RESEARCH PROTOCOL

Investigation of somatosensory predictors of response to pregabalin in painful chemotherapy-induced peripheral neuropathy (CIPN)

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1. SYNOPSIS

Study Title	Investigation of somatosensory predictors of response to pregabalin in painful chemotherapy-induced peripheral neuropathy (CIPN).
Objective	In a prospective, randomized, double-blind, cross-over study, to determine the correlation between mechanical pain threshold (MPT) at baseline and analgesic response to 4-week treatment with pregabalin vs. placebo in patients with painful CIPN.
Hypothesis	Increased mechanical hypersensitivity to pinprick (lower mechanical pain threshold (MPT)) corresponds to better analgesic response to pregabalin treatment in painful CIPN.
Study Period	Planned enrollment duration: Approximately 18 months Planned study duration: 10 weeks per subject; 4 weeks duration per arm, with 2 weeks washout between the two treatments.
Number of Patients	35 evaluable patients with painful CIPN
Study Treatment	Oral pregabalin (or matching placebo) administered BID and titrated to individual response in a dose up to 600mg/day. In subjects with CrCL of 30-60 mL/min, the dose will be limited to 300mg/day.
Study Design	Prospective, randomized, double-blind, placebo-controlled, cross-over study.
Inclusion and Exclusion Criteria	<u>Inclusion criteria:</u> 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, 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. Women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control, abstinence) prior to study entry and for the duration of study participation. 8. Able and willing to sign an IRB-approved written informed consent. <u>Exclusion criteria:</u> 1. Hypersensitivity to pregabalin. 2. Current treatment with pregabalin. 3. Current treatment with a vinca alkaloid (e.g. vincristine, vinblastine), or CIPN that may be associated with previous treatment with a vinca alkaloid. 4. History of diabetes mellitus or a neurological disorder with any

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	previous signs of distal symmetric polyneuropathy. 5. Moderate to severe renal failure (CrCL < 30mL/min, by Cockcroft-Gault formula). 6. ALT (SGPT) or AST (SGOT) > 3 times the upper limit of normal. 7. Planned surgeries or radiation treatment within 10 weeks following study inclusion. 8. Inability to complete pain self-report. 9. Pregnancy or lactation 10. Patients with seizure disorders treated with anticonvulsants 11. Current participation in a trial with another investigational agent unless co-enrollment is approved by the PIs of both studies. 12. Concomitant medication as follows: • Subjects treated with gabapentin or other anticonvulsant for neuropathic pain will be required to taper the medication and discontinue for at least 2 weeks prior to study initiation. • Patients on antidepressant treatment for pain or depression (TCAs, SSRI, SNRIs etc. will be allowed to continue their medications provided they have been on a stable dose for at least 4 weeks before study initiation. No dose regimen changes of antidepressants will be allowed during the study period. • Patients on around-the clock opioid treatment (including tramadol) will be allowed to continue their medication provided they have been on a stable dose for at least 4 weeks before study initiation. The maximum allowed dose of opioid will be equivalent to 60mg oral morphine sulphate. Patients with higher doses will be required to taper down their opioid dose to maximum 60mg oral morphine equivalent, and continue on stable dose for 4 weeks before enrollment in the study. PRN short-acting opioids for painful CIPN treatment will not be allowed. Patients receiving PRN short-acting opioids (with or without acetaminophen) for pain other than CIPN will be allowed up to 4 daily doses, with daily recording of analgesic consumption. • Treatment with NSAIDs will be discontinued at least 2 weeks before study initiation. However, low-dose aspirin (≤325mg/day) will be allowed.
Measurements	<u>Baseline sensory testing:</u> 1. Quantitative sensory testing (QST): Mechanical Pain Threshold, thermal detection and pain thresholds, mechanical detection threshold, pressure pain thresholds, temporal summation (TS), and conditioned pain modulation (CPM).

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	2. Spontaneous pain at baseline on 0-10 Numerical Rating Scale (NRS); 3. Assessment of pain symptoms on Neuropathic Pain Symptom Inventory (NPSI) and Brief Pain Inventory (BPI). 4. Assessment of mood on Depression, Anxiety and Positive Outlook Scale (DAPOS), and assessment of sleep on MOS Sleep Problem Index (SPI) scale. 5. Intraepidermal nerve fiber density and length of a 3-mm skin punch biopsy. <u>Testing for intervention response (repetitive testing):</u> 1. Daily average and maximum pain intensity recording on 0-10 NRS; 2. Scoring of NPSI, BPI, DAPOS and SPI questionnaires at the end of week 4
Statistical Methodology	The primary outcome is the comparison of slopes of the two correlations: baseline MPT vs % pain reduction with pregabalin, and baseline MPT vs % pain reduction with placebo. The two slopes will be compared using t-test. Significant difference between slopes will indicate that baseline MPT correlates with pregabalin response (vs. placebo response) in painful CIPN. As secondary analyses, we will compare mean absolute pain reduction with pregabalin to absolute pain reduction to placebo (both for average daily pain and maximum daily pain), using paired t-test, given normal data distribution. If normal distribution is not obtained, we will use Wilcoxon signed ranked test. In addition, we will perform Fisher exact test to compare the proportion of patients with 50% or more reduction in average pain intensity from baseline with pregabalin vs. placebo. Number needed to treat (NNT) will be calculated from this proportion of responders to pregabalin and placebo.

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2. STUDY PROTOCOL

2.1 Background and Significance

Chemotherapy-induced peripheral neuropathy (CIPN) affects 25-90% of patients treated with anticancer drugs such as platinum compounds, taxanes, and vinca alkaloids, or their combinations. CIPN can be early-onset or late-onset, and its prevalence and severity are affected by age, single chemotherapy dose intensity, cumulative dose, length of therapy, and other factors (Chaudhry, Rowinsky et al. 1994, Polomano and Bennett 2001). The early onset neuropathy associated with chemotherapy is frequently a dose-limiting side effect of these drugs, hindering the ability to achieve high effective doses. Indeed, patients developing CIPN eventually receive lower cumulative doses of chemotherapy (Eckhoff, Knoop et al. 2013, Speck, Sammel et al. 2013) which can increase the rate of anticancer treatment failure. The early-onset neuropathy may transition into a chronic condition, or late-onset neuropathy can appear 2-8 weeks after chemotherapy discontinuation. Some of the patients affected by chronic neuropathy are in remission or cancer-free, and could otherwise be fully functional, yet painful CIPN causes substantial suffering and significantly reduces their quality of life (Hershman, Weimer et al. 2011).

Painful CIPN, as clinical experience and research data suggest, is rather treatment-resistant, and is more challenging to treat compared to other neuropathic pain conditions. Illustrating this challenge is the fact that only one large positive randomized controlled study on the treatment of CIPN has been published (among more than 300 randomized controlled pharmacotherapy trials in various neuropathic pain conditions), demonstrating effectiveness of duloxetine, a serotonin norepinephrine reuptake inhibitor. In a trial of 231 patients with taxane- or paclitaxel-induced CIPN, patients were randomized to treatment with duloxetine 60 mg/day or matching placebo for 5 weeks. The average decrease in pain intensity was greater with duloxetine than with placebo (Smith, Pang et al. 2013). Venlafaxine, another SNRI, has shown efficacy in the possible prevention of oxaliplatin-induced PN, when administered for 10 days after onset of acute neurotoxicity (Durand, Deplanque et al. 2012).

