University of Pittsburgh | Department of Anesthesiology and Perioperative Medicine School of Medicine

Statistical Analysis Plan (SAP)

Title	PACE-UP: Personalized Analgesia through Central Nervous System Engagement to Unlock Performance
Department/Division/Center	Anesthesiology and Perioperative Medicine
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1 Study Overview

Aerobic exercise such as walking is important in preventing joint deterioration and is a first-line treatment for knee osteoarthritis, despite being painful. Indeed, many patients are limited in their ability to walk due to pain. Prior literature and preliminary data from our group indicate that central nervous system (CNS) changes contribute to pain-related walking impairment. While acetaminophen, nonsteroidal anti-inflammatories, opioids, and surgery are often used in knee osteoarthritis, side effects and health risks limit their use in many patient subgroups. In patients with CNS changes, these treatments may be less effective. In patients with nociplastic pain features related to CNS sensitization, knee replacement is associated with worse post-operative pain and functional outcomes. Potentially targeting CNS sensitization, duloxetine is the only CNS-acting medication FDA-approved for knee osteoarthritis pain and given that patient-reported physical function improves with duloxetine, its use may slow joint degeneration and delay the need for knee replacement by improving walking ability. However, no clinical trials have investigated whether duloxetine improves objectively measured physical function. This study will address this gap and lead to future large-scale studies examining knee osteoarthritis endophenotypes, related to CNS pain processing and other pathophysiology, which may aid in identifying patients who respond better to duloxetine versus other existing therapies. Techniques used in the setting of this randomized clinical trial include patient-reported outcomes, physical performance tests, actigraphy, noninvasive brain imaging, and quantitative sensory testing.

1.1 Study Aims

The goal of the current study is to examine whether a centrally acting pain therapy improves walking ability and facilitates activity in patients with pain during walking.

Research question: In knee osteoarthritis (OA) patients with pain during walking, does duloxetine improve walking ability and physical activity?

1.2 Study Hypotheses

1.2.1 Primary Hypothesis

Hypothesis: In knee OA, duloxetine increases 6-minute walk test distance (6MWT) compared with placebo.

Primary Endpoint: 6-minute walk test distance (6MWT)

1.2.2 Secondary Hypotheses

Hypothesis 2.1: In knee OA, duloxetine decreases pain intensity during the 6MWT
Secondary Endpoint: maximum pain intensity (0-10 numeric rating scale, NRS) of the index knee during the 6MWT

Hypothesis 2.2: In knee OA, duloxetine decreases walking-evoked pain during the 6MWT
Secondary Endpoint: pain change during the 6MWT at the index knee (post NRS – pre NRS)

Hypothesis 2.3: In knee OA, duloxetine increases offset analgesia to be more analgesic
Secondary Endpoint: offset analgesia index (quantitative sensory test)

Hypothesis 2.4: In knee OA, duloxetine improves conditioned pain modulation efficiency to be more analgesic
Secondary Endpoint: condition pain modulation index (quantitative sensory test)

Hypothesis 2.5: In knee OA, duloxetine reduces BPI-pain measured with the home pain diary

Hypothesis 2.6: In knee OA, duloxetine reduces BPI-walking interference measured with the home pain diary

Hypothesis 2.7: In knee OA, duloxetine improves patient-reported knee pain and on the KOOS-pain and KOOS-ADL subscales

1.2.3 Exploratory Analyses

Given the mechanistic nature of this study, additional data collection and analyses are planned. This will yield novel mechanistic insights. As with all human neuroscience and clinical research, replication of results from both additional analyses and primary and secondary hypothesis testing will be important.

Mediation analyses will be conducted to explore the relationship between endogenous pain modulation and walking pain and function. With treatment as independent variable, we will examine whether change in offset analgesia mediates the outcomes of 6MWT distance. Three other mediation analyses will be done including testing for mediation effects of change in offset analgesia on 6MWT maximum pain, change in conditioned pain modulation on 6MWT distance, and change in conditioned pain modulation on 6MWT maximum pain.

Additionally, we will explore predictive models of 6MWT pain and distance using data collected prior to randomization. Mechanistic analyses will examine duloxetine (vs placebo) and effects over time regardless of treatment period on physiologic (e.g. noninvasive brain imaging with fNIRS) and biomechanical measures (e.g. wearable sensors) during study tasks including physical function tests and at rest. Effects on activity at home will also be examined using a combination of self-reported metrics and data from wearable sensors.

