painful CIPN group we will also collect duration of painful CIPN.

A pregnancy test will be performed on women of childbearing potential and subjects excluded if pregnant.

The patients will complete the following questionnaires: assessment of pain symptoms on Neuropathic Pain Symptom Inventory (NPSI) and Brief Pain Inventory (BPI), and assessment of mood on Hospital Anxiety and Depression Scale (HADS).

The subjects will undergo quantitative sensory testing (QST) to assess warm and cold detection thresholds, heat and cold pain thresholds, mechanical detection, presence of wind-up (enhanced temporal summation) to pinprick, and we will apply conditioned pain modulation (CPM) protocol (per QST protocol below).

7.2. QST protocol

Quantitative sensory testing will be performed on the dorsal mid-foot. In the painful CIPN group, if asymmetry in pain intensity exists between extremities, QST will be performed in the more painful foot; otherwise the foot will be chosen randomly. The ipsilateral shoulder will serve as control area. A description of the QST procedures follows:

Thermal detection and thermal pain thresholds
Equipment: The Thermal Sensory Analyzer (TSA-II or PATHWAY platform - Medoc, Ramat Yishai, Israel) will be used to determine thermal detection and pain thresholds. This equipment is used globally for functional (psychopysical) assessment of pain and temperature-conducting nerve fibers (C and Aδ fibers).

Method and Background: Using the Thermal Sensory Analyzer, cold and warm detection thresholds (CDT and WDT, respectively), as well as cold and heat pain thresholds (CPT and HPT, respectively) will be determined [13,52]. The thermode with contact area of 9.0 cm^2 is applied to the tested site, and all thresholds are determined by continuous ramping of temperature from 32°C baseline temperature by 1°C/s until the subject presses the ‘stop‘ button. Cut-off temperatures are

0°C and 50°C, to minimize thermal damage to the skin. The baseline temperature to which the thermode returns before each test is 32°C. The average threshold is calculated from three measurements in each area.

Determination of mechanical detection threshold (MDT)
Equipment: A set of standardised von Frey filaments (0.25, 0.5, 1, 2, 4, 8, 16, 32, 64, 128 and 256mN). The contact area of the hairs with the skin is of uniform size (<1 mm^2) and texture.

Methods and Background: Standardised von Frey filaments [12,50] will be used in a modified "method of limits" manner using 3 series of increasing and decreasing stimulus intensities to determine the geometric average as the tactile detection threshold of the affected and unaffected skin areas [4].

Von Frey filaments of different stimulus intensities are used to determine the tactile detection thresholds. A filament eliciting 16mN force* is applied first, followed by filaments of consecutively lower intensity until the patient cannot detect the stimulus being applied. This respective force represents the first threshold value. The order in which the stimuli are applied is then reversed and stimuli of consecutively greater intensity are applied until sensation is detected (this intensity becomes the second value). Again filaments with decreasing intensity are applied until in total 3 upper and lower values of detection are fulfilled from which the mechanical detection threshold can be determined.

* In case the first von Frey filament with an intensity of 16mN is not detected, the next highest intensity filament which can be detected must be used as a starting intensity. However, the relevant force of this stimulus is not documented. Filaments with consecutively lower intensity are applied until the patient cannot detect the stimulus being applied. The procedure is followed as above; until in total 3 upper and lower values of detection are fulfilled from which the mechanical detection threshold can be determined.

Determination of wind-up ratio (WUR)
Equipment: A pinprick stimulus with standardised intensity (#6.10 von Frey filament, approx. 980mN) and a flat contact area of 0.25mm diameter.

Methods and Background: In this test a pinprick (980mN) is first applied singularly. After that a series of 10 identical pinprick stimuli are applied with a frequency of 1 s^{-1} within an area of 1 cm^2. Immediately following the single stimulus and series of stimuli, an evaluation of the sensation must be provided according to NRS (0-10, '0': 'no pain', '10': 'worst pain imaginable'). A ratio is calculated using these values. This procedure shall be repeated twice. A geometric average of the 'wind-up' is calculated from the two ratios [26,36].

Determination of conditioned pain modulation (CPM) efficiency
Equipment: Cold water bath, The Thermal Sensory Analyzer (Medoc, Ramat Yishai, Israel) will be used for CPM paradigm testing.

Methods and Background
CPM testing includes the application of a "test" stimulus without conditioning, and a subsequent application of the same test stimulus with conditioning.

Test stimulus: The thermode is applied at a volar forearm of an individual, with temperature ramping as described above for HPT measurement. The "test" stimulus is individually determined as the temperature that elicits pain intensity of 60 on 0-100 NRS in the individual.

After 15 minutes rest, this stimulus is applied and the subject-reported pain intensity is measured. The procedure is repeated twice.