The mechanisms of nerve injury in CIPN, and the transition from acute to chronic phase of neurotoxicity, are not fully understood. The patients present with peripheral nerve fiber degeneration and hyperexcitability, and one of the leading theories regarding the causation is related to the mitotoxic effects of chemotherapy on somatosensory afferent neurons. Despite differences in mechanisms of action, the different chemotherapy agents share some of the pathological findings in CIPN, e.g. common final pathway of axonal mitochondria damage. In addition, preclinical data suggest that CIPN caused by oxaliplatin and taxanes (paclitaxel and docetaxel) results in overexpression of α2δ subunits of voltage-gated Ca^{2+} channels in the dorsal root ganglion (DRG) and spinal cord dorsal horn in rats. This may imply that modulating neuronal Ca^{2+} channel function by α2δ ligands may be effective in treating CIPN caused by oxaliplatin and taxane compounds. Indeed, in preclinical studies administration of gabapentin (Lynch, Wade et al. 2004, Ling, Authier et al. 2007, Xiao, Boroujerdi et al. 2007) and pregabalin (Peng, Xi et al. 2012, Aoki, Kurauchi et al. 2014) has been demonstrated to successfully reduce mechanical and thermal hypersensitivity in rat models of CIPN induced by these compounds. Although CIPN induced by different drugs may result in a similar symptom pattern, the underlying mechanisms may vary. In mice, treatment with paclitaxel and oxaliplatin increases the DRG expression of α2δ mRNA, while treatment with vincristine does not result in similar changes. In addition, treatment with α2δ subunit ligand gabapentin was effective in mice with paclitaxel and oxaliplatin-induced mechanical allodynia, but not in vincristine-induced mechanical allodynia (Gauchan, Andoh et al. 2009). Thus, preclinical data suggests that α2δ subunit

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of voltage-gated Ca^{2+}channels is a promising target for attenuating oxaliplatin- and taxane- induced CIPN.

Neuronal voltage-gated Ca^{2+}channels as a therapeutic target for treating CIPN remain largely unexplored clinically. Recently published guidelines on CIPN failed to identify any treatment for which robust data exist to support clinical effectiveness (Hershman, Lacchetti et al. 2014). Studies with first-line agents for neuropathic pain treatment, such as gabapentin or tricyclic antidepressants, yielded negative or inconclusive results, and serotonin norepinephrine reuptake inhibitor duloxetine is the only drug for which positive results from a large randomized controlled trial are available.

The only published randomized clinical trial assessing effectiveness of an α2δ ligand gabapentin in CIPN was negative (Rao, Michalak et al. 2007), but it is important to note that patients in this study did not necessarily have pain as inclusion criterion, and these methodological issues may have affected the analgesic outcomes. Recent observational data suggest clinical effectiveness of pregabalin in oxaliplatin- and taxane-induced peripheral neuropathy (Nihei, Sato et al. 2013). Twenty seven patients with oxaliplatin-induced PN, and 28 patients with paclitaxel-induced PN treated with pregabalin were compared to 20 and 25 patients (respectively) treated with other treatments, such as amitriptyline, carbamazepine, clonazepam and vitamin B12. Response rates in both CIPN groups were better in pregabalin-treated subjects that in non-pregabalin treated subjects (40.7% vs 10.0% experienced decrease in neuropathy grade in oxaliplatin-induced PN; 28.6% vs. 12.0% experienced decrease in neuropathy grade in paclitaxel-induced PN). There is an urgent need to find efficacious treatment options for patients suffering from painful CIPN. In neuropathic pain conditions in general, the "one size fits all" pharmacotherapy approach does not seem particularly effective, and the current trend is to move toward more innovative subgroup response and responder analysis methods. Numbers of patients needed to treat (NNT) to achieve substantial pain relief (e.g. ≥50%) compared to placebo in neuropathic pain are between 4 and 7 for most drugs, which is discouraging. However, some data, mainly from ad-hoc analyses of clinical trials, suggest that subgroups of patients with neuropathic pain identified by somatosensory phenotyping, may be more responsive to specific treatments (Campbell, Kipnes et al. 2012, Yarnitsky, Granot et al. 2012, Haroutiunian, Nikolajsen et al. 2013). For example, in an HIV sensory neuropathy study that was negative overall, a subgroup of patients with mechanical allodynia (excessive pain response to pin-prick stimulation) at baseline had a better analgesic response to pregabalin treatment than to placebo (Simpson, Schifitto et al. 2010).

Pregabalin (Lyrica®) is a voltage-gated calcium channel α2δ subunit ligand that has shown efficacy in numerous trials of neuropathic pain conditions. It is currently approved by the FDA for the treatment of several neuropathic pain conditions, including pain associated with diabetic peripheral neuropathy, postherpetic neuralgia, and spinal cord injury. It is also approved for the treatment of fibromyalgia (Lyrica Prescribing Information). The NNT for pregabalin in various neuropathic pain conditions is 7.7, and it is generally safer than the SNRIs in treating neuropathic pain, with a number of patients needed to treat for 1 patient to drop out of study due to adverse effects (NNH) of 13.9 for pregabalin, compared with 11.8 for SNRIs) (Finnerup, Attal et al. 2015). The FDA-approved maximal dose of pregabalin is 600mg/day.

Some animal data supports the effectiveness of pregabalin on mechanical allodynia in chemotherapy-induced or other neuropathies (Hahm, Ahn et al. 2012, Miyazaki and Yamamoto 2012, Aoki, Kurauchi et al. 2014).

Based on the findings of pin-prick hypersensitivity as predictor of response to pregabalin, together with animal data and preliminary clinical data supporting pregabalin efficacy in CIPN, we hypothesize, that increased mechanical hypersensitivity to pinprick (lower mechanical pain threshold - MPT) corresponds to better analgesic response to pregabalin treatment in painful CIPN.

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2.2 Objective

The primary objective of the proposed study is to determine the correlation between baseline mechanical pain threshold (MPT) and analgesic response (% pain reduction) after 4-weeks of treatment with pregabalin vs. placebo in patients with painful peripheral neuropathy induced by oxaliplatin, paclitaxel, and/or docetaxel.

The additional aims of the study are:

1. To investigate whether treatment with pregabalin results in better pain relief compared with placebo in patients with painful CIPN.
2. To identify additional phenotypic predictors of response to pregabalin in CIPN by performing comprehensive baseline somatosensory phenotyping.

2.3 Patient Selection

Thirty-five evaluable patients with painful CIPN will be recruited from the Siteman Cancer Center and the Pain Management Center of Washington University in St. Louis.

2.3.1 Inclusion Criteria

Inclusion criteria:

1. Age >18
2. Distal symmetric pain distribution (both feet, with or without pain in hands).
3. Pain appeared during or up to 12 weeks after treatment with oxaliplatin, paclitaxel, docetaxel or any combination of these.
4. Score of 4 or more on DN4 neuropathic pain questionnaire (Bouhassira, Attal et al. 2005).
5. Pain duration > 2 months.
6. Patient report of average daily pain intensity >3 on 0-10 Numerical Rating Scale (NRS) in the past week.
7. Women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control, abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately.
8. Able and willing to sign an IRB-approved written informed consent.

2.3.2 Exclusion Criteria

Subjects will not be enrolled if any of the following criteria exist:

1. Hypersensitivity to pregabalin.
2. Current treatment with pregabalin.
3. CIPN that may be associated with previous treatment with a vinca alkaloid (e.g. vincristine, vinblastine), or current treatment with a vinca alkaloid.
4. History of diabetes mellitus or a neurological disorder with any previous signs of distal symmetric polyneuropathy.
5. Moderate to severe renal failure (Clcr < 30mL/min).