In the study design (Section 2 below), there are two measures of noninvasive brain imaging, physical function tests, and quantitative sensory tests performed at the phenotyping visit (Study Visit 2) and the baseline visit for the first cross-over period (Study Visit 3). Test-retest analyses will be conducted on relevant measures, including offset-onset balance, a novel measure of pain modulation.

2 Trial Design

Using a double-blind, cross-over, 16-week randomized clinical trial (RCT) of duloxetine versus placebo, this study will investigate whether duloxetine improves walking ability and physical activity in knee osteoarthritis patients (N=85).

Study timeline: There is a screening visit (Study Visit 1) followed by a phenotyping visit (Study Visit 2) which includes comprehensive collection of patient-reported outcomes, physical performance tests, noninvasive brain imaging, and quantitative sensory testing. Eligibility for randomization is determined by significant knee pain severity and walking pain interference measured with a week-long pain diary. Eligible participants are randomized to cross-over order (duloxetine – placebo vs. placebo – duloxetine) and enter into the cross-over RCT phase, which includes a baseline (Study Visit 3 & 5) and follow-up visit (Study Visit 4 & 6) that bracket two treatment periods such that there are four study visits (Study Visits 3-6) collecting primary, secondary, and exploratory outcomes before and after duloxetine and placebo for each participant.

3 Study Population

3.1 Inclusion Criteria

- Knee pain for greater than 6 months.
- Moderate-to-severe knee pain (>3/10) due to osteoarthritis, as defined by American College of Rheumatology and EULAR diagnostic criteria (Zhang 2010, Altman 1986) on >50% of days in the past month.
- KL grade 2-4 (Kellgren and Lawrence 1957) indicating significant degenerative changes on knee X-ray. This will be determined on evaluation of weight-bearing clinical X-rays on review of medical records. In the infrequent situation that the patient does not have an X-ray on review of their medical records, then the patient will obtain weight-bearing knee X-rays as part of the Screening Visit.
- Age: 45-80 years old
- Gender: Males and females will be recruited.
- Language: only English-speaking subjects will be included.

3.2 Exclusion Criteria

- Inflammatory arthritis (e.g., rheumatoid arthritis).
- More intense pain due to another chronic pain syndrome (e.g., fibromyalgia, hip osteoarthritis)
- Significant pain or weakness in the lower extremities due to a neurological condition (e.g., lumbar radiculopathy, paresis due to stroke)
- New, ongoing acute pain
- Recent use of duloxetine defined as any use within the last 3 months - Current use of antidepressants or tramadol - Current routine use of more than 30 mg oral morphine equivalents per day (use of ≤30 mg OME does not exclude the participant).

- New treatment that may help knee osteoarthritis (including medication, exercise, behavioral, or complementary and integrative treatments) started in the last month. Stable use of treatments for greater than 1 month does not exclude the participant.
- Unwillingness to maintain current knee osteoarthritis treatments for the duration of the study. This means that eligible participants must plan for the following: medications and dietary supplements should not change substantially in frequency or dose; behavioral therapy, exercise therapy, and complementary and integrative therapies should be consistent; and there should be no interventional treatments planned for lower extremity musculoskeletal conditions (e.g., joint steroid injections, knee radiofrequency ablation, knee replacement surgery).
- Recent knee intra-articular injection of steroid or another agent (greater than 1 month does not exclude the participant)
- Recent knee radiofrequency ablation (greater than 3 months does not exclude the participant)
- Recent knee arthroscopic surgery (greater than 3 months does not exclude the participant)
- History of knee replacement or open knee surgery on the index knee, defined as the more painful knee on average over the last month.
- Inability to walk or climb stairs without significant assistance (e.g., a one-person assist, use a wheelchair; use of a cane does not exclude the participant).
- Inability to participate in study procedures (e.g., cognitive impairment limiting ability to understand directions, inability to understand and read English)
- Uncontrolled or unstable medical disorder preventing participation in study procedures
- History of brain surgery
- Tattoos on sensory testing sites
- Pregnancy
- History of severe renal impairment defined by GFR < 30 ml/min or, if renal function testing is not available on EMR review, patient-reported history of acute or chronic kidney disease of any severity
- History of chronic liver disease or cirrhosis
- History of angle-closure glaucoma