Conditioning: The conditioning stimulus includes the immersion of the contralateral hand up to the wrist to a thermostat-controlled water bath maintained at 12°C.

The length of the conditioning stimulus is 60 seconds, and during the last 30 seconds of it the test stimulus is applied (at the contralateral forearm) twice, as described above.

The difference between the intensity of pain stimulus with concomitant conditioning and between the intensity of pain stimulus without conditioning is the CPM magnitude. CPM<0 implies efficient descending pain modulation.

If the participant cannot tolerate the cold water hand immersion for 45 seconds or more, the water temperate will be readjusted and the CPM testing repeated.

7.3. DLss testing per the following protocol

We anticipate the total testing will take 40 minutes or less
Subjects will be comfortably seated in a treatment recliner or in a gurney in a private room. Patient and the examiner will wear safety goggles. The door will be closed. The areas of skin to be tested will be shaved to avoid differences in light absorption and heating, because stimulation is non-contact; but strong pigmentation spots, tattoos, and moles will be avoided.

Patients will be reminded that if pain becomes too difficult for them to tolerate, they can stop the testing at any time.

The skin on the dorsum of the foot will be marked with a pen denoting 10mmx10mm squares.

The skin temperature will be monitored by infrared thermometer before the test and several times during testing. We will attempt to maintain a skin temperature of 32-33°C. If skin cools down by more than 1.5 °C from baseline, the skin will be heated with warmed towels/heating pad and maintained to keep target temperature.

Each patient will have an A and C fiber stimulation. Stimulation will be performed on the dorsum of the foot using stimulation previously published parameters to elicit "burning pain," which is from activation of C-fibers and "pinprick" pain from A-fibers:

-*Aδ fiber protocol*: 60-millisecond duration, 980-nm stimuli, 1-mm diameter stimuli
-*C fiber protocol*: 2-second duration, 980-nm stimuli, 5-mm diameter stimuli

Stimulation will begin at 800mA current for C fiber and increased at no more than 100mA

increments based on the subject's response. The current will be increased up to a maximum of 1650 mA for the C-fiber protocol. Stimulation will begin at 1000mA current for Aδ fiber and increased at no more than 200mA increments based on the subject's response. The current will be increased up to a maximum of 4000 mA for the Aδ-fiber protocol. In rare cases, patients may have pain thresholds that exceed the maximum skin temperature considered safe. For these patients, we will use the maximum safe stimulus cut-off (4000 mA for the Aδ fiber, 1650 mA for the C fiber) to calculate the Aδ:C ratio.

The pain threshold will be obtained using the "method of levels", using a series of ascending stimulation from energy that does not evoke sensation and increasing by steps until the last sensation is recorded as a 30-40 on the 0-100 pain scale. (0= no sensation, 10= definite sensation/pain, 100 worst imaginable pain). The procedure will then be repeated three times and the pain threshold will be defined as averaging the readings of the last 3 successive stimulations.

The area of stimulation will be moved by a minimum of 5 millimeters between each stimulation according to the grid.

In a subset of participants, cutaneous flare response will be measured following C-fiber stimulation with the current that elicited the detection response and the pain response. A single stimulation typically causes a local flare response that peaks after 10-20 seconds and diminishes after 90-120 seconds. The flare response will be measured with an imaging camera that will take up to 6 pictures and videos of the affected skin. We will use either Moore Dopplerometer (Moore instruments, Devon, UK) or PeriCAM PSI dopplerometer (Perimed AB, Jarfalla, Sweden) for determining the cutaneous flare response.

Prior Validation of the DLss

Laser stimulation, similar to what is being used in the DLss, has been used in pain clinics and research since 1975 as a diagnostic test. It has been proven to be useful and safe. Laser irradiation simultaneously activates both A delta and C fibers and primarily heat fibers located in the epidermis (up to 50-150 micron depth)[30,35,43].

Diode laser stimulation (the DLss used in this protocol) provides relatively uniform heating of skin from 50 to 600 microns deep, allowing for a distinct, singular burning or singular pricking pain depending on the laser pulse parameters. Experiments using an infrared diode laser conducted in Stanford and another institutions have shown that a short and a long laser pulse produces, respectively, a singular pinprick sensation (representing A-delta stimulation), and a singular burning pain sensation (representing C fiber stimulation) when applied to the dorsal hand skin of healthy subjects and pain patients volunteers. These preliminary results show that diode laser stimulation can safely and selectively activate A delta and C thermo-nociceptors[14,29,46,47].

Diode lasers similar to the one used in this study are FDA approved and are often used in cosmetic medical procedures for hair removal. The lasers used for cosmetic procedures are set at *ten times* the power density of that used in our study.