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6. ALT (SGPT) or AST (SGOT) > 3 times the upper limit of normal.
7. Planned surgeries or radiation treatment within 10 weeks following study inclusion.
8. Inability to complete pain self-report.
9. Pregnancy or lactation
10. Patients with seizure disorders treated with anticonvulsants
11. Current participation in a trial with another investigational agent, unless co-enrollment is approved by the PIs of both studies.
12. Concomitant medication as follows:

- Subjects treated with gabapentin or other anticonvulsant for neuropathic pain will be required to taper the medication and discontinue for at least 2 weeks prior to study initiation.
- Patients on antidepressant treatment for pain or depression (TCAs, SSRI, SNRIs etc. will be allowed to continue their medications provided they have been on a stable dose for at least 4 weeks before study initiation. No dose regimen changes of antidepressants will be allowed during the study period.
- Patients on around-the clock opioid treatment (including tramadol) will be allowed to continue their medication provided they have been on a stable dose for at least 4 weeks before study initiation. The maximum allowed dose of opioid will be equivalent to 60mg oral morphine sulphate. Patients with higher doses will be required to taper down their opioid dose to maximum 60mg oral morphine equivalent, and continue on stable dose for 4 weeks before enrollment in the study. PRN short-acting opioids for painful CIPN treatment will not be allowed. Patients receiving PRN short-acting opioids (with or without acetaminophen) for pain other than CIPN will be allowed up to 4 daily doses, with daily recording of analgesic consumption.
- Treatment with NSAIDs will be discontinued at least 2 weeks before study initiation. However, low-dose aspirin (≤325mg/day) will be allowed.

2.4 Design and Procedures

2.4.1 Study Design

This is a prospective, randomized, double-blind, placebo-controlled, cross-over pilot study in 35 evaluable subjects with painful CIPN. If our hypothesis is confirmed, then patients with lower MPT at baseline will have larger analgesic effect with pregabalin than with placebo.

Registration, Randomization, and Blinding

Subjects that meet all criteria and wish to participate will be assigned study ID # and be randomized for the sequence of treatment for the study sessions. Patients may not start any protocol intervention until registration through the Siteman Cancer Center (SCC) is complete. The following steps will be taken before any study interventions occur: 1) confirmation of patient eligibility, 2) registration of patient in the SCC database, and 3) assignment of study ID #.

According to a computer-generated list of randomization numbers, the sequence of interventions will be determined: pregabalin and then matching placebo, or vice versa. Randomization will be performed by blocks of 8 subjects, without stratification. An unblinded

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investigator will be assigned to match the study number with randomized treatment sequence, and this person will dispense the study medications. This investigator will not be involved at any stage at patient assessment, data collection or analysis. The participants and all other study personnel will be blinded to the treatment sequence allocation. At the end of the study, the subjects will be asked to complete a participant blinding questionnaire, to assess the risk of possible unmasking. As an additional approach to identify inadvertent failure in randomization or patient allocation, a single blood sample will be obtained at the last visit of each session, to determine the presence of pregabalin in plasma in patients allocated to pregabalin, and lack of drug in the placebo arm.

2.4.2 Pre-Study Period

At the screening visit, after obtaining signed informed consent, we will collect patient demographic data including details on cancer type and status, chemotherapy regimen(s) and cumulative doses, history of chronic pain, concomitant diseases and medication, and duration of painful CIPN. A blood sample for CMP will be obtained for determining serum creatinine concentration and liver transaminases (ALT and AST). If subject's lab tests results are available within the past 30 days, we will utilize these without the need of obtaining new renal and hepatic function tests. In addition, the patients will complete the following questionnaires: Brief Pain Inventory (BPI), Neuropathic Pain Symptom Inventory (NPSI), and Depression Anxiety and Positive Outlook Scale (DAPOS). Average and maximum daily pain intensity in the past week will be recorded as baseline pain intensity measure.

The subjects' extremities will be mapped for spontaneous pain, and response to the following sensory stimulation: cold (20°C Thermal Roller, Somedic), warm (40°C Thermal Roller, Somedic), pinprick (# 5.88 Semmes-Weinstein monofilament, 60mN target force, North Coast) and brush (SenseLab 0.5 brush, Somedic) (Finnerup, Sorensen et al. 2007). The probability of neuropathic pain will be graded as possible, probable, or definite according to Treede et al. criteria (Treede, Jensen et al. 2008). The results of sensory mapping will provide the information for the criterion on presence of any objectively tested positive or negative neurological signs in the painful territory. Photographs of the mapped extremities will be taken (in a way not to include any part of the subjects' face or other identifying information), for accurate reproduction of areas of sensory disturbance.

The subjects will undergo a quantitative sensory testing (QST) procedure to determine the MPT parameter (Maier, Baron et al. 2010). MPT will be measured using standardized weighted metal probes (Nervetest, MRC systems) using a modified method of levels (Haroutounian, Nikolajsen et al. 2014, Haroutounian, Nikolajsen et al. 2014). Several other QST measures will be obtained to better characterize the underlying nerve damage, and to provide additional factors to be incorporated in multiple regression analysis of predictors of response to pregabalin in CIPN. Namely, we will determine cold and warm detection thresholds (CDT and WDT), as well as cold and heat pain thresholds (CPT and HPT) with Thermal Sensory Analyzer (TSA-II, Medoc) using the method of limits. We will also assess the existence of paradoxical heat sensations (PHS) with TSA-II device, per standard protocol (Maier, Baron et al. 2010, Haroutounian, Nikolajsen et al. 2014) and will calculate the warm sensibility index (WSI) from the WDT and HPT values (Jensen, Bach et al. 1991). We will also test for temporal summation (TS) to mechanical stimulation (with 256mN pinprick probe), per standard protocol (Haroutounian, Nikolajsen et al. 2014) as a surrogate measure for central sensitization which has been implied to be important in determining effectiveness of $Ca2+$ channel $\alpha2\delta$ subunit ligands.

In addition, we will apply conditioned pain modulation (CPM) protocol to assess the efficiency of individual descending noxious inhibitory pain controls, as this was previously suggested

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to predict response to treatment in neuropathic pain (Yarnitsky, Granot et al. 2012). CPM protocol will test whether concomitant application (conditioning) of a mildly painful stimulus to an upper extremity (heat that elicits individually calibrated pain intensity of 30 on 0-100 NRS), reduces the painful experience from a painful (test) stimulus applied to the contralateral extremity (a thermal stimulus at temperature previously calibrated to elicit pain with intensity of 60 on 0-100 scale). Reduction of reported pain experience to test stimulus during conditioning is a surrogate measure of efficient descending pain modulation.

2.4.3 Study Period

The cross-over study design and the treatment plan are illustrated in Figure 1. Pregabalin (or matching placebo) will be administered for four weeks in each period. The target dose will be increased from 75mg BID to 300mg BID within 7 days (per schedule in Fig. 1), and continued for an additional 22 days.

In case of any grade 2 adverse events (Common Terminology Criteria for Adverse Events – CTCAE version 4.0), the dose will be reduced to the last dose prior to dose increase (i.e. to 150mg BID from 300mg BID; to 75mg BID from 150mg BID). If the adverse effect diminishes to ≤ Grade 1, one attempt to dose escalate will be allowed after 3 additional days. If the dose escalation is not tolerated (i.e. recurrent grade 2 adverse event), the treatment will be continued at the previous (lower) dose until day 28. Any CTCAE ≥ grade 3 will trigger subject withdrawal from the study

At the end of treatment sequence, the dose will be tapered over 3 days (e.g., 150mg BID for 2 days, then 75 mg BID for 1 additional day) followed by 10 days of study drug-free washout (end of week 6). In subjects with CrCL 30-60 mL/min, the target (maximum) dose will be 300mg/day. In these subjects the dose will be tapered over 3 days of 75mg BID dosing. If subject's highest tolerable maintenance dose was 75mg BID, the taper schedule will be 75mg QD over 3 days.