4 Outcomes, Exposures, and Additional Variables of Interest

4.1 Primary Endpoint(s)

Outcome	Description	Specifications
6-minute walk test distance (6MWT)	The primary endpoint of walking ability will be captured with distance walked on the 6MWT, performed at Visits 4 and 6 and compared with baseline walk distance (Visits 3 and 5).	Continuous variable Expected range: 500 feet -2500 feet based on prior study

4.2 Secondary Endpoint(s)

Outcome	Description	Specifications

Maximum pain intensity during the 6MWT	Maximum pain intensity of the index knee during the 6MWT	Continuous variable Numeric rating scale (NRS) Expected range: 0-10 scale
Pain change during the 6MWT at the index knee	Difference in index knee pain intensity (NRS) after 6MWT from before 6MWT	Continuous variable Difference in NRS Expected range: -10 – 10 with positive values reflecting increasing pain intensity during the 6MWT and negative values reflecting decreasing pain intensity during the 6MWT.
Offset analgesia index	Quantitative sensory test using dynamically changing heat stimuli applied to the skin that reflects temporal aspects of endogenous pain modulation. Calculated as a relative within stimulus pain intensity difference (100 * [min – max]) / max; see [1] for details)	Continuous variable Relative difference in computerized visual analogue scale Expected range: -100 – 0 with more negative values reflecting greater offset analgesia
Conditioned pain modulation index	Quantitative sensory test using two painful stimuli on remote body parts to measure spatial aspects of endogenous pain modulation. Calculated as a percent change in pressure pain threshold (PPTh, primary painful stimulus) when the hand is immersed in a cold water bath (second "conditioning" stimulus): 100 * (PPTh during the cold water bath / baseline PPTh). See [2].	Continuous variable Relative difference in pressure pain threshold Expected range: 0 – 1000 with more positive values reflecting greater conditioned pain modulation. See [1] for application to study population.
Brief Pain Inventory - Pain (BPI-pain)	Measures index knee pain on a 0-10 scale. Mean of daily BPI-pain responses collected over a one week period	Continuous variable Expected range: 0-10 scale with higher values reflecting more intense knee pain
Brief Pain Inventory – Pain Interference - Walking Ability	Single-item measuring how pain has interfered with walking ability in the last 24 hours. Mean of daily BPI-pain interference – walking ability responses collected over a one week period	Continuous variable Expected range: 0-10 scale with higher values reflecting more pain interference with walking ability.
Knee Injury and Osteoarthritis Outcome Score	A 42-item patient reported questionnaire assessing five knee-related areas: pain (9 items), symptoms (7), daily activities (17), sports/recreation (5), and quality of life (4).	Continuous variable Scores are converted to a 0-100 scale. Expected range: 0-100 scale Scores of 0 indicate extreme pain and scores of 100 represent no symptoms/perfect knee health.

4.3 Additional Variables (covariates) of Interest

Age, gender, race/ethnicity, body mass index, and others may be included as covariates in multivariable models constructed to test hypotheses above.

5 Randomization Scheme

Randomization will be assigned in a 1:1 ratio of duloxetine-placebo or placebo-duloxetine using a permuted block design with block sizes of 4 and 6.

6 Masking/Blinding

In randomized clinical trials it is important to mask the research team and participants to the interventions to which the study participant has been randomly assigned. Not doing so may introduce bias in the assessment of the outcomes.

7 Adherence to treatment

The number and proportion of patients who did not receive the treatment they were allocated to will be reported.

8 Statistical Analysis Plan

8.1 Demographic and Clinical Characteristics ("Table 1")

Descriptive statistics will be presented and examined for all relevant measures among all participants of the RCT. Examination of distributional assumptions for all study measurements will be performed, and transformations will be considered on the basis of distribution diagnostics and outlier analysis. Categorical variables will be summarized by frequencies and percentages. Continuous variables will be summarized using standard measures of central tendency and dispersion, using the mean and standard deviation for normally distributed continuous data, and median and interquartile range for non-normally distributed continuous data.