Over 115 subjects have been tested with the DLss. No patient has withdrawn from DLss testing due to pain. Two patients had pin-tip sized skin discolorations that resolved in the course of a week.

STUDY CALENDAR

Study Visit Day - Required Assessment
Informed Consent
Inclusion/exclusion criteria
Pregnancy test (if needed)
Demographic data collection
Spontaneous Pain Intensity 0-10 NRS
Duration of Painful CIPN (painful CIPN group only)
Brief Pain Inventory (BPI) questionnaire
Hospital Anxiety and Depression Scale (HADS) questionnaire
Neuropathic Pain Symptom Inventory (NPSI) questionnaire
Quantitative Sensory Testing (QST)
DLss testing
Adverse effect(s) – monitored during the study visit day

8. OBSERVATIONS AND MEASUREMENTS

8.1. Outcome Measurements

8.1.1 Outcome Measure 1

The primary outcome measure of this study is the difference in the "Aδ:C pain threshold ratio" for patients with painful CIPN vs controls who did not develop painful CIPN, as measured using the DLss.

The "Aδ:C fiber threshold ratio" is calculated using the Aδ-fiber and C-fiber pain thresholds determined using the protocol outlined in **section 7.3**.

This is not a safety outcome.

8.1.2 Outcome Measure 2

The key secondary outcome measure of this study is the correlation between the "Aδ:C fiber threshold ratio" and the severity of painful CIPN.

We will generate a Spearman correlation coefficient for the "Aδ:C fiber threshold ratio" and the severity of painful CIPN reported on the neuropathic pain symptom inventory (NPSI).
This is not a safety outcome.

8.2. Analysis plan

The Aδ:C pain threshold ratio will be recorded for each eligible and evaluable patient. We will calculate the average value of this parameter for each group (A and B) on a logarithmic scale. Then we will use a standard statistical two side t-test to compare the "Aδ:C fiber threshold ratio" in the painless CIPN group to the painful CIPN group. We hypothesize that the painful CIPN group will have statistically significantly higher value "Aδ:C pain threshold ratio" than those without.

To assess whether the "Aδ:C fiber threshold ratio" is associated with the severity of CIPN, we will conduct linear regression analysis of the Aδ:C fiber threshold ratio vs the neuropathic pain symptom inventory (NPSI).

8.3. Sample size

The power analysis of the preliminary data presented here was conducted by Alex McMillan (biostatistician - Stanford). The analysis was done on the natural logarithm scale and converted back using anti-logarithms. The geometric mean (GM) ratio of chemotherapy was 1.58 times the GM for healthy group. The GM in the diabetic group was 1.38 times the GM of the healthy group. The most variable group (Diabetics) had a standard deviation (log scale) of 0.33. We used a slightly more conservative value of SD=0.4 for our sample size calculations.

To achieve 80% power to detect a 1.4 fold Aδ:C pain threshold ratio in patients with painful versus painless CIPN (with SD=0.4, two-sided t-test, α=0.05,), 17 subjects will be required in each group. We will enroll 20 subjects per group to account for unexpected variability. As it is a single-day visit, we do not expect drop outs, or participants lost to follow-up.

However, if we are unable to recruit 20 patients per group on time, we will we will request a 12 month no-cost extension from the NIH/NCI.

9. MANAGEMENT OF INTERCURRENT EVENTS

9.1. Adverse Experiences

The subjects will be monitored for evidence of adverse events. Patients will be prompted to report any adverse effects throughout the study visit day, and by telephone any time after the study visit. All adverse events will be reported and followed until satisfactory resolution. The description of the adverse experience will include the time of onset, duration, severity, etiology, relationship (none, unlikely, possible, probable, highly probable), and any treatment required.

The assessment, grading, and reporting of Adverse Events (AEs) will be followed according to the guidelines outlined below:

Adverse Events

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website: http://www.hhs.gov/ohrp/policy/advevntguid.html

Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Pre-approval of all protocol exceptions must be obtained prior to the event.

Reporting to the Human Research Protection Office (HRPO) and the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

9.2. Potential Risks

9.2.1. Potential risks from thermal testing

Risk of injury related to thermal pain testing is minimal. Thermal testing is widely used and safe. While thermal testing does produce pain, risks to the individual are minimal, because 1) the pain is

transient in nature and generally subsides immediately after the procedure; 2) subjects are instructed that they may stop any procedure at any time with no adverse consequences; and 3) the level of pain experienced by subjects is below their tolerance level. With thermal stimulation there is a very slight risk of a burn, but this is minimized by the following: 1) positive lockout of stimulus parameters above 50°C; and 2) the stimulator has built in a shut-down system to prevent the delivery of prolonged or high intensity stimuli. Both TSA-II and Q-Sense have FDA 501(k) clearance (K922052).