The subjects will return on day 28±2 days [prior to washout period] for Visit 2 to turn in the 4 questionnaires which were completed on day 28 (week 4a), sensory mapping, PK blood sample and a blood sample for renal and hepatic function tests will be obtained. After the washout period (end of week 6), the subjects will return to Washington University Medical Center for Visit 3, prior to second treatment sequence. The subjects will undergo QST and CPM procedures (Fig. 1), and complete the appropriate questionnaires (SPI, NPSI, BPI, DAPOS). Thereafter, the second 4-week treatment sequence will be scheduled for weeks 7-10, according to the same titration schedule as sequence 1. Three day dose tapering-down will be practiced at the end of second treatment sequence in the same manner as was performed for the first treatment. The subjects will return for the final assessment visit [Visit 4] approximately 3 days after end of week 10 (end of 3-day taper). Safety and efficacy data, a PK blood sample and a blood sample for renal and hepatic function tests will be obtained, turn in 4 questionnaires which were completed on day 28 (week 4b), sensory mapping, and the optional skin biopsy in subjects who agreed, will be obtained at Visit 4.

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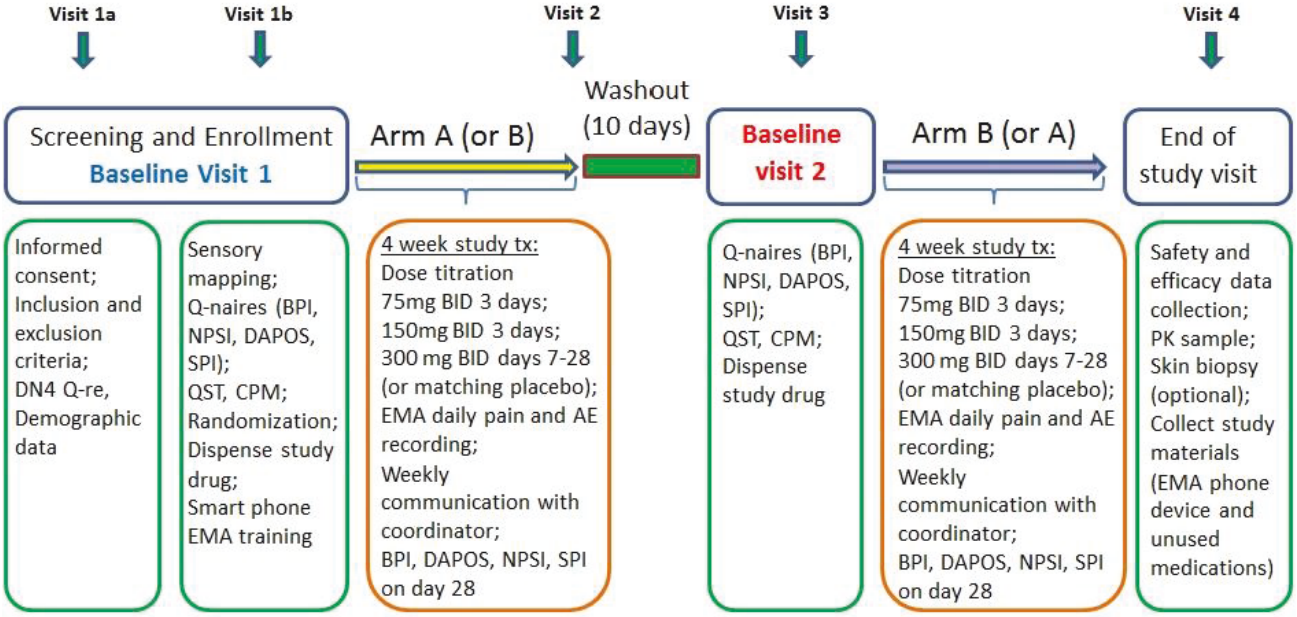
Figure 1.



Legend: BPI – brief pain inventory; NPSI – neuropathic pain symptom inventory; DAPOS – depression, anxiety and positive outlook scale; SPI – sleep problem index; QST – quantitative sensory testing; CPM – conditioned pain modulation. Tx – treatment.

Rescue Analgesia

Study subjects will be allowed to take oral acetaminophen (paracetamol) as rescue analgesic at a dose of 1 gram up to 4 times/day. FDA-approved 500mg acetaminophen tablets will be purchased and provided by the investigators. If the patient has been receiving PRN short-acting opioids (with or without acetaminophen) for non-CIPN pain, up to 4 daily doses will be allowed during the study, with daily recording of analgesic consumption. The total amount of acetaminophen (from opioid-acetaminophen combination and PRN acetaminophen alone) will not exceed 4 grams/day. In subjects with liver disease (AST or ALT increased, but less than 3 times the upper normal level), the total daily dose of acetaminophen will be limited to 2 grams. Based on these considerations, each patient will receive individual written instructions regarding PRN analgesic use, and will be required to report it appropriately.

Outcomes Assessment:

Clinical Efficacy

Patients will complete an electronic diary with daily report of their average and maximum daily pain intensity, recorded every evening before bedtime. The percentage of pain reduction for primary outcome will be assessed from the difference between baseline average pain intensity and the average pain intensity of last 5 days of treatment (day 24-28), on 0-10 NRS.

Secondary efficacy outcomes will include: 1) The difference between baseline maximum pain intensity and the maximum pain intensity of the last 5 days of treatment, on 0-10 NRS; 2) The proportion of patients achieving 50% or more pain relief with pregabalin vs. placebo treatment; 3) Difference in Pain Severity and Pain Interference scales of the BPI, assessed at baseline and day 28 of treatment. The daily use of rescue analgesia (oral acetaminophen) will also be recorded.

Table 1. Data assessment schedule

Required Assessments	Screening and Enrollment Baseline Visit	Week 1a	Week 2a	Week 3a	Week 4a	End of Treatment A (Day 28 +/- 2)	Wash-out	Study crossover visit	Week 1b	Week 2b	Week 3b	Week 4b	End of Study Visit (Day 30 +/-3)
Study visits	Visit1 (a/b)*					Visit2		Visit3					Visit 4
Informed consent	✓												
Pregnancy (uring Hbhcg) test for women	✓												
Comprehensive Metabolic Panel (CMP) blood test**	✓					✓							✓
Inclusion/exclusion criteria	✓												
Randomization	✓												
Demographic data collection	✓												
Sensory Mapping	✓					✓		✓					✓
BPI questionnaire	✓				✓			✓				✓	
NPSI questionnaire	✓				✓			✓				✓	
DAPOS questionnaire	✓				✓			✓				✓	
SPI questionnaire	✓				✓			✓				✓	
QST	✓							✓					
CPM	✓							✓					
Dispense study drug	✓							✓					
Pain intensity 0-10 NRS	✓	daily	daily	daily	daily			✓	daily	daily	daily	daily	✓
Adverse Event assessment		daily	daily	daily	daily				daily	daily	daily	daily	✓
Telephone communication		weekly	weekly	weekly	weekly				weekly	weekly	weekly	weekly	
Blinding questionnaire***		✓											
Pill count								✓					✓
Blood sample (PK)						✓							✓
Skin biopsy (optional)													✓

**Visit 1 may be conducted over 2 visits to complete all assessments if required for subject tolerability or convenience*
*** A blood sample for CMP will not be obtained if recent (<30 days) results are available.*
**** Blinding questionnaire to be administered via EMA device on Day 5 of study drug treatment*

Subject Blinding Perception

On Day 5 of the first treatment cycle, subjects will answer a brief question related to their randomization of drug vs placebo using the smartphone device.