For all aims, the statistical analyses will begin by comparing the baseline characteristics of the samples by randomization sequence (duloxetine-placebo versus placebo-duloxetine). Chi-square test or Fisher's Exact test will be used to determine differences in categorical baseline characteristics between the two groups. Two-sample t-test or Wilcoxon rank-sum test will be used to determine differences in continuous baseline characteristics between the two randomization orders.

8.2 Analysis Plan for Primary Hypothesis

For the primary hypothesis, the intention-to-treat (ITT) principle will be used for the primary comparison in the randomized controlled trial (RCT) of duloxetine versus placebo. Thus, all participants who enter the RCT will be included in the analysis. If adherence to the protocol is lower than expected a per protocol analysis will be conducted as a sensitivity analysis. The study will be conducted using a two-sided alternative hypothesis and $\alpha=0.05$.

The primary hypothesis will determine the effect of duloxetine versus placebo on walking performance as measured by 6-minute walk test distance (6MWT). We hypothesize that duloxetine increases 6-minute walk test distance (6MWT) compared with placebo. The comparison of the primary outcome, 6-minute walk test distance, will be using linear mixed effects model, which accounts for within participant correlation across periods. Fixed effects will include sequence (duloxetine-placebo versus placebo-duloxetine), period (period 1 versus period 2), and treatment (duloxetine versus placebo). Random effect will include participant nested within sequence to model repeated measures on the same participant. We will assess carryover effect by examining the sequence by period interaction effect. A statistically significant interaction effect

will indicate a potential carryover effect. If violations of the assumptions are identified, transformations will be investigated, or nonparametric models will be used. Kenward-Roger degrees of freedom method will be used for accurate type I error in small sample crossover trial. Linear mixed effects model will also be used to control for baseline characteristics not balanced through random assignment.

The analysis for the primary outcome will then be repeated on a per protocol sample as a sensitivity analysis, excluding participants in either intervention arm who had a recorded protocol deviation during the course of the study.

8.3 Analysis Plan for Secondary Hypotheses

The same analytical approach used for the primary outcome will be implemented for the continuous secondary endpoints (e.g., KOOS-pain, KOOS-ADL, etc.). Unadjusted and Benjamini-Hochberg adjusted p-values will also be presented to account for multiple hypothesis testing (i.e., multiple comparison correction).

8.4 Analysis Plan for Exploratory Analyses

Mediation analysis will be performed using standard techniques such as those described by [3]. Physiologic and biomechanical analyses will be performed using standard techniques in the field similar to [4-7]. For test-retest reliability of the continuous variable, offset-onset balance (novel QST measure), we will use two-way mixed model intraclass correlation coefficient (absolute agreement).

9 Sample Size and Power

The sample size calculation was based on the study primary endpoint. 6-minute walk test (6MWT) distance power analysis – using minimal clinically important difference (MCID) and standard deviation (SD) for change in 6MWT after knee replacement. In cardiopulmonary trials, 6MWT has no placebo effect. The MCID for 6MWT after knee replacement = 70m (SD 90 m) (King2022). Using an ANOVA 2x2 cross-over design testing for the difference in means between the duloxetine period and placebo period, 66 participants allow detection of a moderate effect (Cohen's d of 0.5) at a power of 0.8. Accounting for drop out during the RCT phase and ensuring at least 66 participants with complete data, we propose randomizing 75 participants into the RCT portion and enrolling 85 participants total to meet these accrual goals.

10 Missing Data

Missing data can plague any study and negatively impact the study's ability to address the specific aims. The best solution to the problems caused by missing data is the elimination or reduction of missing data. To prevent, or minimize the amount of missing data, we will strive for due diligence in careful data collection, data management and quality control procedures. When missing data occur, we will recommend imputation approaches based on the missing pattern (e.g., missing completely at random or missing at random).

Missing data will be investigated in an effort to understand how they are missing: (1) missing completely at random (MCAR), (2) missing at random (MAR) or (3) non-ignorable. Little's test will be performed and if the test indicates that the complete case data set is a random sample, we will continue without imputing missing values. If Little's test indicates that the data set of complete cases is not a random sample, we will report the point estimates and their 95% confidence intervals (CI) by applying a worst/best scenario imputation for the missing values. If the worst/best case analyses allow for the same conclusion, we will not perform multiple imputations. However, if

the worst/best case imputation provides different conclusions, multiple imputations will be performed, creating ten imputed data sets under the assumption of missingness at random.

11 References

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