9.2.2. Potential risks from DLss

There is a remote risk of skin injury or burning by laser stimulation when used for pain testing, which occurs by overheating of skin surface. This laser irradiation penetrates the skin fairly deeply, and does not allow overheating of the skin's surface. We also use a short, concentrated pulse; this will activate nerve fiber but is not long enough in duration to cause tissue damage. We have defined pain threshold levels for testing as moderate only, and are therefore only exposing subjects to the minimal stimulation that causes brief, moderate pain.

Over 115 subjects have been tested with the DLss. No patient has withdrawn from DLss testing due to pain. Two patients had pin-tip sized skin discolorations that resolved in the course of a week.

9.2.3 Other Potential Risks

No psychological risks to subjects are envisioned. Subjects may experience a loss of confidentiality. Investigators will keep subjects' participation confidential to the extent permitted by law. However, it is possible that others may become aware of subjects' participation in this study and may inspect and copy records pertaining to this research. Some of these records could contain information that personally identifies subjects.

9.3. Procedures to Minimize Potential Risks

Studies are conducted in the Washington University Pain Center under the supervision of the PI. The PI is trained and experienced in performing research in human subjects.

Inclusion and exclusion criteria, monitoring, and the clinical protocol are designed to ensure that risks are minimal. Subjects are informed that participation is voluntary and they may refuse to participate and may withdraw from the study at any time without penalty. A pregnancy test will be performed on women of childbearing potential and subjects excluded if pregnant. Subjects will be told that in the event of a physical injury as the direct result of study procedures, they will be cared for by a member of the investigating team at no cost, within the limits of the Washington University compensation plan.

With regard to confidentiality; 1) all subjects will be assigned a study ID number, 2) Samples will be kept confidentially. They will be coded, with a key to the code linking code numbers to names kept at a separate location, under lock and key. 3) The link to identifiers will be destroyed at the end of the study. 4) Data will be stored under lock and key (office, file cabinet) and only the investigators and research team will have access. If data are published, there will be no link to identifiers. Study data will not be revealed to any organization, individuals other than the subjects, or the subjects themselves. 5) Study data will not be entered in subjects' medical records.

9.4. Data and Safety Monitoring Plan

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual, separated by cohort
- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities, separated by cohort
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines.

10. HUMAN SUBJECTS RESEARCH

10.1. Protection of Human Subjects

The study will be conducted under appropriate Washington University Institutional Review Board protocols and consent forms approvals. The study will be conducted under the supervision of the PI, a GCP-certified pharmacist with several years of experience in the conduct of human studies, including CIPN subjects.

10.2. Sources of Materials

Subjects will be recruited from Outpatient Clinics of Siteman Cancer Center and Washington University Pain Management Center.

Data on comorbidities and concomitant medication use are provided by subjects as well as retrieval of medical records. Urine specimen will be obtained from women of childbearing potential for a pregnancy test. Other data including baseline quantitative sensory testing are obtained exclusively for research purposes.

10.3. Recruitment and Informed Consent

Participants will be recruited primarily through Siteman Cancer Center and Washington University Pain Management Center, or referred by the corresponding physicians. In addition, we will post flyers and recruit participants through the Research Participant Registry. Interested subjects will contact the investigators. Subjects will be given verbal (initially) and then written descriptions of the study aims, procedures, risks, and benefits, and will be required to give written informed consent. A member of the investigative team provides all study descriptions, informed consent, and answers all questions. Subjects are informed verbally and in writing that participation is voluntary and they may refuse to participate and may withdraw from the study at any time without penalty.

10.4. Potential Benefits of the Proposed Research to the Subjects and Others

Participants will not benefit directly from this research but other people might benefit from this study because it may lead to a new approach for early detection of CIPN.

10.5. Inclusion of Women

Studies actively encourage the participation of women in the research. As a matter of operational policy, our studies routinely and deliberately attempt to include equivalent numbers of women and men. However, the nature of the current study precludes enrollment of a set number of female or male patients since the main criteria for inclusion is painful chemotherapy-induced peripheral neuropathy. The taxane compounds that may cause CIPN are typically used for the treatment of breast cancer. As a consequence, we expect that the majority of the study participants are, indeed, women. Women of childbearing potential are not excluded from our research protocols.

10.6. Inclusion of Minorities

All of our studies actively encourage the participation of minorities in the research. Our minority recruiting typically matches the demographic composition of the Washington University community from which subjects will be recruited (78% white, 21% Black, <1 % Hispanic).

10.7. Inclusion of Children

Children <18 years will <u>not</u> be studied in this investigation, because the types of cancers treated with taxanes and oxaliplatin (typically breast cancer and colorectal cancer) are uncommon in this population. Including children may expose them to an unnecessary risk without the benefit of generalizability of the results.

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