Safety

Voluntary reporting of any adverse effects (AEs) will be recorded daily in the electronic diary. In addition, possible AEs will be addressed during weekly communications with the subjects. The

subjects will be able to contact the PI or study coordinator with regard to any AE, and moderate to severe AEs will be addressed and treated, if necessary. Any serious or unexpected AE will be promptly reported to the IRB and to the QASMC (refer to Section 3.0). All subject withdrawals from the study (for any reason) will be documented and the reasons for withdrawal carefully recorded.

Compliance

Compliance will be enhanced by weekly scheduled telephone communication with the subject by the study coordinator, and compliance assessment will be performed by pill count at the end of each study sequence. The electronic diaries have a built-in trigger reminder that will be set both for taking the study medication and for completing pain and AE assessment, according to patient preferences.

Pharmacokinetics

A single blood sample (approx. 5cc) will be collected at the end of each treatment period (approx. day 28 for each drug randomization assignment) to determine plasma level of pregabalin.

Electronic data collection

The pain intensity and AE data will be collected via a validated smart phone-based program, administered as a diary provided to each subject on a locked smart phone. This approach (Ecological Momentary Assessments, EMA) is currently in use with other Washington University in St. Louis IRB-approved protocols. The phones will be provided to subjects by the study team and run only the EMA program. The subjects will receive daily triggers for data assessment, with reminder cues until it is completed. The daily assessment will measure the average and maximum pain intensity (on 0-10 NRS), reported once a day before bedtime and will include voluntary AE report. The diary will also include daily information on the amount of rescue analgesia consumed by the patient. Data are automatically uploaded to a secure Cloud based server and transmitted to a WU firewall protected network server. Subject data will be reviewed approximately weekly by the research team. Only the research team will have access to the password for retrieval of data from the smartphone device. All data will be saved using a linked study ID number. There are no patient identifiers saved to the device.

Skin Biopsy

Subjects will be asked to undergo a 3mm skin punch biopsy in the lower leg (10 cm above the lateral malleolus) to be performed at the final study visit. This is to explore possible correlations between changes in small nerve fiber morphology and the treatment response to the study drug. The measures obtained from the skin biopsies include intraepidermal nerve fiber density (IENFD) and length density (NFLD) of PGP9.5 – stained small fibers. This skin biopsy is a standard procedure using aseptic technique, after local infiltration with lidocaine and will be performed by a trained health care professional. Refusal to undergo this procedure does not exclude subjects from participation in the research study. This procedure is voluntary.

2.4.4 Minimization of Bias

There will be no specific sex, ethnic or racial background for enrollment. Placebo arm is introduced to increase the ability to differentiate between the pharmacologic effects of pregabalin and

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other possible effects. The study is designed as a cross-over in order to minimize inter-subject variability. The investigators and the subjects will be blinded to treatment allocation sequence.

2.4.5 Observations and Measurements

2.4.5.1 Primary Outcome Measures

Primary outcome:

Correlation between MPT at baseline and reduction in spontaneous pain intensity (% reduction on 0-10 NRS) at the end of 4-week treatment. The slopes of the correlation obtained from pregabalin vs. placebo will be compared.

2.4.5.2 Secondary Outcome Measures

Secondary outcomes:

1). Absolute change in pain intensity from baseline to 4 weeks with pregabalin vs. placebo, and from that data – the proportion of patients achieving 50% or more reduction in pain intensity.

2). Change from baseline to week 4 of the following items assessed by questionnaires: NPSI, BPI, DAPOS, and SPI.

3). Exploratory analysis to investigate potential correlation between various QST variables (WDT, CDT, HPT, CPT, WSI, TS, CPM) and pain reduction with pregabalin vs placebo.

4) Exploratory analyses to investigate potential correlation between skin biopsy parameters (IENFD, NFLD) and pain reduction with pregabalin vs. placebo.

2.4.6 QST protocol

Quantitative sensory testing will be performed on the dorsal mid-foot. 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:

Determination of mechanical pain threshold (MPT)

Equipment: A set of standardized weighted metal probes (Nervetest, MRC systems) exerting pressure of 8, 16, 32, 64, 128, 256 and 512 mN.

Methods and Background:

The standardized metal probes will be used in a modified method of levels manner, 3 series of increasing stimulus intensities to detect the mechanical pain threshold. Beginning with an applied force of 8mN, stimuli increase in intensity until the sensation induced by increased pressure can be described as 'painful'. The corresponding force is used to represent the first MPT value. The procedure is then repeated a total of 3 times and until a total of 3 values are obtained, from which the mean MPT is determined.

Thermal detection and thermal pain thresholds

Equipment: The Thermal Sensory Analyzer (TSA-II platform - Medoc, Ramat Yishai, Israel) will be used to determine thermal detection and pain thresholds. This equipment is used globally for functional assessment of pain and temperature-conducting nerve fibers (C and A-delta fibers).
Method and Background: Using the thermal sensory analyzer, cold and warm detection thresholds

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(CDT and WDT, respectively), as well as cold and heat pain thresholds (CPT and HPT, respectively) will be determined (Fruhstorfer, Lindblom et al. 1976, Yarnitsky, Sprecher et al. 1995). 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. The warm sensibility index (WSI) will be calculated by the following formula:

$$WSI = \frac{\text{HPT (°C)} - WDT\ (°C)}{\text{HPT (°C)} - \text{baseline temp. (32°C)}}$$

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 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 (Baumgartner, Magerl et al. 2002).

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 standardized weighted metal probes (Nervetest, MRC systems) exerting pressure of 256mN.

Methods and Background: In this test a pinprick (256mN) 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 will be repeated 3 times. A geometric average of the 'wind-up' is calculated from the two ratios (Price, Hu et al. 1977, Magerl, Wilk et al. 1998).

Determination of conditioned pain modulation (CPM) efficiency

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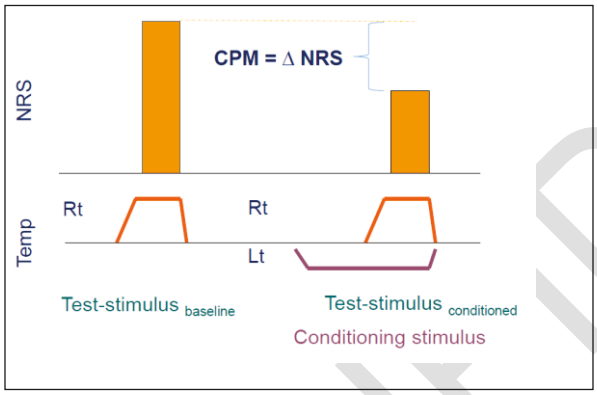
Equipment: The Q-Sense Thermal Analyzer (Medoc, Ramat Yishai, Israel) will be used for CPM paradigm testing.

Methods and Background

The Q-Sense works on the same principle as TSA-II, which is used for determining the thermal thresholds. However, Q-Sense is equipped with two 3x3cm Peltier thermodes – one used as conditioning stimulus, and the other used as test stimulus. The intensity of the conditioning stimulus will be determined individually by temperature that elicits pain intensity of 30 on 0-100 NRS. The intensity of test stimulus will also be determined individually, at the temperature that elicits pain intensity of 60 on 0-100 NRS.

The conditioning stimulus will be applied at left shoulder, while the test stimulus will be applied at the right shoulder. The length of the conditioning stimulus is 60 seconds, during the last 30 seconds of which the pain stimulus is applied. The stimuli are applied according to the diagram in Fig.2 below – and the difference between the intensity of pain stimulus without conditioning and between the intensity of pain stimulus with concomitant conditioning is the CPM magnitude. CPM>0 implies efficient descending pain modulation.

Figure 2. CPM paradigm testing.



2.4.7 Statistical Methods

The primary outcome is the comparison of slopes of the two correlations: baseline MPT vs % pain reduction with pregabalin, and baseline MPT vs % pain reduction with placebo. Percent pain reduction will be assessed from the difference in average pain intensity at baseline and the average daily pain intensity on days 24-28 of each treatment phase, obtained from the patient diaries. The two slopes will be compared using t-test. Significant difference between slopes will indicate that baseline MPT correlates with pregabalin response (vs. placebo response) in painful CIPN, regardless of differences in average pain response between pregabalin and placebo.

As secondary analyses, we will compare mean absolute pain reduction with pregabalin to absolute pain reduction to placebo (both for average daily pain and maximum daily pain), using paired t-test, given normal data distribution. If normal distribution is not obtained, we will use Wilcoxon signed ranked test. In addition, we will perform Fisher exact test to compare the proportion of patients with 50% or more reduction in average pain intensity from baseline with pregabalin vs. placebo. Number needed to treat (NNT) will be calculated from this proportion of responders to pregabalin and placebo. These secondary analyses are aimed at detecting group differences in response to pregabalin vs. placebo.

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In exploratory analysis, we will examine the correlations between the various demographical and QST variables (WDT, CDT, HPT, CPT, WSI, TS, CPM) and pain reduction with pregabalin vs placebo, for generating hypotheses for additional predictors of response to pregabalin in CIPN. We will also perform exploratory multiple regression analysis to evaluate how various QST factors perform in combination as treatment response predictors.

2.4.8 Sample Size

The sample size calculation was performed for detecting a significant difference between correlations on % pain reduction vs. MPT slopes of pregabalin and placebo in linear bivariate regression. Assuming slope and SD data based on our preliminary results of MPT vs. response with another drug, lidocaine (Haroutiunian, Nikolajsen et al. 2013); to detect 0.3 (SD=0.5) difference in correlation coefficients between the two slopes, with 85% power and alpha=0.05, 27 participant are required in this cross-over design. We will aim to recruit 35 patients to account for possible 20% dropout rate.

2.4.9. Dealing with missing data

If less than 10% data on primary outcomes are missing, we will perform our primary analysis per protocol, and perform sensitivity analysis using intent to treat (ITT) approach, where missing pain intensity data will be imputed using last observation carried forward (LOCF) method. If more than 10% primary outcomes data are missing, we will use mixed-effects model imputation method [full information maximum likelihood (Little, D'Agostino et al. 2012)], which considers the reasons for missingness, and is more appropriate for reducing bias.

2.4.10 Clinical Procedures and Laboratory Tests

Due to the risk of decreased clearance of pregabalin in participants with moderate to severe renal insufficiency, active surveillance to prevent drug accumulation will be performed. Plasma creatinine will be determined before study enrollment and only patients with a CrCL $\geq$ 30 [by Cockcroft-Gault formula]) will be eligible. If renal insufficiency (CrCl <30 mL/min occurs, the subject will be withdrawn from the study. If CrCl 30-60 mL/min, the maximum dosage will be 300mg/day, per Lyrica® prescribing information.

<u>Concomitant analgesic medications:</u>

- Subjects treated with gabapentin or other anticonvulsant for neuropathic pain will be required to taper down the medication and to discontinue it at least 2 weeks prior to study initiation.
- Patients on antidepressant treatment for pain or depression (TCAs, SSRI, SNRIs etc. will be allowed to continue their medications provided they have been on a stable dose for at least 4 weeks before study initiation. No dose regimen changes of antidepressants will be allowed during the study period. Patients requiring dose regimen changes will be withdrawn from the study.
- Patients on around-the clock opioid treatment (including tramadol) will be allowed to continue their medication provided they have been on a stable dose for at least 4 weeks before study initiation. The maximum allowed dose of opioid will be equivalent to 60mg oral morphine sulphate. Patients with higher doses will be required to taper down their opioid dose to maximum 60mg oral morphine equivalent, and continue it for 4 weeks before enrollment in

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the study. PRN short-acting opioids for painful CIPN treatment will not be allowed. Patients receiving PRN short-acting opioids (with or without acetaminophen) for pain other than CIPN will be allowed up to 4 daily doses, with daily recording of analgesic consumption.

- Treatment with NSAIDs will be discontinued at least 2 weeks before study initiation. However, low-dose aspirin (≤325mg/day) will be allowed.

2.4.11 Drug procurement and storage

The study drug (pregabalin 75mg capsules) and a matching placebo will be provided by Pfizer Inc. The study drug and placebo are identical and indistinguishable in terms of physical appearance. Both medications will be stored in an OVCR approved narcotics cabinet in the Human Subjects Study lab in the Department of Anesthesiology. An unblinded research nurse coordinator will maintain the study drug accountability log and be unblinded to study drug assignment. The study drug and placebo will be labeled using subject study initials and Study ID number and dispensed by the unblinded nurse coordinator, who is not involved in any other part of the study and does not otherwise interact with participants during the study. The study drug prescription will include dosage instructions and pager number for the PI and study coordinator.

3.0 MANAGEMENT OF INTERCURRENT EVENTS

3.1 Adverse Experiences

The subjects will be monitored for evidence of adverse events. Patients will be prompted to report seven specific adverse events (somnolence, dizziness, edema, nausea, headache, dry mouth and blurry vision) on their EMA device daily throughout the study period. Patients will be required to report any adverse effects in their daily diary, and to report any potentially serious adverse effects to the study team. 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 to the study drug (none, unlikely, possible, probable, highly probable), and any treatment required.

As the study drug is supplied by Pfizer and this investigator-initiated trial is supported by Pfizer ASPIRE grant program, any serious adverse events will be reported to Pfizer via the completion of "Investigator-Initiated Research Serious Adverse Report Form", according to the Safety Reporting Reference Manual.

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.

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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

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event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

All the prescribing information, including clinical pharmacology, adverse effects, pharmacokinetics, dosage forms, storage, stability are included in the attached appendix – Lyrica USPI document.

3.2 Premature Discontinuation

If a subject chooses to discontinue treatment during the first treatment phase, he/she has the possibility either to withdraw from the study, or to continue the second phase of the study after a 2 week washout period.

Subjects will be withdrawn if they undergo a major surgery during the study period (any surgery that requires 2 or more days of hospitalization).

Subjects will be withdrawn from the study if during the study period they are placed on chemotherapy regimen that includes a taxane, a platinum compound or a vinca alkaloid.

Subjects will be withdrawn from the study if they require an increase in their other analgesic drugs, including chronic around-the-clock opioid dose; or need more than 4 PRN daily doses of a short-acting opioid.

Subjects will be withdrawn from the study in case of any of the following adverse events:

- Angioedema
- Hypersensitivity
- Suicidal behavior or ideation
- Peripheral edema in patients with New York Heart Association (NYHA) Class III or IV heart failure (in non-cardiac patients the peripheral edema due to pregabalin has not been associated with cardiovascular complications).

Subjects will be withdrawn if they experience any Grade 3 or higher adverse effect, if the investigator decides that discontinuation is in the best interest of the subject, or the subject requests withdrawal from the study.

3.3 Potential Risks

3.3.1 Potential risks from pregabalin

Potential adverse effects usually associated with pregabalin include dizziness, somnolence, xerostomia, headache, visual disturbances, peripheral edema and abnormal gait (all less than 15% prevalence).

Other common (>10% prevalence) adverse effects may include fatique, ataxia, tremor and weight gain. Less common (5-10% prevalence) adverse effects reported with pregabalin include abnormal thinking, confusion, euphoria, speech disorder, pain, insomnia and constipation. Rare but potentially serious adverse effects reported with pregabalin use are: angioedema, suicidal thoughts, allergic reaction, infection, hypertension, hypotension, amnesia, hypoglycemia, vomiting, thrombocytopenia, and nystagmus.

Subjects will be educated regarding the possible adverse effects, and these will be monitored through weekly screening of patient-reported dairy and weekly telephone follow-ups with the patient.

3.3.2 Potential risks from thermal testing

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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).

3.3.3 Potential risks from skin biopsy

Potential risks associated with 3-mm skin punch biopsy include local pain, infection, and minor bleeding. The pain is transient in nature, and local infiltration with lidocaine typically eliminates the pain. The procedure is performed by a trained health care professional using aseptic technique with sterile skin biopsy kit to minimize the risk of infection. Subjects will be educated regarding the possible risks, and are informed that providing a skin biopsy is voluntary, and refusal will not affect their participation in the study.

3.3.4 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.

3.4 Procedures to Minimize Potential Risks

Studies are conducted in the Washington University Clinical Research Center under the supervision of the PI and the co-investigator. The PI is trained and experienced in performing research in human subjects. The co-investigator is board certified oncologist with extensive experience in managing cancer patients.

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

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3.5 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
- 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
- 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.

4. HUMAN SUBJECTS RESEARCH

4.1 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, and co-PI, a board-certified oncologist with extensive clinical and human research experience.

4.2 Sources of Materials

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

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Data on comorbidities and concomitant medication use are provided by subjects as well as retrieval of medical records. Specimens include blood obtained for determining kidney and liver function for study eligibility, and for determining pregabalin plasma concentration. Urine specimen will be obtained from women of childbearing potential for a pregnancy test. Skin biopsy specimen (optional) is obtained from the foot for determining small nerve fiber density in the skin. Other data including baseline quantitative sensory testing are obtained exclusively for research purposes.

4.3 Recruitment and Informed Consent

Participants will be recruited primarily through Siteman Cancer Center and Washington University Pain Management Center, referred by the corresponding physicians. In addition, we will post flyers and recruit participants through Volunteers for Health organization. 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. Placebo administration is a part of the study, and the subjects will be informed that they will be receiving placebo at one of their study periods. 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.

4.4 Potential Benefits of the Proposed Research to the Subjects and Others

The potential benefit to the study subjects is a relief of their chronic pain associated with CIPN. There are limited data suggesting that pregabalin may be effective in this group of patients. Potential understanding of which patients are more likely to respond to pregabalin may lead to improved patient outcomes from which the study subjects and other subjects suffering from painful CIPN may potentially benefit in the future. The society may benefit from a new approach of optimizing treatment of neuropathic pain.

4.5 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.

4.6 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).

4.7 Inclusion of Children

Children <18 yr 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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5. REFERENCES

Aoki, M., Y. Kurauchi, A. Mori, T. Nakahara, K. Sakamoto and K. Ishii (2014). "Comparison of the effects of single doses of elcatonin and pregabalin on oxaliplatin-induced cold and mechanical allodynia in rats." Biol Pharm Bull **37**(2): 322-326.

Baumgartner, U., W. Magerl, T. Klein, H. C. Hopf and R. D. Treede (2002). "Neurogenic hyperalgesia versus painful hypoalgesia: two distinct mechanisms of neuropathic pain." Pain **96**(1-2): 141-151.

Bouhassira, D., N. Attal, H. Alchaar, F. Boureau, B. Brochet, J. Bruxelle, G. Cunin, J. Fermanian, P. Ginies, A. Grun-Overdyking, H. Jafari-Schluep, M. Lanteri-Minet, B. Laurent, G. Mick, A. Serrie, D. Valade and E. Vicaut (2005). "Comparison of pain syndromes associated with nervous or somatic lesions and development of a new neuropathic pain diagnostic questionnaire (DN4)." Pain **114**(1-2): 29-36.

Campbell, C. M., M. S. Kipnes, B. C. Stouch, K. L. Brady, M. Kelly, W. K. Schmidt, K. L. Petersen, M. C. Rowbotham and J. N. Campbell (2012). "Randomized control trial of topical clonidine for treatment of painful diabetic neuropathy." Pain **153**(9): 1815-1823.

Chaudhry, V., E. K. Rowinsky, S. E. Sartorius, R. C. Donehower and D. R. Cornblath (1994). "Peripheral neuropathy from taxol and cisplatin combination chemotherapy: clinical and electrophysiological studies." Ann Neurol **35**(3): 304-311.

Durand, J. P., G. Deplanque, V. Montheil, J. M. Gornet, F. Scotte, O. Mir, A. Cessot, R. Coriat, E. Raymond, E. Mitry, P. Herait, Y. Yataghene and F. Goldwasser (2012). "Efficacy of venlafaxine for the prevention and relief of oxaliplatin-induced acute neurotoxicity: results of EFFOX, a randomized, double-blind, placebo-controlled phase III trial." Ann Oncol **23**(1): 200-205.

Eckhoff, L., A. S. Knoop, M. B. Jensen, B. Ejlertsen and M. Ewertz (2013). "Risk of docetaxel-induced peripheral neuropathy among 1,725 Danish patients with early stage breast cancer." Breast Cancer Res Treat **142**(1): 109-118.

Finnerup, N. B., N. Attal, S. Haroutounian, E. D. McNicol, R. Baron, R. H. Dworkin, I. Gilron, M. Haanpaa, P. Hansson, T. S. Jensen, P. R. Kamerman, K. Lund, R. A. Moore, S. N. Raja, A. S. Rice, M. C. Rowbotham, E. Sena, P. J. Siddall, B. H. Smith and M. Wallace (2015). "Pharmacotherapy for neuropathic pain in adults: systematic review, meta-analysis and NeuPSIG recommendations." The Lancet Neurology **in Press**.

Finnerup, N. B., L. Sorensen, F. Biering-Sorensen, I. L. Johannesen and T. S. Jensen (2007). "Segmental hypersensitivity and spinothalamic function in spinal cord injury pain." Exp Neurol **207**(1): 139-149.

Fruhstorfer, H., U. Lindblom and W. C. Schmidt (1976). "Method for quantitative estimation of thermal thresholds in patients." J Neurol Neurosurg Psychiatry **39**(11): 1071-1075.

CONFIDENTIAL

Gauchan, P., T. Andoh, K. Ikeda, M. Fujita, A. Sasaki, A. Kato and Y. Kuraishi (2009). "Mechanical allodynia induced by paclitaxel, oxaliplatin and vincristine: different effectiveness of gabapentin and different expression of voltage-dependent calcium channel alpha(2)delta-1 subunit." Biol Pharm Bull **32**(4): 732-734.

Hahm, T. S., H. J. Ahn, S. Ryu, M. S. Gwak, S. J. Choi, J. K. Kim and J. M. Yu (2012). "Combined carbamazepine and pregabalin therapy in a rat model of neuropathic pain." Br J Anaesth **109**(6): 968-974.

Haroutiunian, S., L. Nikolajsen, T. F. Bendtsen, A. D. Kristensen and T. S. Jensen (2013). Contribution of peripheral sensory input in maintaining chronic neuropathic pain and predictors of response to intravenous lidocaine. 4th International Congress on Neuropathic Pain. Toronto, ON, Canada.

Haroutounian, S., L. Nikolajsen, T. F. Bendtsen, N. B. Finnerup, A. D. Kristensen, J. B. Hasselstrom and T. S. Jensen (2014). "Primary afferent input critical for maintaining spontaneous pain in peripheral neuropathy." Pain **155**(7): 1272-1279.

Haroutounian, S., L. Nikolajsen, T. F. Bendtsen, N. B. Finnerup, A. D. Kristensen, J. B. Hasselstrom and T. S. Jensen (2014). "Primary afferent input critical for maintaining spontaneous pain in peripheral neuropathy." Pain.

Hershman, D. L., C. Lacchetti, R. H. Dworkin, E. M. Lavoie Smith, J. Bleeker, G. Cavaletti, C. Chauhan, P. Gavin, A. Lavino, M. B. Lustberg, J. Paice, B. Schneider, M. L. Smith, T. Smith, S. Terstriep, N. Wagner-Johnston, K. Bak and C. L. Loprinzi (2014). "Prevention and management of chemotherapy-induced peripheral neuropathy in survivors of adult cancers: american society of clinical oncology clinical practice guideline." J Clin Oncol **32**(18): 1941-1967.

Hershman, D. L., L. H. Weimer, A. Wang, G. Kranwinkel, L. Brafman, D. Fuentes, D. Awad and K. D. Crew (2011). "Association between patient reported outcomes and quantitative sensory tests for measuring long-term neurotoxicity in breast cancer survivors treated with adjuvant paclitaxel chemotherapy." Breast Cancer Res Treat **125**(3): 767-774.

Jensen, T. S., F. W. Bach, J. Kastrup, A. Dejgaard and J. Brennum (1991). "Vibratory and thermal thresholds in diabetics with and without clinical neuropathy." Acta Neurol Scand **84**(4): 326-333.

Ling, B., N. Authier, D. Balayssac, A. Eschalier and F. Coudore (2007). "Behavioral and pharmacological description of oxaliplatin-induced painful neuropathy in rat." Pain **128**(3): 225-234.

Little, R. J., R. D'Agostino, M. L. Cohen, K. Dickersin, S. S. Emerson, J. T. Farrar, C. Frangakis, J. W. Hogan, G. Molenberghs, S. A. Murphy, J. D. Neaton, A. Rotnitzky, D. Scharfstein, W. J. Shih, J. P. Siegel and H. Stern (2012). "The prevention and treatment of missing data in clinical trials." N Engl J Med **367**(14): 1355-1360.

CONFIDENTIAL

Lynch, J. J., 3rd, C. L. Wade, C. M. Zhong, J. P. Mikusa and P. Honore (2004). "Attenuation of mechanical allodynia by clinically utilized drugs in a rat chemotherapy-induced neuropathic pain model." Pain **110**(1-2): 56-63.

Magerl, W., S. H. Wilk and R. D. Treede (1998). "Secondary hyperalgesia and perceptual wind-up following intradermal injection of capsaicin in humans." Pain **74**(2-3): 257-268.

Maier, C., R. Baron, T. R. Tolle, A. Binder, N. Birbaumer, F. Birklein, J. Gierthmuhlen, H. Flor, C. Geber, V. Huge, E. K. Krumova, G. B. Landwehrmeyer, W. Magerl, C. Maihofner, H. Richter, R. Rolke, A. Scherens, A. Schwarz, C. Sommer, V. Tronnier, N. Uceyler, M. Valet, G. Wasner and R. D. Treede (2010). "Quantitative sensory testing in the German Research Network on Neuropathic Pain (DFNS): somatosensory abnormalities in 1236 patients with different neuropathic pain syndromes." Pain **150**(3): 439-450.

Miyazaki, R. and T. Yamamoto (2012). "The efficacy of morphine, pregabalin, gabapentin, and duloxetine on mechanical allodynia is different from that on neuroma pain in the rat neuropathic pain model." Anesth Analg **115**(1): 182-188.

Nihei, S., J. Sato, M. Kashiwaba, T. Itabashi, K. Kudo and K. Takahashi (2013). "[Efficacy and safety of pregabalin for oxaliplatin- and paclitaxel-induced peripheral neuropathy]." Gan To Kagaku Ryoho **40**(9): 1189-1193.

Peng, P., Q. Xi, S. Xia, L. Zhuang, Q. Gui, Y. Chen, Y. Huang, M. Zou, J. Rao and S. Yu (2012). "Pregabalin attenuates docetaxel-induced neuropathy in rats." J Huazhong Univ Sci Technolog Med Sci **32**(4): 586-590.

Polomano, R. C. and G. J. Bennett (2001). "Chemotherapy-evoked painful peripheral neuropathy." Pain Med **2**(1): 8-14.

Price, D. D., J. W. Hu, R. Dubner and R. H. Gracely (1977). "Peripheral suppression of first pain and central summation of second pain evoked by noxious heat pulses." Pain **3**(1): 57-68.

Rao, R. D., J. C. Michalak, J. A. Sloan, C. L. Loprinzi, G. S. Soori, D. A. Nikcevich, D. O. Warner, P. Novotny, L. A. Kutteh, G. Y. Wong and G. North Central Cancer Treatment (2007). "Efficacy of gabapentin in the management of chemotherapy-induced peripheral neuropathy: a phase 3 randomized, double-blind, placebo-controlled, crossover trial (N00C3)." Cancer **110**(9): 2110-2118.

Simpson, D. M., G. Schifitto, D. B. Clifford, T. K. Murphy, E. Durso-De Cruz, P. Glue, E. Whalen, B. Emir, G. N. Scott and R. Freeman (2010). "Pregabalin for painful HIV neuropathy: a randomized, double-blind, placebo-controlled trial." Neurology **74**(5): 413-420.

Smith, E. M., H. Pang, C. Cirrincione, S. Fleishman, E. D. Paskett, T. Ahles, L. R. Bressler, C. E. Fadul, C. Knox, N. Le-Lindqwister, P. B. Gilman, C. L. Shapiro and O. Alliance for Clinical Trials in (2013). "Effect of duloxetine on pain, function, and quality of life among patients with chemotherapy-induced painful peripheral neuropathy: a randomized clinical trial." JAMA **309**(13): 1359-1367.

CONFIDENTIAL

Speck, R. M., M. D. Sammel, J. T. Farrar, S. Hennessy, J. J. Mao, M. G. Stineman and A. DeMichele (2013). "Impact of chemotherapy-induced peripheral neuropathy on treatment delivery in nonmetastatic breast cancer." J Oncol Pract **9**(5): e234-240.

Treede, R. D., T. S. Jensen, J. N. Campbell, G. Cruccu, J. O. Dostrovsky, J. W. Griffin, P. Hansson, R. Hughes, T. Nurmikko and J. Serra (2008). "Neuropathic pain: redefinition and a grading system for clinical and research purposes." Neurology **70**(18): 1630-1635.

Xiao, W., A. Boroujerdi, G. J. Bennett and Z. D. Luo (2007). "Chemotherapy-evoked painful peripheral neuropathy: analgesic effects of gabapentin and effects on expression of the alpha-2-delta type-1 calcium channel subunit." Neuroscience **144**(2): 714-720.

Yarnitsky, D., M. Granot, H. Nahman-Averbuch, M. Khamaisi and Y. Granovsky (2012). "Conditioned pain modulation predicts duloxetine efficacy in painful diabetic neuropathy." Pain **153**(6): 1193-1198.

Yarnitsky, D., E. Sprecher, R. Zaslansky and J. A. Hemli (1995). "Heat pain thresholds: normative data and repeatability." Pain **60**(3): 329-332.

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